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From Life Molecules to Global Health

José Fragata
(Coordinator)

PRINCIPIA

FROM LIFE MOLECULES TO GLOBAL HEALTH

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NOVA University Lisbon

FROM LIFE MOLECULES TO GLOBAL HEALTH

PRINCIPIA

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FOREWORD

Amélia de Mello Foundation

In the context of the celebrations of the 150 years of the birth of Alfredo da Silva, arose the opportunity to carry out a study in the area of health in which the Rectory of the UNL – University of Lisbon played the leading role.

The Vice-Rector, Professor José Fragata, prosed an approach with the objective of making a project that would incorporate a global and fully holistic vision of topics, that would not only present works that could embrace an exhaustive listing of topics, but most importantly, would cover a vast team of professors and researchers at UNL, thus giving the readers who are interested a very complete perspective of the state of the art of the Academy's contribution related to the level of research and practices linked to medicine.

This seems to us that one can perceive the most focused themes and the potential impact for society in general and its sustainability in an individual vision and in a logic of what should be followed, and which responsible behaviours adopt to lead to a quality of life coherent with the commitments for an ethically responsible «perpetuity».

Therefore, it is worth noticing that this piece of work fully achieves the objectives that were initially defined with Professor José Fragata, to whom we express our deepest gratitude for this magnificent demonstration of the produced knowledge and the teamwork that is apparent in the texts now available. We would also like

to extend our gratitude to the vast UNL team which collaborated in the execution of this desideratum.

The Amélia de Mello Foundation, heir of a culture and commitment focused on People, an example of which is the CUF Hospital created in 1945, by supporting the publication of this book reinforces its commitment to responsibility and continued support for the initiatives it has supported since 1964 in the areas of Education and Health.

This Mission, created by the Founder Manuel de Mello, husband of Amélia, daughter of Alfredo da Silva, is thus duly consecrated, which is a source of pride for our José de Mello Families.

3 November 2021

PREFACE

António Rendas

It is a great pleasure, and honour, for me to write this forward message to the book entitled: "From life molecules to global health", a genuine footprint of NOVA Health, an institutional platform that reflects the commitment of NOVA University towards biomedical, clinical, and health research, based on bidirectional pathways linking bench, bedside, and human population.

Research and action cutting across disciplines and scientific areas is essential to human life survival in the twenty-first century but it is a very difficult task, as the current health crisis, created by the COVID-19 pandemic, is demonstrating every day.

While scanning through the chapters I could not help to remember two paintings with the same title: "Le déjeuner sur l'herbe" painted with a small interval, around three years, by Édouard Manet and Claude Monet, in the late nineteenth century. The landscapes where both meals take place represent a balance between lights and shadows, populated by human figures, controversial in Manet and formal in Monet. Relating science to nature, in its multiple facets, has been the dream of humankind, from disruptive to fashionable approaches but always with an insatiable curiosity and fearless work. This was the main flavour that I sensed from all the chapters of the book, in addition to the obvious scholarly competence.

In a more contemporary setting, the content of the book is online with the most advanced European initiatives being implemented transnationally, in recent

years, to tackle health problems using a multi-system approach. Such is the case of the LifeTime Initiative (1) which aims to track, understand, and target human cells during the onset and progress of complex diseases. At the time of the mentioned publication, the TimeLine Initiative included more than 90 research institutions, 80 companies, several funding agencies, and national academies. In 2019, the initiative was awarded a Coordination Support Action by the European Commission to develop a Strategic Research Agenda (SRA) for a large-scale, long-term initiative with a road map to implement cell-based interceptive medicine in Europe, in the next decade. It is a very ambitious project that is making its first steps but with two special distinctive assets: first, it relies on already existent resources and attempts to connect them in close network, second, it returns to the cellular approach to disease, now enriched with all the new capabilities labelled as single-cell technologies which are generating the first reference atlases of healthy tissues and organs and are revealing the extraordinary diversity of cell subtypes and functionally distinct cell states. It is expected that this omics approach, particularly spatial transcriptomics, will include information on the locations of diseased cells, their molecular structure, and aberrant cell to cell communication. This methodology will then be applied to hundreds of thousands of patient cells using imaging methodologies and machine learning. Furthermore, the third component of the initiative will develop patient-derived experimental disease models, including organoids to study tissue-tissue and organ-organ interactions. The dimension of this project is immense, but it is only by planning at such a level that Europe will remain competitive on a global scale. The Life Initiative also includes plans to interact with industry and to promote innovation together with addressing ethical and legal issues. Last, but not least, it will develop training activities based on a multi-professional educational approach with an inclusive philosophy involving researchers, clinicians, technical staff, managers, patients, and the lay public. In synthesis, it will contribute to consolidating a European health ecosystem and can be used as the benchmark for national, regional, or institutional projects with similar global approaches to health.

NOVA Health was launched as an ambitious project aiming to mobilize the existing resources and leverage further initiatives, coming from different scientific areas within NOVA University, related to health, in multiple dimensions This book, written a few years later, reflects how the initiative matured and became a success recognized by the response of the NOVA academic community expressed, amongst other signs, in chapters written by multiple authors coming from different academic units. Another special trait that deserves recognition is the high, sometimes

exceptional, quality of the research performed, unique to the NOVA Health and Biomedical ecosystem. It is also very clear the great growth potential of some areas if a more in depth multi and interdisciplinary approach is pursued in the future.

The quality of human life is a constant balance between the structure and the function of our molecules, cells, organs, and whole body, both internally and externally. Learning more about these complex relations requires outstanding research and a global vision of human health, from molecules to quality of care and to the social determinants of quality of life. In these times of turmoil, I wish to congratulate the Rector João Sàágua and the Vice-Rector José Fragata, and through them all the authors of the book, not only for having kept the ship floating but also for maintaining a successful route.

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INTRODUCTION

José Fragata
João Sàágua

It is our great privilege to introduce this book entitled *From Life Molecules to Global Health*, that is, indeed, a roadshow of NOVA's greatest contributions to biology and health.

NOVA has a strong commitment to health sciences, an effort that encompasses all of our faculties and spans from the basic structure of life to the global health of populations. Our contributions to healthcare are naturally aligned with UN developmental goals and extend from cutting edge research to innovative teaching, while seeking solutions that might be of value to communities.

We have challenged our researchers to contribute to this "life construct book" providing evidence of their most prominent research. This book includes contributions relating to the basic structure of life, bioengineering, normal living, human diseases and global health, as well as topics belonging to the "other face of Medicine" – ethics, business, economics, medical education, data science and digital technology.

Our academic community responded promptly to the request for producing this amazing science display that truly represents the strength of the University in Health Sciences.

We thank them for their individual and group contributions and congratulate them proudly for making part of NOVA's ever growing health elite.

Sustainable and good quality healthcare is only possible through knowledge advancement and technical innovations, these being led by the constant translation between fundamental sciences and the clinical reality that reaches out to populations and communities. One should remember that healthcare innovation is only truly effective when it extends to everyone, particularly those in lowest rung of economic development, then becoming fair and remaining sustainable. That is what this book is all about.

From reading this book, one can extract three main inspirational ideas:

First, the strength of academy, second, the merits of scientific integration and finally, the need for a holistic approach to the science and practice of healthcare.

This book reflects the power of our University, not only by the robustness of its scientific production, the extension or the integration of the fields it covers, but also by the capacity of our scientists to respond and deliver as a group – group that ultimately works to improve the health of everyone and all.

Also, this book demonstrates the extraordinary merits of science integration and scientific cooperation among our researchers, their research centres and faculties. This has allowed the majority of sections in this book to be written under partnerships between NOVA research units, a practice that extends far beyond the contents of the book and represents the strong production capability of science at NOVA.

Finally, it expresses the imperious need to approach healthcare from a holistic viewpoint – *from life molecules to bed side* – as this is the only way to get the full picture, to address sustainable innovation and respond to continuous change.

Presently and for the future, health of individuals will depend more and more on personalized DNA-chain interplays, while, at the present, we are fighting the impact of largescale pandemics by RNA manipulation strategies and, ultimately, produce vaccines in record time... However, sustainable healthcare solutions also need to be mindful of economics and business knowledge, also keeping in line with ethics and retaining human empathy and compassion. Only by using this holistic view, thinking healthcare globally but acting locally, will health challenges be addressed in this ever changing and challenging world.

This book perspires the leading vision of health sciences at NOVA – a vision that fosters healthcare innovation through science, using technology and surfing the digital, while never relinquishing the humanity that is inherent to the art of medicine and inseparable from healthcare.

We heartily thank Fundação Amélia de Mello for kindly sponsoring this book and, once more, we thank the authors for their timely and outstanding contributions.

CHAPTER I

THE STRUCTURE OF LIFE

A

MOLECULES OF LIFE – STRUCTURE OF PROTEINS

Maria João Romão
Margarida Archer
Eurico J. Cabrita
Cláudio M. Soares

1. INTRODUCTION

Cells are made up of many molecules, well-orchestrated to perform essential functions and give rise to what we call Life. Molecules are the building blocks of life and crucial for the well function of living cells, where tens of thousands of biomolecules are found. These are usually classified in two general groups: Biomacromolecules (e.g. proteins and nucleic acids) with a high molecular mass that are relatively unstable when isolated from the native environment and low molecular mass organic molecules that are smaller and chemically more robust (e.g. amino acids, nucleotides, fatty acids and carbohydrates).

Biological macromolecules are polymers of simple monomeric building blocks. Nucleic acids (DNA and RNA) are remarkable molecules that include only 4 types of monomers (4 chemical "letters"), containing the genetic information and playing a key role in protein biosynthesis. Proteins are much more complex molecules, constructed by a chain of 20 (or more, in certain organisms) different amino acids residues organized in a specific order (sequence). They are the most abundant and functionally versatile macromolecules in the cell playing key roles in essential biological processes from signal transduction, transport and metabolic pathways, to structural and storage roles. These physiological functions involve interactions among different proteins as well as binding to other biomolecules.

Since function is intrinsically related with structure, understanding protein function in health and disease strongly depends on the knowledge of the three-dimensional structures of the intervening biological macromolecules at an atomic level. This is a tremendous task that corresponds to the identification and location of thousands to millions of atoms (e.g., ~10,000 in hemoglobin, ~90,000 in the 50S ribosome and ~4000,000 in the HIV capsid). Thanks to enormous advances in Structural Biology that took part in the second half of the XXth century, we can nowadays solve this problem for essentially all types of molecules, which led to a remarkable revolution in Biological and Medical Sciences. To this date (April 2021) ~177,000 3D structures are freely available at a worldwide archive for macromolecular structures data, the Protein Data Bank (www.rcsb.org/).

2. INTEGRATED STRUCTURAL BIOLOGY AND ITS IMPACT TO SCIENCE

Integrated Structural Biology is an area of knowledge in full expansion, marked by an accelerated pace with which new structures of proteins and nucleic

acids are determined. These structures have been disclosed by X-ray crystallography, Nuclear Magnetic Resonance (NMR) and single particle analysis Cryo-Electron Microscopy (Cryo-EM). The integration of these techniques is crucial to provide more information on the structural and functional aspects. All three techniques are nowadays used to solve 3D structures of biological macromolecules and, depending on the problem under study, integrated studies are the preferred choice. For example, NMR techniques are particularly important for the analysis of protein-protein and protein-ligand interactions, while X-ray crystallography would be more adequate to elucidate atomic details of enzyme active sites and provide insights into their reaction mechanisms. Cryo-EM realm is more toward large biomolecules and biomolecular complexes.

These techniques, X-ray crystallography, NMR spectroscopy and Cryo-EM, require access to very sophisticated equipment and dedicated infrastructures. These infrastructures are usually networks of research institutions at national and international level that provide open access to high-end technologies to European researchers. Instruct-ERIC is a pan-European research infrastructure in structural biology that offers services and access to large Research Infrastructures (RI) in Europe. Portugal is an Affiliate Centre of Instruct-ERIC through the Portuguese Centre for Integrated Structural Biology (PCISBIO, coordinated by M. Arménia Carrondo) and the scientific community has periodic access to Synchrotron facilities.

Researchers at NOVA play crucial roles at the Portuguese Roadmap of RI, which include the National NMR Network (PTNMR, with nodes in ITQB-NOVA and FCT-NOVA, coordinated by Eurico Cabrita) and the National Advanced Electron Microscopy Network for Health and Life Sciences (CRYOEM-PT) proposed by NOVA in 2019.

Structural Biology techniques have a tremendous impact in many scientific areas. Over the past century, many scientists have made seminal scientific achievements directly related to (or involving the use of) crystallography and were awarded numerous Nobel Prizes (for details see https://www.iucr.org/people/nobel-prize). As an example, to highlight the continuing importance of crystallography, the year 2014 was proclaimed by the United Nations General Assembly as the International Year of Crystallography (IYCr). At FCT-NOVA, IYCr2014 celebrations comprised a series of lectures that included the visit of 3 Nobel laureates: Robert Huber, Nobel Prize in 1988 for discovering the structure of the bacterial photosynthetic reaction center; Ada Yonath, Nobel Prize in 2009 for decoding the molecular complexity of the ribosome, and Brian Kobilka, Nobel Prize in 2012 for elucidating the structure and function of G protein-coupled receptors.

2.1. Advanced Technologies and Methodologies

X-ray Crystallography is by far the most used technique to elucidate the three-dimensional structure of biological macromolecules (88% of the structures currently deposited in the PDB were characterized using this technique). It is first required to grow crystals of the macromolecule whose structure is to be determined (Figure 1). The crystal is then irradiated by an X-ray beam that interacts with the electron clouds of the atoms in the crystal and gives rise to a complex pattern of diffracted beams which are recorded by a detector as spots (the diffraction pattern). Encoded in this pattern is information about the positions of all the atoms in the crystal. Additional experiments and specific computational programs are needed to calculate an electron density map, from where an atomic model of the biomolecule is built. This model is then refined against the experimental data until a good quality 3D structure is obtained (1–3).

The recent technological breakthroughs in electron microscopy has led to a "resolution revolution" in **single particle cryo-Electron Microscopy (cryo-EM**). It only needs low amounts of non-crystalline material, a major advantage when compared to X-ray crystallography. 3D structures solved by this technique are being reported at an unprecedented pace, in particular membrane proteins and large macromolecular assemblies that are resilient to crystallise, leading to a new era in structural biology. Electrons can be used to "look" at protein structures, as proteins scatter electrons approximately four orders of magnitude stronger than X-rays, and electrons can be accelerated in high voltage electric fields (~300 kV) to wavelengths (10^{-12} m) that are much smaller than the distances between atoms in protein structures. Moreover, electrons can be focused with electro-magnetic lenses, so electron microscopes can be built to make images with atomic-level details.

Nuclear Magnetic Resonance (NMR) spectroscopy is a non-destructive technique that plays a major role in the determination of structures, dynamics and interaction of proteins and other biological macromolecules. NMR spectroscopy is based on the magnetic properties (spin) of atomic nuclei such as ^{1}H, ^{13}C,^{15}N, ^{31}P or ^{19}F. In a typical NMR experiment samples are placed inside a powerful magnet in order to allow the nuclear spins or magnetic moments of the sample to align with the magnetic field, then an electronic coil surrounding the sample generates a radiofrequency pulse that moves the spins out of alignment with the magnetic field. When the spins return to their alignment with the magnetic field they generate a

response signal that can be detected by the same electronic coil. Differences in response of the sample nuclei are related to the nearby electronic density and influence of other nuclei and provide detailed information about molecular structure, dynamics, and more. The manipulation of nuclear magnetization in different NMR experiments provides a way to estimate distances between nonbonded protons, making NMR spectroscopy a general method to obtain a large number of internuclear distances in 3D space. Sophisticated methods of NMR data analysis assisted with molecular dynamics programs allow to assess various conformers and their relative energies that, through an iterative process, led to a final 3D model of high quality based on the experimental NMR conformational restraints (3).

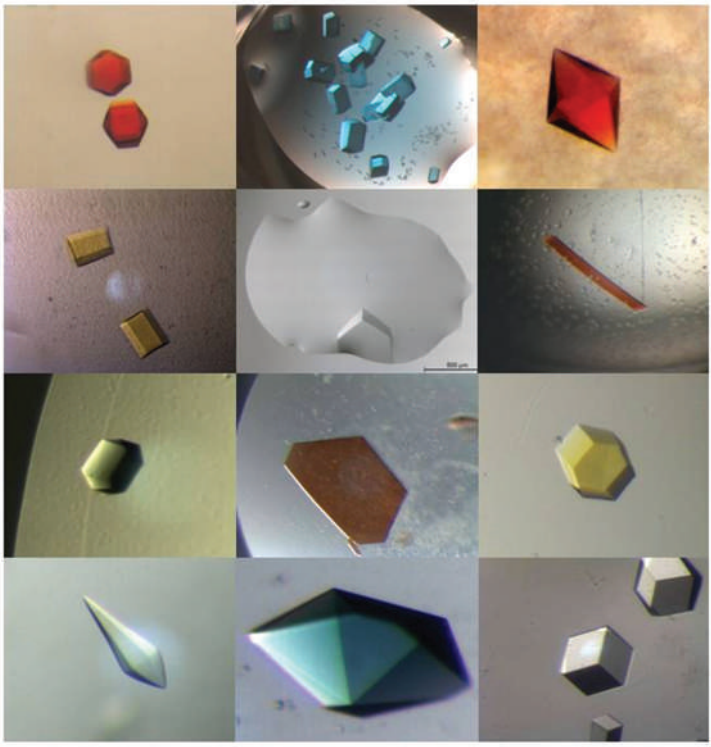

Figure 1 – Images of protein crystals obtained by NOVA researchers.

The 3D structures obtained by X-ray crystallography, cryo-EM and NMR, provide detailed information on the overall architecture, specific atomic interactions, flexibility regions, conformational changes occurring upon ligand binding, effects of point mutations in the protein fold and their repercussion on its function,

as well as insights into the catalytic sites and reaction mechanism of enzymes. The knowledge of accurate molecular structures is also an important prerequisite for structure-aided drug design.

Computational Structural Biology. Experimental structural biology has a computational sibling, these two approaches being complementary and allowing the enlargement of the knowledge of biology and biomedicine at the atomic and molecular levels.

A multitude of approaches exist in computational structural biology, ranging from quantum chemistry methodologies, chemoinformatics methods, molecular mechanics/dynamics simulation (including docking methods), electrostatic methods, protein structure prediction and others. While quantum mechanics would theoretically be sufficient to describe the behaviour of atoms and molecules, and their choreography to generate what we call Life, their general applicability is hampered by methodological and computational limitations. In fact, the treatment of large molecular systems such as biomolecules in a realistic way demands huge amounts of computational power. For these reasons, approximations are often needed, and these will depend on the problem being analysed.

2.2. Structural Biology at NOVA

NOVA gathers the largest number of researchers in Portugal dedicated to Structural Biology. Since the foundations of CTQB/ITQB NOVA by António Xavier, Structural Biology was elected as a strategic area and a Macromolecular Crystallography Unit (MXU) was then created and led by Maria Arménia Carrondo. This Unit has grown ever since and presently harbors 5 independent groups (Maria Arménia Carrondo, Margarida Archer, Pedro Matias, Carlos Frazão and Colin McVey) at ITQB NOVA. At ITQB NOVA the CERMAX (Centro de Ressonância Magnética António Xavier) was set with the sole high-field NMR spectrometer in the country operating at a proton frequency of 800 MHz, particularly suitable for biomolecular NMR research. Currently ITQB NOVA hosts 2 independent research groups in NMR (Ricardo Louro and Tiago Cordeiro) and 3 independent groups working in Computational Structural Biology (Cláudio M. Soares, António M. Baptista and Manuel N. Melo)

At FCT-NOVA, Structural Biology is carried out by the Macromolecular Crystallography group headed by Maria João Romão (that includes 2 independent researchers, Ana Luisa Carvalho and Teresa Santos-Silva) and by the NMR group headed by Eurico Cabrita (that includes the independent researcher Filipa Marcelo).

Other groups working in NMR are headed by Carlos Salgueiro (heme proteins lab), Sofia Pauleta (microbial stress lab) and Anjos Macedo. João Aires de Sousa heads a group working in Chemoinformatics.

As part of the development and growth of the NOVA crystallography groups is worth underlining the mentoring by the Nobel Laureate Robert Huber to Maria João Romão (post-doc as AvHumbold fellow) and to Margarida Archer and Teresa Santos-Silva (during their PhDs). In 2000, Robert Huber was awarded the Doctor Honoris Causa degree by NOVA.

Researchers from NOVA are internationally recognized and have leading roles in the coordination of nodes of the National Roadmap on Research Infrastructures (see above); coordination of the Block Allocation Group (BAG) of Synchrotron Radiation users at the ESRF (Pedro Matias); in representing Portugal in the ESRF Council and Scientific Advisory Committee of ALBA (Maria João Romão); and in the coordination of the International PhD program on NMR (Eurico Cabrita). Also, worth highlighting, NOVA is the only University in the country where Structural Biology is part of the curricula and taught at the BSc (Biochemistry) and MSc (Biochemistry; Biochemistry for Health; Biotechnology) courses.

2. RELEVANT CONTRIBUTIONS FROM NOVA

The FCT-NOVA and ITQB-NOVA teams have been pioneering in integrating scientific expertise in Structural Biology to address questions related with challenging mechanisms of disease, develop tools and provide clues for therapeutic targets. Some highlights from published and on-going research are provided in this chapter, to illustrate the impact of protein structures in health and medicine. Due to space limitation only selected publications from NOVA researchers will be included, where more references on the subject can be consulted.

2.1. Aberrant protein glycosylation and cancer

Tumor-glycome has been recently highlighted as a novel immune checkpoint and attractive target for new and tailored cancer immunotherapies. In this context, human macrophage galactose-type lectin (MGL), expressed on immune cells, specifically interacts with tumor-associated glycans that carry an end-standing *N*-acetylgalactosamine moiety (GalNAc), inducing immune responses in cancer immunity.

During the last years the NMR group at FCT-NOVA has been actively involved in unveiling the structure-functional features of MGL-glycan recognition process (4–6).

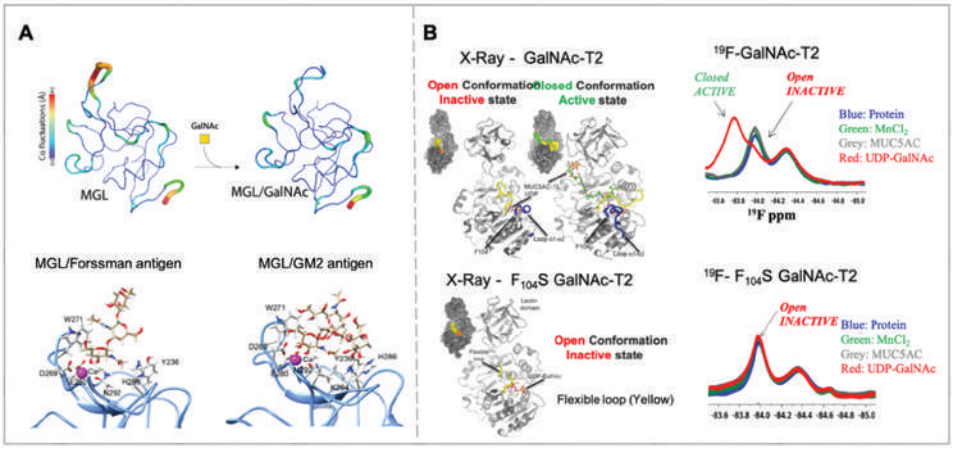

Figure 2 – A. 3D view of recognition of GalNAc-containing ligands by MGL-CRD. Top. Atomic fluctuation analysis of MGL-CRD apo and GalNAc complexed state derived from MD simulations. Bottom panel: Representative frame obtained from MD simulations of MGL-CRD complexed with Forssman and GM2 tumour-associated antigens. B. Top left. GalNAc-T2 in complex with UDP (PDB entry 2FFV), flexible loop (yellow) in open conformation (inactive state), and UDP/MUC5AC-13 (PDB entry 5AJP), with flexible loop in closed conformation (active state). Top right. 19F NMR spectra of GalNAc-T2 as function of UDP-GalNAc, MUC5AC and MnCl2 additions. Bottom left. Crystal structure of the F104S GalNAc-T2 mutant bound to UDP-GalNAc. Bottom right. 19F NMR spectra of F104S.

The NMR assignment of the $^{1}H,^{15}N$ backbone resonance of the carbohydrate recognition domain of MGL (MGL-CRD) in the free and GalNAc complexed states indicate that GalNAc binding induces strong conformational and dynamic changes on the MGL-CRD structure. Furthermore, NMR binding studies with distinct α- and β-GalNAc-tumour associated epitopes, show that a different degree of interactions, between GalNAc-containing ligands and MGL takes place, reliant on GalNAc moiety presentation (Figure 2A). These results stress the conclusion that the MGL structure is sensitive to the precise structure of the GalNAc-containing ligand and might explain the ability of MGL to produce distinct immune responses depending on the nature of GalNAc ligand. Our work suggests that by modulating the structure/dynamics of MGL-CRD with structurally distinct GalNAc-ligands it can be possible to modify the MGL-specific signaling outcome in immune cells to boost anti-cancer immunity (4).

2.2. Protein mutation and disease mechanism

The large family of polypeptide N-acetylgalactosamine (GalNAc) transferases (GalNAc-Ts) is responsible to initiate the posttranslational modification of many cell-surface proteins by transfer of GalNAc to serine and threonine residues in proteins. In this perspective, in close collaboration with Hurtado-Guerrero's group at Universidad Zaragoza, FCT-NOVA has also contributed to unravel the structural, dynamics and recognition features of the glycosylation mechanism behind catalysis by GalNAc-Ts (7–10). One of the examples relied on the study of the mutation in GalNAc-T2 (F104S), located distant from the catalytic center that inhibits glycosylation and is associated to low levels of high-density lipoprotein cholesterol (HDL-C) in humans. With this work we elucidated the molecular basis of why mutant F104S is inactive by adopting a multidisciplinary approach that combines X-ray crystallography, molecular modeling, and NMR spectroscopic techniques (7).

NMR spectroscopy using the wild type protein and the mutant in the presence of a peptide substrate demonstrated that the mutation induces loss of binding of the substrate. Analysis of the crystal structure of the F104S mutant revealed that the mutation causes instability in a flexible loop possibly involved in switching the enzyme between active and inactive states (Figure 2B). This was confirmed by labelling the protein with a ^{19}F structural probe in the flexible loop and by performing ^{19}F-NMR experiments. These experiments clearly show the inability of the F104S mutant to achieve the active conformation and concomitantly to glycosylate peptide substrates (Figure 2B). Indeed, ^{19}F-NMR spectroscopy experiments with WT enzyme reveal that binding of the enzyme with the UDP-GalNAc donor substrate is the key to change the conformation of the loop from inactive to active state. Therefore, our study demonstrates that GalNAc-T2 follows an induced-fit mechanism, in which UDP-GalNAc is essential to activate the enzyme. Since the flexible loop is conserved in all GalNAc-T families, the described behavior can be extended for other *O*-glycosylation processes. In summary, this study provides fundamental insights into the molecular catalytic mechanism of the large family of GalNAc-Ts and how these enzymes orchestrate protein *O*-glycosylation with implication in disease mechanisms.

2.3. Epitope mapping of antibodies for cancer and Alzheimer's Disease therapeutics

Passive immunotherapy strategies do not rely on the human body's immune system to fight diseases and include the administration of immune system

components, such as antibodies, to target foreign entities like cancer cells or amyloid fibrils. The development of better and more specific antibodies requires a detailed knowledge of the recognition mechanism and binding epitope.

At FCT-NOVA we have been applying different NMR techniques in order to characterize the epitope of monoclonal antibodies (mAbs) providing a structural rationale for their affinity, with particular relevance for the development of optimized therapeutics.

In the first example, the chemical epitopes of cancer related mAbs have been studied following a combined multidisciplinary approach integrating synthetic chemistry methods, mAb generation, microarrays, NMR, and computational methods. This methodology allowed us to identify the molecular elements of the recognition region of antigens for two different families of cancer-related mAbs (anti-MUC1 and anti-Tn). The use of STD-NMR techniques was fundamental to highlight that the amino acid sequences of the *O*-glycosylated antigens modulate the affinity of the anti-MUC1 mAb, while for the anti-Tn mAbs it is the sugar residue and the underlined amino acid that modulates the binding. This detailed information can be used for the design of tailored Tn-based vaccines like MAG-Tn3 (11).

Recently, in a study related with Alzheimer's Disease (AD) performed in collaboration with STABVida company, we have reported the epitope mapping of a novel murine anti-Aβ mAb that was developed with a therapeutic purpose (STAB-mAb). By performing a series of NMR $^{1}H,^{15}N$ HSQC-based titrations of Aβ(1–40) and Aβ(1–42) peptides with the antibody we proposed a possible mechanism through which the STAB-mAb antibody inhibits and reverts the formation of fibrils thus providing a structural rationale for its high affinity and for its mechanism of action (12).

2.4. Drug discovery and xenobiotic metabolism

Drug and xenobiotic metabolism are of great concern for the pharma industry in the process of developing new drugs. While cyt P450-mediated metabolism is well controlled, many cyt P450-resistant drugs are often modified by the liver enzyme aldehyde oxidase (hAOX1), which prominence was recognized only in the last decade. The capacity of hAOX1 to metabolize a wide diversity of drugs has been responsible for unexpected drug metabolism, for promoting drug interactions, for affecting drug's efficacy and ultimately, for leading to several failures in clinical trials. hAOX1 belongs to the xanthine oxidase (XO) family of molybdenum-dependent enzymes (13) and has a very complex metabolic profile, being a very promiscuous enzyme, able to perform surprisingly diverse reactions (14).

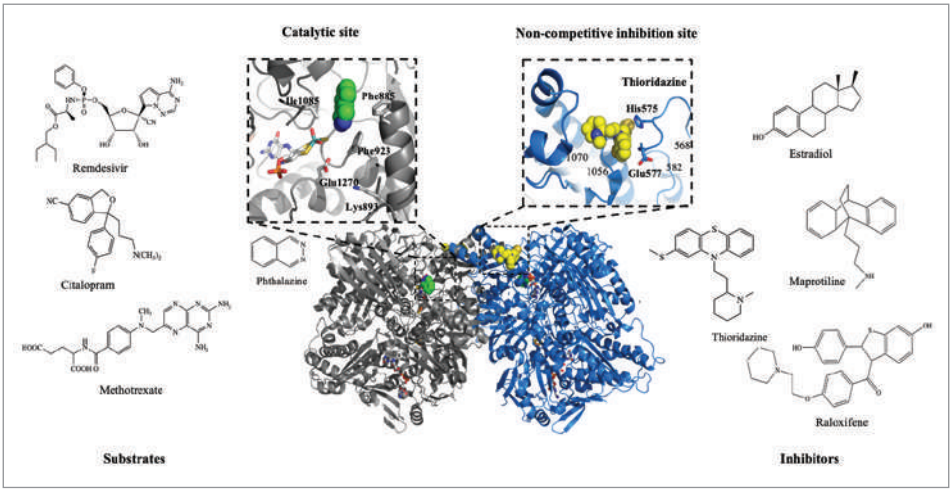

Figure 3 – Overall structure of the hAOX1 homodimer in complex with the substrate phthalazine (green) and the inhibitor thioridazine (yellow). Left: closeup view of phthalazine binding site. Molecules represented illustrate the diversity of drugs that are AOX substrates; Right: close-up view of thioridazine allosteric binding pocket. Molecules represented are examples of drugs that are AOX inhibitors (16).

Researchers from NOVA, reported in Science (1995) the first structure of a XO-related enzyme (15) that led to the first structure-based proposal for the enzymatic mechanism. In 2015, the FCT-NOVA team, in collaboration with Silke Leimkuehler (Potsdam University) determined the first structure of another member of the XO family, that of the human liver detoxifying enzyme Aldehyde Oxidase (hAOX1) (Figure 3) (16), that included the discovery of a novel inhibition mechanism. The clarification of the 3D structure of free and drug-bound forms was an important advancement in the field of drug discovery and development. Three pharma companies approached us (Merck, Eisai and Pfizer) and CDA agreement was defined with Eisai.

In fact, every year newly approved drugs are found to be hAOX1 substrates and we continue to contribute to the basic understanding of how hAOX1 functions as a drug metabolizing enzyme to help drug discovery efforts to develop new, better and safer drugs and to develop predictive models for drug/drug interactions.

2.4. CO Based Therapeutics with Organometallic Pro-Drugs (CORM)

Carbon Monoxide is constantly produced in living organisms during the destruction of heme by heme oxygenase. CO is a signaling molecule that mediates

many physiologic events with anti-apoptotic, anti-inflammatory and anti-thrombotic activity, among others. When applied through inhalation, CO has clear therapeutic effects that justified its entry in human clinical trials. Reducing the administered dose of CO, the use of prodrugs designed to deliver CO specifically to the site of disease, presents obvious advantages over inhalation.

The development of CO-releasing molecules (CORMs) as pharmaceutical agents at ITQB NOVA, (led by Carlos C. Romão) had a profound impact and recognition in the medical community. Understanding the interactions CORMs with biological systems is crucial for the design of pharmaceutical CORMs, which will ultimately circulate in the protein-rich plasma of humans.

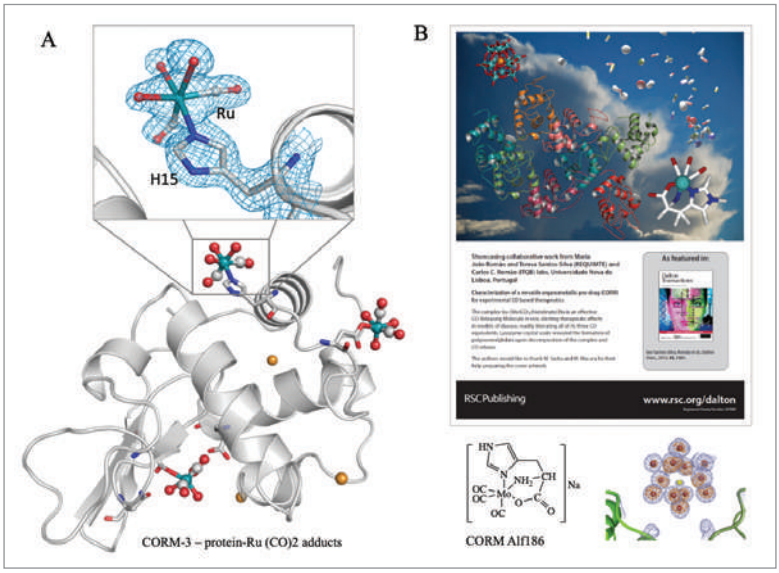

Figure 4 – Interaction of CO Releasing Molecules (CORM) with proteins: (A) CORM-3 covalently bound to His15 of lysozyme reveals the formation of protein-Ru (CO)2 adducts (17)). (B) Cover page of the characterization of a versatile organometallic pro-drug (CORM Alf186) for experimental CO based therapeutics (18).

At FCT-NOVA studies with several serum proteins of the reaction of the Ru containing CORM-3 identified the rapid formation of protein-Ru (CO)2 adducts (Figure 4-A) (17). These findings helped to explain *in vivo* data with CO-hemoglobin data and were of significance for the design of improved CORMs for therapeutic uses. As a follow-up, studies were pursued with another CORM (ALF186), a candidate therapeutic molecule for the delivery of pre-established amounts of CO at

a controlled rate (18). The interaction of ALF186 with proteins analysed by X-ray crystallography, showed that, under normoxic conditions, phosphomolybdate (from the decomposition of ALF186) was formed in the protein crystal (Figure 4-B). The therapeutic effects of ALF186 shows that is in a close mimic of CO inhalation therapy. Its reactivity with serum proteins and the generation of decomposition products in biological media have contributed to the knowledge of this important class of pro-drugs and assist the development of a second generation of drug-like CORMs.

2.5. Antibiotic resistance

The increasing threat of antibiotic resistant bacteria underlines the need to find drugs with a narrow range of action, in comparison to β-lactams. Gram-positive bacteria homeostasis and antibiotic resistance mechanisms are dependent on the intricate architecture of the cell wall, where amidated peptidoglycan plays an important role. The amidation reaction is carried out by the bi-enzymatic complex MurT-GatD, and its implication in antibiotic resistance mechanisms, has been studied in the group of Rita G. Sobral at FCT-NOVA and Herminia Lencastre at ITQB, with whom the crystallography group has collaborated.

The FCT NOVA team has disclosed the first crystal structure of the glutamine amidotransferase member of this complex, GatD from *Staphylococcus aureus* with a glutamine molecule close to the active site funnel. *In vitro* functional studies using ^{1}H-NMR spectroscopy showed that *S. aureus* MurT-GatD complex has glutaminase activity even in the absence of lipid II, the MurT substrate (19). These results provided significant insights into the molecular basis of the so far unknown amidation mechanism.

In addition, the structure of the whole complex MurT-GatD from *S. aureus* was crystallised and the 3D structure solved at FCT-NOVA. In collaboration with the IBET team, functional and kinetic data on the amidation reaction contributed to a much better insight into structure and function correlations, reinforcing the relationship between MurT-GatD complex assembly and enzymatic activity (Leisico et al, to be submitted) (Figure 5).

These results are of great value to understand the mechanistic diversity used by pathogenic bacteria in peptidoglycan amidation and serve as exploratory platforms to test promising antimicrobial compounds that specifically target the enzymatic activity and the assembly of the MurT-GatD complex provided additional information.

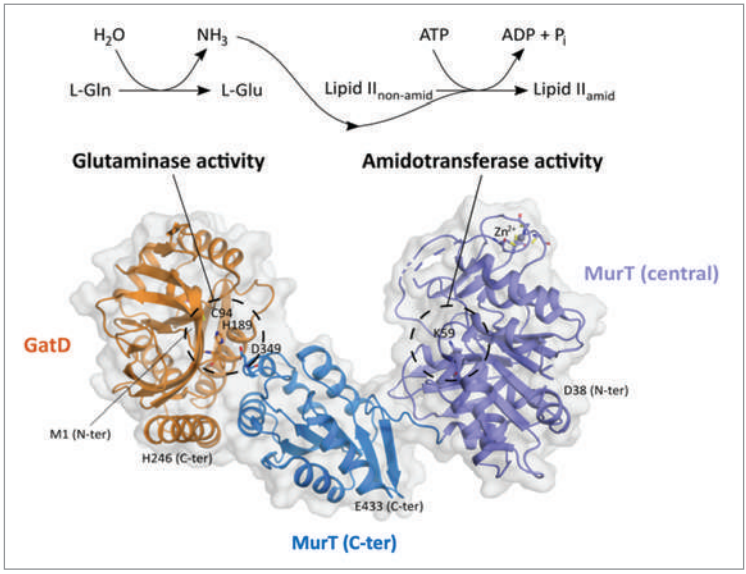

Figure 5 – Representation of the *Staphylococcus aureus* MurT-GatD complex crystal structure, responsible for catalyzing the peptidoglycan precursor lipid II amidation (Leisico F, Ana C. F. Paiva, Morlot C, Zapun A, Sousa P, Bandeiras TM, Mertens, Ludovice AM, Romao MJ, Sobral RG, and Santos-Silva T. Functional Insights into Peptidoglycan Amidation by the Essential MurT-GatD Complex. to be *submitted*)

2.6. Human DNA tumour virus

About a fifth of all human cancers worldwide are caused by infectious agents. The study of oncogenic viruses has been essential to our present knowledge of cancer biology. Tumourigenesis is considered to be a multistep process and infection with the DNA tumour viruses can manipulate one or more of these steps that finally lead to cancer. A human DNA tumour virus, called Kaposi's sarcoma-associated herpesvirus (KSHV), has been consistently identified in Kaposi's sarcoma tumours. Amongst the key viral proteins coordinating KSHV infection, is the Latency associated nuclear antigen protein, LANA. KSHV, like other human herpesviruses, establishes a biphasic life cycle referred to as dormant or latent, and productive or lytic phases. Latent infection predominates in KSHV infected tumour cells in which the linear viral genome undergoes circularization and chromatization. LANA is essential for latency and is expressed in all KSHV infected tumours. KSHV genomes persist in large nuclear bodies (foci) in latently infected tumour cells and the C-terminus of LANA, a DNA binding domain, mediates dimerisation, transcriptional repression, and targeting to nuclear bodies.

Researchers from ITQB NOVA led by C. McVey joined efforts with teams from IMM UL (P. Simas) and Harvard Medical School USA (K.M. Kaye) to further investigate the role played by LANA. The crystal structure of LANA DNA binding domain (DBD) revealed a dimer structure (20) (Figure 6) and showed bending and rotation about the oligomerisation interface when binding to viral terminal repeat (TR) DNA (21). We have demonstrated that LANA exhibits a biphasic binding mode to LANA binding site LBS3 site that is essential for replication (21). Interestingly, KSHV LANA (kLANA) functionally substitutes for murine gammaherpesvirus 68 LANA (mLANA), allowing kLANA investigation *in vivo* (22).

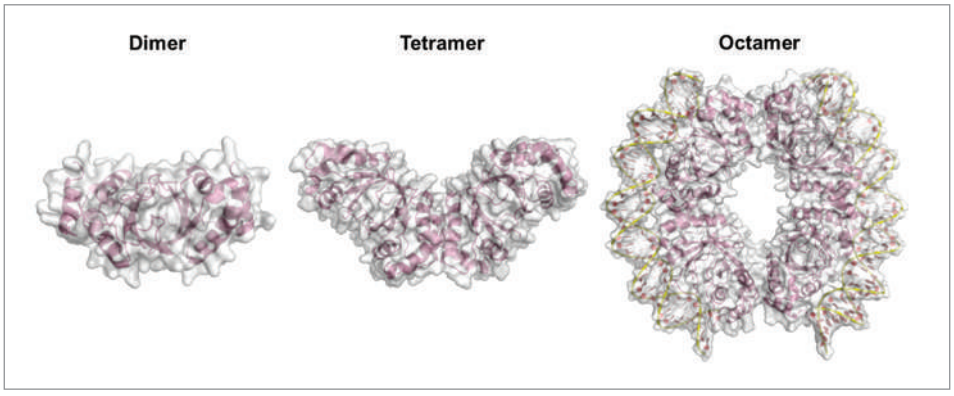

Figure 6 – KSHV LANA DBD forms oligomeric structures that have flexibility to bend viral DNA.

2.7. Molecular chaperone to proper protein assembly

Many cellular pathways, either in normal or disease backgrounds, require the association of already folded proteins to form multi-protein machines to perform specific tasks inside the cell. To ensure the correct assembly of these protein complexes, the cell uses a toolbox that works as a "Quaternary chaperone". Despite their low ATPase activities in *vitro*, RuvBL1 and RuvBL2 are believed to function as molecular motors, using ATP to perform a mechanical force to help in the assembly or disassembly of client multiprotein machineries. They belong to a large family called AAA+ that assemble into hexameric rings (23).

The list of RuvBL clients is long and includes DNA damage sensing complexes, oncogenic transcription factors and metabolic energy sensing complexes (23). All of these examples are associated with disease, especially cancer, where the RuvBL proteins, their client complexes, or both are exploited to benefit and support

cancerous cell growth and division, cell mobility, and immortality. The numerous client proteins that depend on RuvBL proteins for assembly led scientists to discover that, with slight modifications, the cell can adapt this toolbox for different needs. In practice, this means that different adaptor and helper proteins can associate with RuvBLs to assemble a specific target complex.

Researchers from ITQB NOVA and iBET led by Pedro Matias and Tiago Bandeiras started working with RuvBL proteins in 2005 through a collaboration with Schering Pharma company (Berlin), which resulted in the structural determination by X-ray crystallography of full-length RuvBL1 (24), RuvBL2 (25) and a truncated form of RuvBL1/RuvBL2 complex (26) that revealed for the first time its hetero-hexameric nature (Figure 7). More recently, this work has evolved towards the structure elucidation by Cryo-EM of several complexes involving RuvBL proteins in collaboration with French research groups.

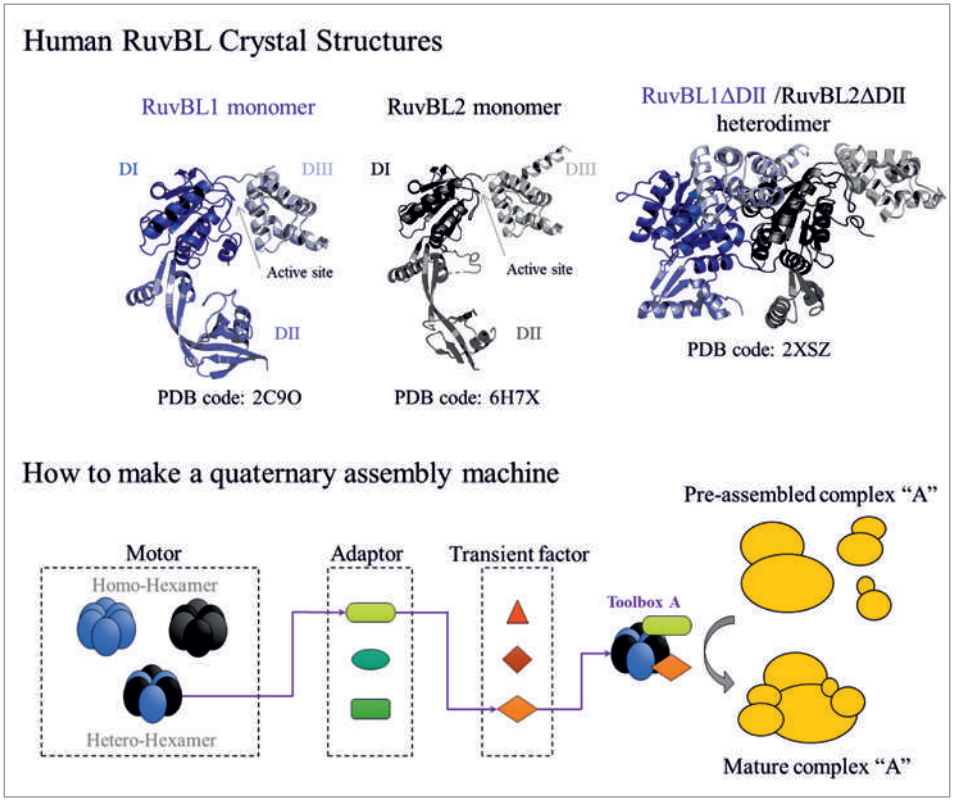

Figure 7 – Crystal structures of RubVLs (1,2 and complex) and a schematic diagram of the assembly mechanism

2.8. Targeting cell wall synthesis in Mycobacteria to fight tuberculosis

Tuberculosis (TB) remains a leading cause of mortality from a single infectious organism (above HIV/AIDS), killing over 1.5 million people and infecting more than 10 million worldwide each year (WHO report, 2019). The rise of multidrug-resistant tuberculosis threatens to derail decades of progress in controlling the disease. Ending TB epidemic by 2030 is among the health targets of the United Nations Sustainable Development Goals. The growth and virulence of *Mycobacterium tuberculosis* is related to the very low permeability of its cell envelop. Therefore, the identification and characterization of enzymes involved in its biosynthesis has revealed to be attractive anti-tuberculosis drug targets.

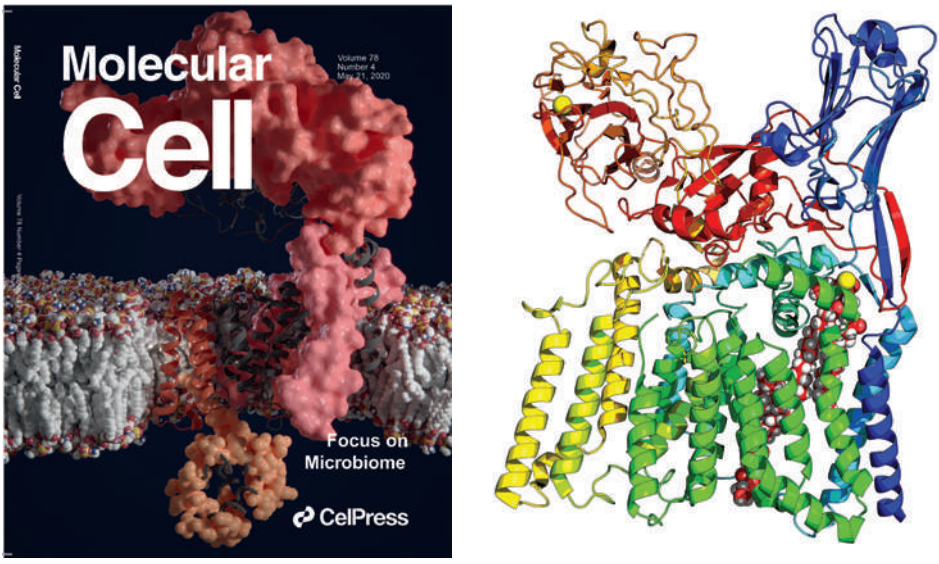

Figure 8 – Cover page of Molecular Cell journal with an artistic image of AftD (reference (28)) and cartoon representation of the overall architecture of EmbB with ligands (reference (27))

Researchers from ITQB NOVA (Margarida Archer Lab) joined with a team from Columbia University (Filippo Mancia), New York, USA to elucidate the structures of arabinofuranosyltransferases (AraT). These enzymes build the lipidated polysaccharides of the mycobacterial cell envelope. We have so far characterized by cryo-EM the 3D structures of two members from AraT family, AftD and EmbB (27,28) (Figure 8). Visual inspection of the structures reveal their overall protein architectures composed by a soluble domain with three carbohydrate binding modules and a membrane domain with a conserved GT-C glycosyltransferase fold.

Remarkably, AftD is unexpectedly bound to an acyl carrier protein, which prompted us to further investigate its role. Moreover, mutations on these integral membrane proteins are known to confer resistance to anti-tuberculosis drugs. The atomic models of AftD and EmbB contribute to better understand the catalytic action and drug resistance mechanisms of these proteins and may open avenues to explore novel drugs to treat tuberculosis infection.

Computational structural biology

Next, we will describe some of the applications in health of computational structural biology by laboratories of NOVA. For reasons of brevity, this description will be mostly focused on the last decade.

2.9. Computational drug design/virtual screening

Computational drug design/virtual screening are now mainstream in the development of pharmaceuticals. The most common applications consist in the inhibition of a given protein involved in a disease by a small molecule. Examples include the inhibition of biological processes in pathogenic bacteria, inhibition of cancer cells growth, among others. The Lab of João Aires de Sousa (FCT-NOVA), together with experimental Labs, has been quite active in the application of QSAR (Quantitative structure–activity relationship) models to design antitubercular agents (29,30) and anticancer agents resistant cancer cells(31). QSAR models are very powerful approaches, which use molecular descriptors that correlate with a given activity effect. QSAR models are trained on molecules with a known effect and allow the prediction of the effect of new molecules, which can then be synthesised and tested *in vitro* and *in vivo*. These approaches are complementary to molecular docking methods, which can predict the interaction between small molecules and protein active sites. These molecules can be retrieved from large databases and then docked into an enzyme's active site, allowing the estimation of the interaction energy; the best molecules can then be experimentally tested.

2.10. Molecular dynamics simulation approaches

Molecular mechanics/dynamics aim at simulating molecular systems by dealing with the approximated physical interactions between atoms and integrating their motion using classical mechanics. This is a serious approximation, since molecular systems live in a quantum world, but a necessary one for computational efficiency. For this reason, these methods are restricted to treat atoms and not their

subatomic constituents, but this is adequate for many applications. These are clearly the most widely used methodologies in biomolecular modelling in the health field.

Simulating pH effects in peptides and proteins

The Lab of António M. Baptista (ITQB NOVA) has been dealing with simulations with small endogenous peptides, such as the dipeptide kyotorphin (32) (Tyr-Arg) and potential drugs derived from it (33), performing normal molecular dynamics simulations and more sophisticated approaches, like molecular dynamics simulations that take into account the effects of the pH (constant pH molecular dynamics simulations) (34). The objective was to study the partition and interactions of the molecule at the water-lipid interface in order to infer about the therapeutical mechanisms. Also using constant pH molecular dynamics methods, the Lab of Antonio M. Baptista studied prion proteins (Figure 9) (35,36) and the pulmonary surfactant protein C (37,38).

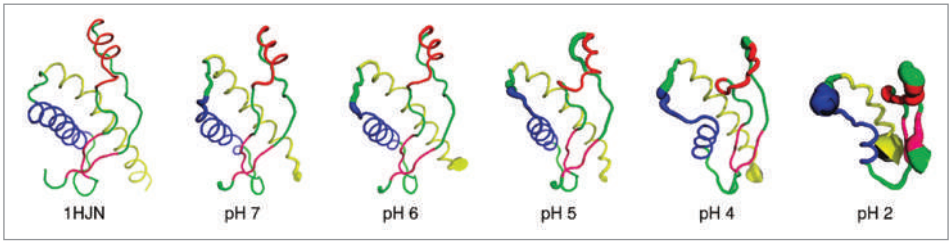

Figure 9 – Simulated behaviour of the human prion protein at different values of pH (image reprinted (adapted) with permission from reference reference (35). Copyright (2010) American Chemical Society).

Coarse grained models of antimicrobial peptides

The Lab of Manuel Melo (ITQB NOVA) is specialised in a specific kind of molecular dynamics simulation **– Coarse grained** (CG) models – where groups of atoms are represented by larger particles, which allows the treatment of larger biomolecular systems for longer periods of time, using the same computational resources. This Lab has been studying Antimicrobial peptides (39), trying to understand their pore forming mechanisms and contributing for the design of molecules capable of fighting microbial disease.

Simulating mechanisms in ABC transporters

The Lab of CM Soares (ITQB NOVA) has been using molecular dynamics simulation calculations to study several problems related with health, alone or in

collaboration with experimental laboratories. One example is the long standing work on the understanding of molecular mechanisms in ABC transporters (40–46). ABC transporters are membrane molecular machines that help in the permeation of substrates across membranes with the concomitant hydrolysis of ATP. This Lab has been studying conformational changes that explain the molecular mechanisms of mechanical work propagation through the molecular structure and substrate permeation. Of direct impact on health are the studies (44,46) on the cystic fibrosis transmembrane conductance regulator (CFTR) channel, which is an ABC transporter involved in chloride transport in epithelia, being connect with a dramatic genetic disease – cystic fibrosis – which results from mutations on CFTR generating a compromised channel.

Computational virology

Viral membrane fusion with eukaryotic cells is a fundamental process in infection by membrane virus. The Lab of CM Soares, together with experimental collaborators, is trying to understand membrane fusion and fusion proteins located in the membrane of several viruses, namely Influenza, Zika, Denge, Parainfluenza and, more recently, SARS-CoV-2. In Influenza, it is established that membrane fusion is prompted by the low pH of the late endosome, which induces conformational changes in the fusion protein hemagglutinin, exposing the fusion peptide, which inserts into the host cell membrane leading to infection. Extensive studies with fusion peptide (47–49) shed light into the conformational determinants of membrane perturbation (Figure 10).

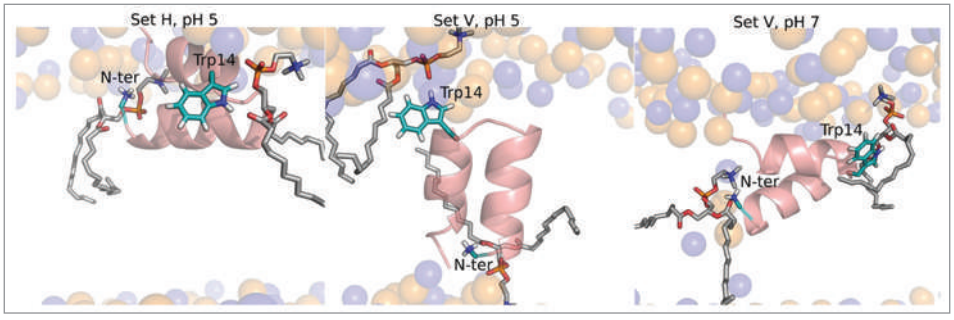

Figure 10 – Constant pH molecular dynamics simulation of the hemagglutinin fusion peptide in a phospholipid membrane, studying different conformations at different pH values (reference (49)).

SARS-CoV-2 appeared at the end of 2019 and created the COVID-19 pandemic, with devastating consequences worldwide. NOVA's scientists decided to

study this virus and its infection mechanism using all the technical expertise available. At ITQB NOVA and FCT NOVA a scientific task force was assembled by researchers, ranging from biochemists to biologists. Structural and computational biology (including the Labs of M Melo and CM Soares), are studying diverse fundamental proteins of this virus and of the human cells. The spike protein from SARS-CoV-2, responsible for membrane fusion, is one of the targets (Figure 11) being studied by experimental and simulation studies. Of particular importance for infection is its attachment to the eukaryotic receptor ACE2. Understanding the mechanics and thermodynamics of this interaction (Figure 11) is of fundamental importance to understand the infection mechanism, the effect of emerging variants and their role in evading antibodies.

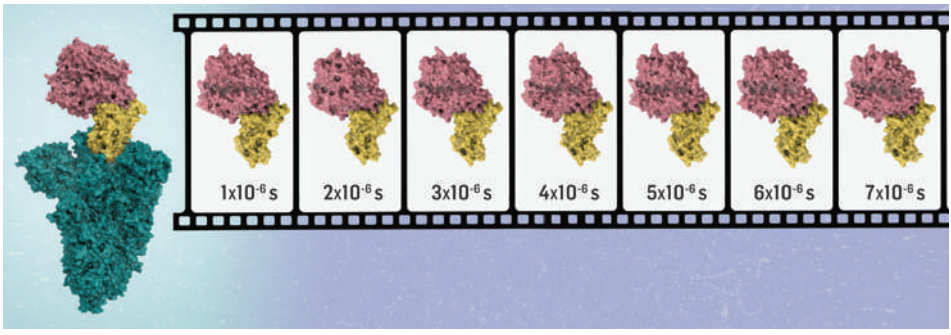

Figure 11 – The topography of SARS-CoV-2 infection in computer simulations. The image on the left represents the spike protein (S-protein) (in green, with the RBD – receptor binding domain in yellow) of the SARS-CoV-2 virus, bond to the human receptor ACE2 (in brick colour). The film strip contains several conformations of ACE2 bond to the RBD, obtained by molecular dynamics simulation, allowing the study of the dynamics and of the effect of mutations from various emerging variants of the virus (Soares *et al.* unpublished results, with the design by Luis Morgado).

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B

INFORMATION CODES OF LIFE- RNA & DNA

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INTRODUCTION

The nucleic acids RNA and DNA are the information codes of life and therefore they are essential molecules. They encode the language of life, which is ubiquitous in all the living organisms and necessary for replication, transmission, and conservation of the genetic information. From virus to man all living beings have the same nucleotides, the letters that promote life, but the words are different and each organism is a book written in an universal language. Science keeps deciphering this information; but rapid advances in technology have been showing that much more combinations are possible and what was initially though to be silent is also providing much information. Furthermore, the environment sets labels in the RNA and DNA and that extra information can be transmitted and promote adaptation to different conditions. In the Human genome less than 2% produces proteins and non-coding sequences are linked to various biological processes and human diseases. Quality control processes are crucial to avoid errors but sometimes errors can lead to biological diversity, which promotes evolution and survival. We know the information codes of life, and if we learn better how to read, write and erase them we can certainly progress from these life molecules to a Global Health.

STATE OF THE ART REVIEW

DNA was first considered the molecule of heredity, and all the types of RNA were considered as molecules wich decodify the information and transmit the message and produce the proteins. However, this idea has been changing and much leads to think that all started with an RNA world. RNA can also carry genetic information (it can be replicated, e.g. many viruses like the SARS-CoV2 which lead to this pandemic crisis of COVID-19 is a RNA virus); RNA can be catalytic and perform enzymatic reactions (natural ribozymes e.g. Class I introns, RNase P); some RNA molecules are among the most highly conserved bio-molecules and are common to all living organisms; RNA building blocks are precursors of DNA building blocks in present living organisms (e.g. the RNA base Uracil is the precursor of the DNA base Thymine); RNA primers are needed to replicate DNA (e.g. Okasaki fragments).

The advance of technologies, namely RNAseq showed that a plethora of new RNA species were being synthesized from what was previously known as the “silent

and dark matter of DNA". These are called non-coding RNAs because they do not encode for proteins, but they are regulators and have shown to be important for the homeostasis of the organism, development and adaptation to different stresses, leading to health or disease.

Knowing the DNA and RNA sequence is crucial and the detection of some specific mutations allows their connection with specific diseases. Studies at the genomic level allow detection of horizontal gene transfer between different species and comparative studies of different strains of a same species may lead to the identification of specific genes related to certain virulence traits. Since nucleic acids are essential for survival there are many molecular mechanisms involved in their protection and repair.

Transcription factors direct RNA Polymerases to the locations where they start synthesizing RNAs. Then ribonucleases process the RNAs to their functional size, and they degrade the defective RNAs control their right levels needed for survival. RNA chaperones help in these processes. Therefore, transcription factors and ribonucleases are determinant for Health and Disease. Some diseases are caused by problems with control of transcriptional regulation (for example, cancer) and various drugs target transcription factors.

Modern technologies are detecting DNA and RNA in real time, and this is quite important to define what is happening in time and space. Furthermore, their detection can also be applied for molecular diagnosis of certain pathogenic agents (e.g. detection of RNA from SARS-CoV2). The study and application of modified nucleosides can contribute to many medicinal applications, namely organometallic nucleosides can be applied in medicinal chemistry and molecular recognition.

The continuous breakdown and re-synthesis of mRNAs allows for the production of new proteins and best explains the adaptation to changing environments. RNAs differ in their susceptibility to degradation due to differences in their sequence and structure. The enzymes that cleave RNA are called ribonucleases (RNases). These ribonucleases are responsible for the maturation, degradation, and quality control of all types of RNA. Therefore abnormal function of ribonucleases is directly related with a plethora of diseases. Furthermore, RNases and non-coding RNAs can be used for diverse biotechnology purposes, such as the CRISPR-Cas system which lead to the 2020 Nobel Prize in Chemistry, and is widely used as a genome editing tool in a myriad of applications. The RNases present, RNA chaperones and a plethora of functional non-coding RNAs contribute to the network of post-transcriptional control of gene expression.

RELEVANT CONTRIBUTIONS FROM NOVA

DNA, Genes and Genomes

The DNA is usually present in chromosomes and genome sequencing is very important to find specific mutations which lead to disease. For instance, the group of Miguel Viveiros at IHMT NOVA, has been investigating the role played by specific DNA mutations in resistance to antimicrobials. In particular, they are interested in the characterization of the responses at the molecular level and its relation to the development of multidrug resistance phenotypes. Their studies focused on two main bacterial pathogens; *Mycobacterium tuberculosis*, the agent of tuberculosis and staphylococci, important agents of infections (1-3). These studies were the basis for the development of WGseq based genotypic and phenotypic Drug susceptibility tests and are now being extended to other pathogens.

Plasmids are a form of extrachromossomal DNA and play a key role in the genetic plasticity and survival. Although many *Staphylococcus aureus* plasmids have been described, still few studies portray the plasmid content of a given *S. aureus* population, like the study performed in a joint collaboration of Miguel Viveiros and Isabel Couto from IHMT NOVA (2).

Chlamydia trachomatis is an obligate intracellular bacterial pathogen that causes ocular and genital infections. Serovars A-C cause trachoma, serovars D-K cause non-invasive genital infections and serovars L1-L3 cause lymphogranuloma venereum (LGV). Serovars A-K infect superficial columnar epithelial cells whereas LGV strains are invasive and spread to lymphatic tissues. All *C. trachomatis* genome sequences exhibit >98% of identity and a high degree of synteny. Therefore, the determinants of the different types of infection (invasive or non-invasive) and tissue tropism (eyes, genitals, and lymph nodes) must rely on few genes only present in some strains. The group of Jaime Mota at UCIBIO, FCT NOVA, showed that subtle variations in the expression of a subset of virulence genes (*inc*), among *C. trachomatis* strains may contribute for the unique tropism and invasiveness of LGV strains (4).

Horizontal gene transfer (HGT) is the exchange of genetic material between organisms of different species that coexist in time and space. In prokaryotes, it is well established that HGT is as major driver of evolutionary innovation but the importance of this mechanism in eukaryotes is less consensual. Recently, the group of Paula Gonçalves at UCIBIO, FCT NOVA, have shown that HGT played a major role in remodelling of metabolism in a yeast lineage (the W/S clade) comprising approximately 100 species. In this lineage, individual genes and even entire operons were

acquired from bacteria endowing the recipient species with metabolic capabilities entirely new to yeasts or reinstating functions that were lost in the course of evolution. The analyses showed that HGT is also detectable in other fungal lineages, including those harbouring pathogenic fungi (5, 6).

The group of Célia Romão at ITQB NOVA, aims to unveil the molecular mechanisms which confer radiation resistance by focusing on the model organism *Deinococcus radiodurans*, a bacterium which has extreme resistance to different types of radiation. To address this question, they have been studying the DNA-protecting proteins under starved conditions (Dps). These proteins are able to shield the DNA from the degradation promoted by the reactive oxygen species, to store iron and manganese, and are involved on the homeostasis of antioxidant manganese-complexes (7).

The group of Tiago Cordeiro at ITQB NOVA studies the Proliferating Cell Nuclear Antigen-associated factor p15, which acts as a regulator of DNA repair during replication. This process corrects damage to the DNA caused by environmental factors, such as radiation. When over-produced, p15 is directly related to tumor progression. It is known that, upon DNA damage, p15 becomes ubiquitylated at two sites (aka dmUbp15). Ubiquitination is a chemical modification that targets proteins to degradation but may also affect their biological function. This group is investigating the impact of non-degradative ubiquitination in the dynamics and binding properties of p15 that might be relevant for the onset and progression of cancer. Their work unveiled that the two ubiquitin moieties form transient dimers that bind Dnmt1 methyl transferase (8). The latter is an enzyme that chemically modifies DNA, ensuring the replication of inherited epigenetic patterns (Fig 1).

The group of Elin Moe at ITQB NOVA studies activities for DNA repair. Endonuclease III (EndoIII) is a DNA glycosylase which removes oxidation damaged bases from DNA in the Base Excision Repair (BER) pathway and is important for genome maintenance across all kingdoms of life. The extremophile *Deinococcus radiodurans* possesses three genes encoding EndoIII enzymes (EndoIII1, 2 and 3). They have shown that small structural modifications in these enzymes have major impact on function, and result in an extended genome maintenance mechanism (9).

Furthermore, the group of Smilja Todorovic at ITQB NOVA has shown that biophysical methods can reveal unique information on these Endonuclease III enzymes that undertake search and repair of specific DNA damages in cells (10). This novel evidence indicates that *in vivo* the Fe-S cluster could be redox-activated by species

other than DNA, possibly as a part of a signaling mechanism in DNA damage search, and demands for a revision of the currently proposed mechanism of EndoIII.

RNA, Expression, and Technologies

The group of Catarina Pimentel at ITQB NOVA, has recently be involved in a venture into the field of molecular diagnosis and they have established an inexpensive colorimetric assay, based on RT-LAMP, that efficiently detects SARS-CoV-2 RNA in saliva samples (Figure 2). This has led to a recent patent.

The group of Ana Petronilho (ITQB NOVA) has been centered in the study of modified nucleosides for medicinal applications (11, 12). They have recently examined the reactivity of 7-methylguanosine, the so-called mRNA cap0, which is highly relevant for transcription of RNA. 7-methylguanosine shows a higher acidity when compared to guanosine, due to the transient formation of an ylide. They were able to isolate this transient ylide by reacting 7-methylguanosine with platinum (0). This new compound is highly stable, and the measurement of its base paring ability shows no significant effect of metalation on the formation of Watson Crick base pairs (11, 12). This result opens a plethora of possibilities for the application of organometallic nucleosides in medicinal chemistry and molecular recognition.

The group of Zach Hensel at ITQB NOVA uses microscopes to observe single RNA molecules in living bacterial cells (Fig 3). Bacteria must precisely control growth and react to environmental changes in order to compete with other organisms for limited nutrients and survive challenges from viruses and antibiotics. Yet, bacteria have very low numbers of some components—only a single DNA molecule and only a few mRNA molecules at a time encoding any specific protein (13). Low numbers are intrinsically "noisy" with variation in the concentration of critical components. Their goal is to observe this "noise" in gene expression by labelling single mRNA and protein molecules so that they glow under the microscope. Then they can count them one by one and track how they move within the cell.

The group of Sérgio Filipe at UCIBIO, FCT NOVA, has contributed to the development of genetic tools that permit the expression of fluorescent derivatives of target proteins in *Streptococcus pneumoniae*, a Gram-positive bacterial pathogen of clinical interest (14). These tools were further used in other Gram-positive bacteria, as they ensure an accessible ribosome-binding site in the mRNA that encodes pneumococcal proteins with a fluorescent protein linked to their N-terminal ends.

The group of Isabel Sá-Nogueira at UCIBIO, FCT NOVA is focused on the control gene expression of carbohydrate metabolism and transport in bacteria.

The main area of interest is to understand how the transcriptional and translational regulatory networks interact with other cellular components. This, involves the analysis of the mechanisms through which the cell senses nutrient availability and transmits that information to the level of gene expression. Their findings, in the Gram-positive model organism *Bacillus subtilis*, illustrate different mechanisms of transcription and translation control involving distinct players: DNA-looping formation, transcription factors, RNA-polymerase roadblocking, and inhibition of translation by regulatory RNA (15-18). Sá-Nogueira's group described for the first time, in Gram positive bacteria, that ABC-type I importers of sugars share a common energy-coupling component (Nucleotide-binding-domain, NBD), named multitask ATPase (19). More recently, their studies showed a novel ability of multitask ATPases, their functional interspecies exchangeability among Firmicutes including several human pathogens of clinical relevance such as, *Streptococcus pneumoniae, Staphylococcus aureus and Clostridioides difficile* (20). ABC type I importers are not present in humans and could be targets for antibacterial drugs.

The group of Claudina Rodrigues-Pousada and Catarina Pimentel at ITQB NOVA works in the mechanisms involved in homeostasis control when yeast cells are exposed to different environmental cues. The function of Yap transcription factors in stress response is investigated. They have been investigating how pathogenic (*Candida* spp.) and non-pathogenic (*Saccharomyces cerevisiae*) yeasts cope with toxic levels of metals, with emphasis on those pathways that are regulated at the transcriptional level. They unveiled several lines of defense against metal toxicity coordinated by a family of transcription factors – the Yap family – and studied how stress signals are transduced to the Yap members (21). They also showed that metals and antifungals can act synergistically against opportunistic yeasts and that genetic reprogramming plays a crucial role in these processes. Based on their findings, using an interdisciplinary approach bridging chemistry and biology, compounds were designed combining azole and metal-binding groups and their antifungal activity was studied (22).

RNA levels can regulate protein synthesis and cellular growth. The laboratory of Cecilia M. Arraiano in ITQB NOVA focuses on all types of RNA and the Control of Gene Expression mediated by transcription factors, ribonucleases, RNA chaperones and other regulators. Below are examples of their relevant contribution in these topics of research and their connections to global health.

Arraiano's group has discovered that the conserved stress response Protein protein BolA is a transcription factor with important implications for microbial

survival and biofilm production. For instance they have shown that BolA Influences Fitness and Promotes *Salmonella* enterica Serovar *Typhimurium* Virulence (23).

Transcription termination is a critical step in the control of gene expression. Using a combination of experimental and bioinformatic approaches, the group of C.M. Arraiano has first described the role of *Salmonella* non-coding sRNA SraL in the control of the expression of the important transcription termination factor Rho (24). This was very important since the interaction region in both RNA species corresponds to a very-well-conserved sequence in enterobacteria, which may indicate that this regulation also occurs in other bacterial organisms. A plethora of small non-coding RNAs (sRNAs) contribute to the network of post-transcriptional control of gene expression and this group has also studied several other sRNAs, their regulation and mode of action identifying their preferred targets.

Members of Arraiano's group are also interested in the use of synthetic biology to reprogram bacteria for biotechnology use, have contributed to establish modules to stabilize RNAs (Fig 4, (25), and developed a plasmid tool which uses tailor-made sRNAs to control the expression of target mRNAs (26)- in collaboration with the group of Lígia Saraiva also from ITQB NOVA).

Furthermore Arraiano's group at ITQB NOVA is internationally known for its expertise on ribonucleases (RNases). Recently they have applied their knowledge to characterize the mode of action of the SARS-CoV2 nsp14 ribonuclease, which is crucial for the replication of this RNA virus. The group of Cecília Arraiano joined efforts with the ITQB NOVA group of Cláudio Soares and together they have discovered novel targets for intervention against COVID-19 (27).

Ribonucleases (RNases) can act as determinant virulence factors in many pathogens, and they actively contribute to microbial survival inside the host, leading to virulence, persistence and antibiotic resistance (Fig 5; (28). For instance this laboratory showed that ribonucleases affect motility, biofilm formation, and antibiotic resistance in *Enterobacteriaceae* (29, 30).

They have also shown that ribonucleases such as RNase R can also affect the metabolism of ribosomal RNAs and translation in *Streptococcus pneumoniae* (31). Furthermore, they discovered that RNase R and the RNA-binding protein Hfq are important for ribosome biogenesis and Hfq also affects translation fidelity (32, 33).

Important findings over the last few years have shed light into eukaryotic RNA degradation by members of the RNase II/RNB family of enzymes and their importance in Health and Disease (Fig 5). Years ago, they have published in *Nature*

the structure of the first member of this family of enzymes – Cecilia M. Arraiano and Arménia Carrondo ITQB NOVA, were the co-corresponding authors (34). Humans encode three members of this family of enzymes: Dis3/Rrp44; Dis3L1; and Dis3L2. These enzymes have been shown to be involved in important cellular processes, such as mitotic control, and associated with human disorders like cancer (35).

DIS3/Rrp44 enzyme represents the catalytic subunit of the multiprotein complex called exosome. This RNase is related with many diseases, namely multiple myeloma (Fig 5). In a collaborative effort this group has shown that DIS3 isoforms vary in their endoribonuclease activity and are differentially expressed within haematological cancers (36). The group of Cecília Arraiano was the first to show that DIS3L2 acts independently of the exosome and has a distinctive preference for uridylated RNAs (37). DIS3L2 degrades many different transcripts and they showed that it also affects natural nonsense-mediated mRNA decay targets in human cells (38). This DIS3L2 ribonuclease has been related with stem cell proliferation and cancer and recently they demonstrated that Dis3L2 regulates cell proliferation and tissue growth through a conserved mechanism (39).

IMPACTS ON SCIENCE AND SOCIETY

Unveiling the radiation resistant mechanisms will impact on new methodologies that can be applied to different areas, such as health and environment. Some main areas of interest are: protection of organisms from the deleterious effects of radiation, for instance in radiotherapy; and bioremediation of toxic compounds, for instance from waste water.

If damaged bases in our genome are not repaired, they can cause mutations, replication errors and persistent DNA damage, which ultimately can lead to cancer and premature aging. These damages are repaired in the highly conserved BER pathway. Mammalian DNA repair systems are complex and in order to obtain a better understanding of molecular mechanisms underlying DNA repair, it is important to study prokaryotic systems.

Development of genetic tools allowing the expression of fluorescent derivatives of target proteins contributed to the determination of the sub-cellular localization of proteins that permit *S. pneumoniae* bacteria to propagate within the infected host.

C. trachomatis infections affect millions of people worldwide and can lead to blindness or sterility. While antibiotic therapy is usually effective, chlamydial genital infections often go unnoticed and antibiotics have undesirable side-effects. Development of vaccines are in progress. An alternative to antibiotic therapy is next-generation precision antimicrobials such as virulence blockers or anti-host drugs. The study on the sequence variability and expression of *inc* genes reveal basic aspects of *C. trachomatis* molecular pathogenesis which can contribute to the development of new prophylactic and therapeutic measures.

Invasive fungal infections are life threatening and existing antifungal drugs are not completely effective due to undesirable side effects and resistance emergence. Distinct research groups at NOVA address using a different methodology. A better understanding of horizontal gene transfer in fungi will contribute to elucidate mechanisms that may help explain the distribution of virulence determinants in populations of pathogenic fungi. On the other hand, research on YAP transcription factors has shown that the use of strategies that specifically perturb fungal metal homeostasis can be seen as promising antifungal approaches.

Living organisms sense changes in nutrient levels and trigger an adequate response to these extracellular signals that enable organisms to adapt successfully for survival. The elucidation of the mechanisms involved in the control gene expression of carbohydrate metabolism and transport in bacteria has been contributing to the general knowledge of the regulatory processes that govern the cellular metabolism. Nowadays it is generally accepted that transcription and translation control also contribute to metabolic homeostasis in complex organisms. Perturbations in the regulatory mechanisms of gene expression may lead to the progress of metabolic diseases.

The inexpensive colorimetric assay, based on RT-LAMP, that efficiently detects SARS-CoV-2 RNA in saliva samples, has great potential to be widely used in the rapid diagnostic of COVID-19 in a large range of settings such as schools, airports, music festivals.

A goal of the microscopy work is to image mRNA production and degradation in real time. This will make it possible to observe processes such as how some bacteria can evade antibiotics by controlling degradation of an mRNA to pause cell growth. This knowledge can be used to improve antibiotic therapy, or natural gene regulation systems can be used and modified in synthetic biology applications.

The formation of 7-methylguanosine at the 5 end of mRNA (capping) in eukaryotes is a fundamental step in transcription, required to initiate translation

and to regulate eukaryotic protein synthesis, pre-mRNA splicing and nucleocytoplasmic transport. Capping is also utilized by viruses to evade cellular defense mechanisms. To unveil unknown aspects of the reactivity of mRNAcap0 contribute to expand its medicinal applications.

It is widely known that the disorders on mRNA degradation have many implications on Human Disease.

Furthermore, the 2020 Nobel Prize in Chemistry was given to the discovery of the CRISPR/Cas system has shown that the study o non-coding RNAs and ribonucleases can lead to innumerous applications in Health and Biotechnology. The pandemic COVID-19 world crisis was caused by an RNA virus, and new technologies have rapidly developed RNA-based vaccines which are showing to be quite effective in controlling the problem. These are just two examples to show that it urges to learn more about RNA, RNases and the control of gene expression, since they can have a huge impact on Science and Society. It is excellent that NOVA has expertise in this area and keeps at the forefront of the international science in these topics.

ONGOING RESEARCH

RNA and DNA research at NOVA will further advance and expand based on the topics here described.

Integrated structural and functional studies at NOVA are rapidly advancing in the characterization of new genes, transcription factors, RNAs and ribonucleases, and other enzymes that can control major cellular functions in diverse organisms. Their studies certainly have many important implications for intervention in Health Sciences. For instance, ongoing research has discovered drugs which inhibit the SARS-CoV2 ribonucleases, and they have shown to halt the replication of this virus. This will have a great impact in new therapeutics against COVID-19 pandemic crisis and other infections with related viruses.

Synthetic Biology can design and implement novel approaches to tailor the mode of action of RNAs, DNA regulatory elements, promoters, RNA- and DNA-binding proteins. This can contribute to rapid advances in RNA- and DNA-directed technologies.

Novel strategies based on DNA and RNA are currently being devised to find new anticancer agents, anti-fungal and anti-bacterial targets and drugs.

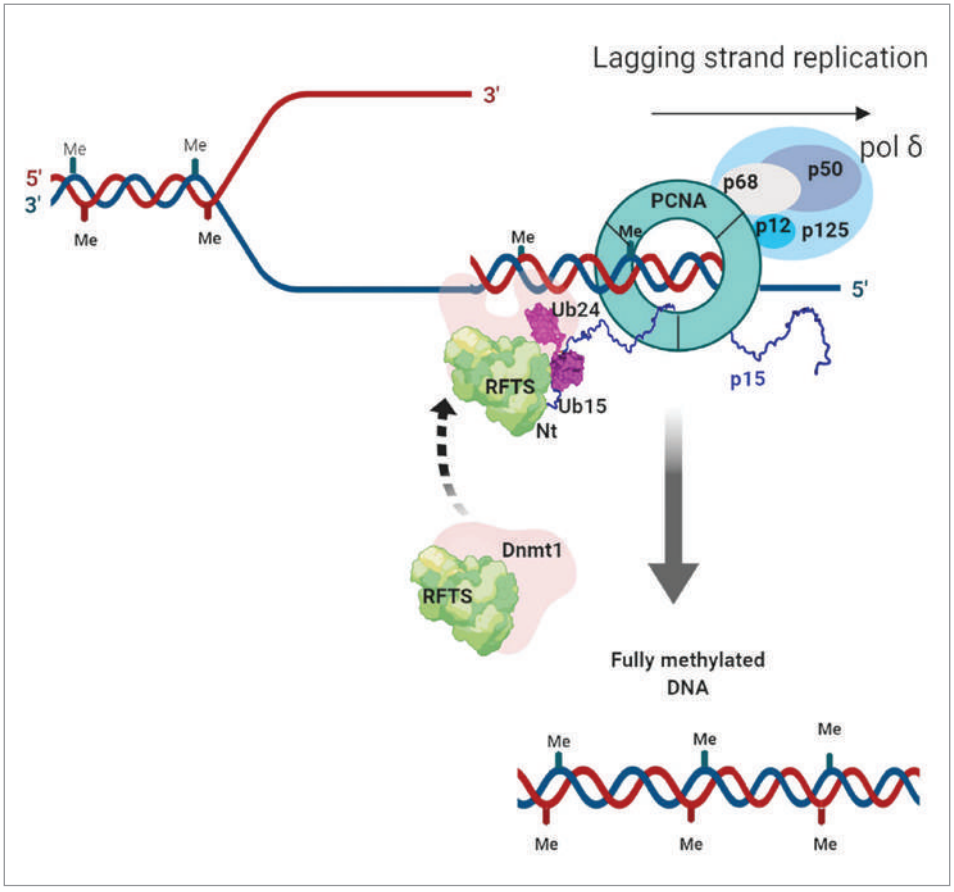

Figure 1 – Proposed model of dmUbp15 binding to RFTS domain of Dnmt1. dmUbp15 is recognized by the RFTS domain of Dnmt1 and is recruited to the replication fork. The binding might induce the rearrangement of Dnmt1, leading to the opening of the active site and methylation of the newly synthesized DNA strand.

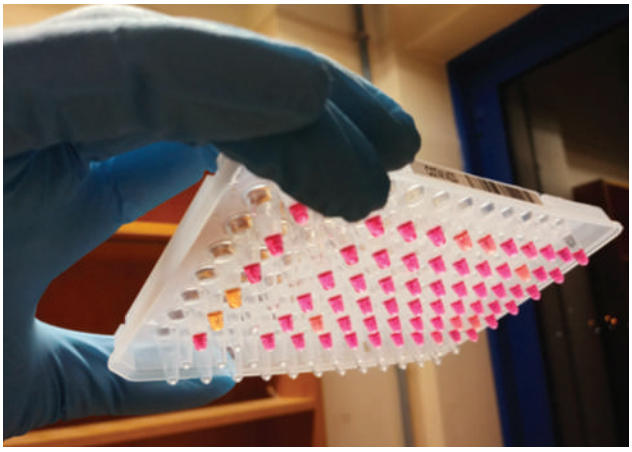

Figure 2 – Typical result of a saliva test based on RT-LAMP (reverse transcription loop-mediated isothermal amplification) for SARS-CoV-2. The pink and yellow colors indicate a negative or positive test, respectively.

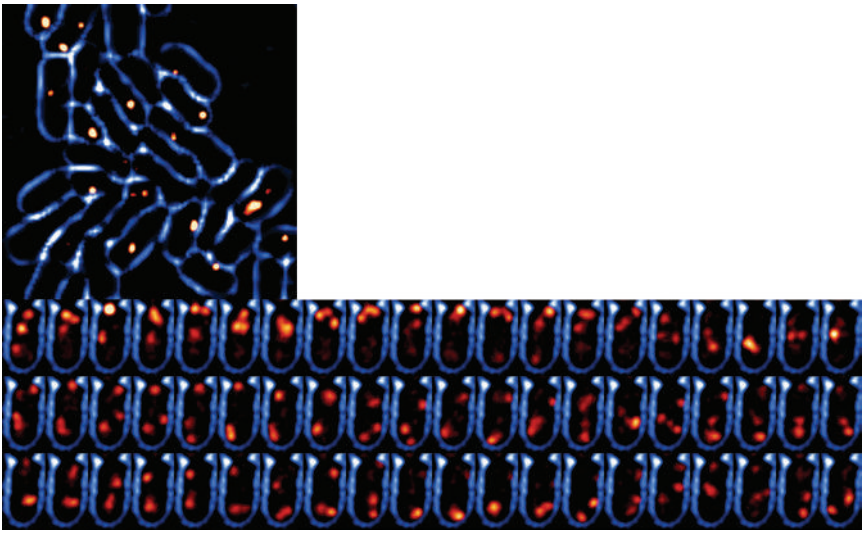

Figure 3 – Single mRNAs detected in living *Escherichia coli* cells by microscopy. Single mRNA molecules were labeled in a colony of E. coli cells by a fluorescent protein (orange spots) and often observed to appear near the cell membrane (blue). A movie of mRNA spots in a single cell with images taken every 2.5 seconds shows that single mRNAs move throughout the cell. Top: A colony of *E. coli* cells Bottom: One cell imaged every 2,5 seconds.

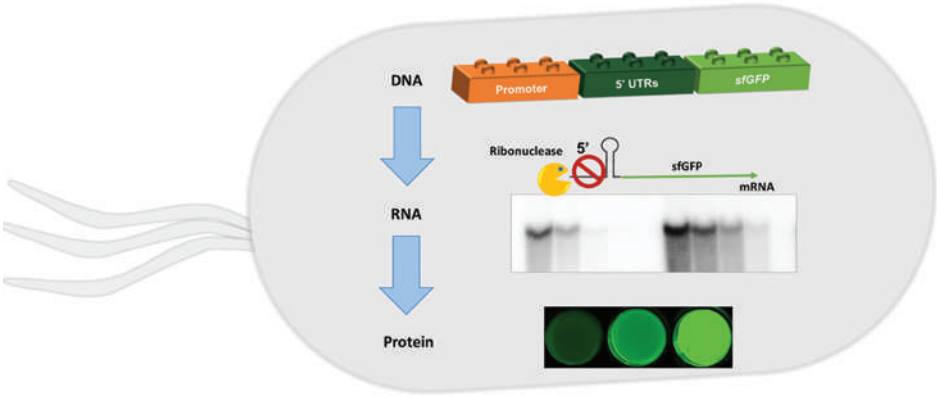

Figure 4 – Modulating heterologous gene expression with portable mRNA-stabilizing 5'-UTR sequences. In this work it was explored the role of the mRNA 5'-Unstranslated Regions (UTRs) as a way to increase protein production, Some natural 5'-UTR sequences can confer stability to heterologous messages because they protect them from ribonuclease cleavage. A group of Standard European Vector Architecture (SEVA) plasmids were constructed using the superfolder GFP as reporter. The only difference among them were the stabilizing 5'UTR elements and the outcome was inspected by Northern Blot analysis and fluorescence quantification. The results showed that by keeping transcription fixed and using different 5'-UTRs we could make mRNA decay the limiting constituent of the overall gene expression flow. The set of secondary structures described herein could be most useful genetic gadgets for increasing protein expression with a reduced physiological cost.

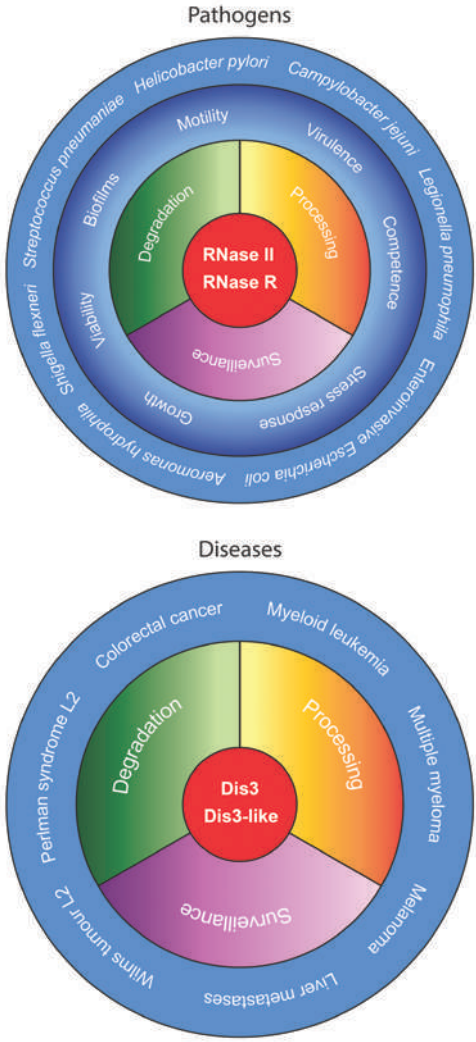

Figure 5 – RNase II family proteins and their role in pathogenesis and disease. At the top, the circle indicates human pathogens whose physiology has been shown to be affected by mutations in RNase II and/or RNase R. At the bottom, the circle indicates human diseases that have been related with mutations in the Dis3 or Dis3L2 proteins. The sections in the outer circle do not correlate with those of the inner circles.

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MICROBES FIGHTING BACK & ANTIBIOTIC RESISTANCE

Raquel Sá-Leão
Luís Jaime Mota

INTRODUCTION

Life emerged on Earth 3.5 to 4 billion years ago. Prokaryotes (bacteria and archaea) dominated the planet for at least one billion years, until the appearance of the first microbial eukaryotes (unicellular protozoa). Earth was therefore already populated by a large diversity of microbes when animals and the first human ancestors appeared, ~700-800 and ~5-7 million years ago, respectively. From the early days of interaction between bacteria and predatory protozoa, microbes have adapted to higher life forms, which resulted in the present-day multitude of host-microbe interactions, ranging from beneficial to harmful to the host. For example, human life depends on the microbiota, the trillions of microbial cells that use our bodies as host and that help us in many ways, such as to digest food or to develop our immune system. On the other hand, although most microbes cause no harm, a few cause infectious diseases. Indeed, infectious diseases are major causes of human morbidity and mortality worldwide and are associated with massive health and economic costs. Microbial infections of animals and plants affecting livestock and crops have an additional environmental and economic impact.

Throughout History, and left alone viral infections, humans have been facing several types of infections: e.g., plague, tuberculosis, meningitis, pneumonia, aspergillosis, sleeping sickness, or malaria. This is because microbes developed mechanisms that enable them to resist the sophisticated human defences and responses to infection. The relatively recent introduction of antibiotics in clinical use, in the 1940s, was a major improvement in the ability of humans to control bacterial infections. Microbes, however, rapidly evolved and several became resistant to antibiotics and, in the 1980-90s, it became clear that antibiotic resistance had become a major public health problem which is, nowadays, even greater.

In this scenario, it is of obvious relevance to study how host-microbe interactions can result in disease, and how antimicrobials and antimicrobial resistance impact on the control and treatment of infectious diseases. In this Chapter, we will survey the most relevant research contributions of NOVA University Lisbon aiming at developing novel tools to detect, prevent, and treat microbial infections.

STATE OF THE ART REVIEW

The body of human and other mammals can detect, resist, and kill infectious microbes. The skin and the mucosal epithelia that lines the respiratory, intestinal,

and urogenital tract, function as physical barriers but also possess additional antimicrobial characteristics, such as the resident microbiota (preventing colonization by pathogens), iron chelators, lysozyme (which degrades bacterial cell walls), antimicrobial peptides, secreted antibodies, and underlying immune cells. The innate immune system includes phagocytic cells capable of detecting, engulfing, and destroying microbes, which are helped by extracellular proteins (complement and cytokines) that stimulate phagocytic activity or have intrinsic microbe destroying properties. The killing capacity of phagocytes is associated with oxidative and nitrosative killing, which depends on the release of reactive oxygen and nitrogen species at the site of infection or into the phagosome (the vacuolar compartment containing engulfed microbes), and with non-oxidative killing, which requires the fusion of vesicles containing various types of hydrolytic enzymes with the phagosome. If the innate immune system cannot handle an infection, a more specialized defense system, the adaptive or acquired immune system, comprising proteins (antibodies) and T cells, can then target specific microbes.

To establish an infection, pathogens must then encode and produce proteins enabling them to adhere and invade host barriers, and to disarm the recognition and killing capacities of phagocytes and of other immune cells. For this, many pathogens form complex and multilayered bacterial communities (biofilms). Another general mechanism is the secretion of microbial proteins into the surface of the pathogen, into the extracellular milieu, or directly into host cells using specialized protein transport systems. Pathogens also often hide the specific molecules (for example, bacterial peptidoglycan) that are recognized by immune cells. In the case of bacteria, this can be achieved, e.g., by the production of a polysaccharide capsule or by modifications of the cell wall.

As many microbes can overcome mammalian barriers and immune defenses and cause devasting infections, humans have successfully developed vaccines, antibiotics and other antimicrobial compounds that significantly improved the health of humans and other animals.

While antimicrobials are used to treat infections, vaccines are used to prevent them. Vaccines, are therefore, an ideal solution to combat infectious diseases. They usually contain live attenuated (weakened or inactivated) forms of the infectious agent or they may contain only parts of the infectious agent. In both cases, the host will recognize these foreign bodies as non-self and mount an immune response to it. The result is that the host will become immune to the infectious agent without having disease. In addition, for most vaccines, if the majority of the individuals is

vaccinated, transmission of the infectious agent can be stopped resulting in herd protection for the population. The success of vaccines' use has been enormous. During the last century, together with the availability of potable water, mass vaccination was a main cause of decline in premature human death. The use of vaccines led to eradication of smallpox in 1979 and has enabled control of 14 major diseases.

In the 1940's, widespread use of antibiotics in clinical practice met a great success: a swift treatment of wide range of infections became possible. Diseases that, until then, often resulted in severe sequelae or death could now be easily cured. A general feeling that infectious diseases were no longer a problem – as antibiotics would treat them – was established and medical research focused mostly in other areas. Unfortunately, bacteria resistant to antibiotics emerged and spread worldwide. Antimicrobial resistance is nowadays an enormous problem as it can severely impair treatment options. Multidrug resistant bacteria, i.e., bacteria that are resistant to several classes of antibiotics, are particularly worrisome.

Antibiotics target essential steps of the bacterial cell cycle such as synthesis of cell wall, protein synthesis and DNA synthesis. They usually have a broad spectrum of action as their targets are common to many bacteria. As a result, antibiotic treatment, not only affects the etiological agent of infection, but also several other bacteria of the human microbiota. This stress, which is imposed on the commensal microbiota often disrupts it, causing dysbiosis and providing the ground for opportunistic pathogenic bacteria to expand. In addition, antibiotics select bacteria that are able to thrive in their presence contributing to dissemination of antibiotic resistance.

At NOVA University Lisbon, several research groups are focused on how to fight infectious diseases agents. The next sections summarize their key contributions so far.

RELEVANT CONTRIBUTIONS FROM NOVA

Various research laboratories from NOVA have been addressing how microbes can circumvent host defense mechanisms, and human interventions such as the use of vaccines, antibiotics, and pollution. The spectrum of the studies is wide, in terms of the pathogen (different medically relevant bacterial species, fungi, and protozoa; **Table 1**), of the virulence mechanism and of the targeted mammalian host defense process **(Figure 1)**, and of different aspects related with antimicrobial resistance **(Figure 2)**.

Microorganism[a]	Diseases in humans	NOVA researchers
Chlamydia trachomatis	A bacterial pathogen causing widespread ocular and urogenital infections that can result in blindness or sterility.	Jaime Mota
Clostridioides difficile (C. diff.)	Bacteria causing intestinal diseases linked to antibiotic therapy, ranging from mild diarrhea to life-threatening conditions.	Adriano Henriques
Entamoeba histolytica	Anaerobic protozoan pathogen associated with intestinal and extraintestinal infections.	Miguel Teixeira
Helicobacter pullorum	An avian bacterium that causes gastroenteritis, intestinal bowel, and hepatobiliary diseases.	Miguel Teixeira
Mycobacterium tuberculosis	Bacteria causing tuberculosis.	Ana Varela Coelho, Isabel Couto and Miguel Viveiros
Neisseria gonorrhoeae	Bacteria causing common sexually transmitted disease (gonorrhoea) affecting millions of people worldwide.	Sofia Pauleta
Plasmodium spp.	Malaria-causing parasite.	Duarte Barral and Miguel Seabra
Staphylococcus aureus[b]	Common bacterial commensal that is also a successful and dangerous pathogen, causing different types of infections associated with antibiotic resistant strains.	Hermínia de Lencastre, Lígia Saraiva, Maria Miragaia, Mariana Pinho, Rita Sobral, Sérgio Filipe
Staphylococcus epidermidis[b]	Commensal bacteria of the skin microbiota that in debilitated individual is the most frequent cause of medical device-associated infections.	Ana Varela Coelho, Hermínia de Lencastre, Isabel Couto and Miguel Viveiros, Maria Miragaia
Streptococcus dysgalactiae subsp. *dysgalactiae* (SDSD)	Considered as a bovine pathogen but in the last years human infections have been reported.	Alexandra Fernandes and Ilda Sanches[c]
Streptoccocus pneumoniae	Bacteria frequently found in the nose and throat, but which can cause severe illness in people with a weakened immune system.	Hermínia de Lencastre, Raquel Sá-Leão, Sérgio Filipe

Table 1 – Examples of microorganisms that are studied, or whose proteins are studied, at NOVA University Lisbon regarding the topics of virulence and antibiotic resistance.

[a] *Escherichia coli* has been used as model organism to analyse virulence protein homologs.

[b] Other staphyloccocal species (*S. haemolyticus, S. hominis, S. sciuri, S. saprophyticus*) have also been studied.

[c] Deceased

Microbial subversion of innate immune defenses

A first line of protection against infections is the host microbiota. However, in individuals with a compromised immune system, or in which the normal microbiota has been disrupted, some components of the microbiota can cause serious infections and/or the microbiota is no longer protective. For example, *Clostridioides difficile* (*C. diff.*; formerly known as *Clostridium difficile*) is the leading causative agent of a range of intestinal diseases linked to antibiotic therapy resulting in a disruption of the intestinal flora. The group of Adriano Henriques has been studying the critical role of *C. diff.* spores in its infectious cycle (1). As a strict anaerobe, *C. diff.* relies on its ability to form oxygen-resistant spores for host-host transmission. Disruption of the gut microbiota creates conditions for the germination of the spores in the intestine, with the release of toxins leading to the lysis of intestinal cells and gut inflammation. *C. diff.* also forms new spores in the gut that will be released in the environment and can infect new hosts. The group of Adriano Henriques has provided critical insights on how *C. diff.* spore formation and germination are regulated (2, 3), which is essential to understand *C. diff.* transmission and pathogenesis.

Staphylococcus epidermidis is an example of a normal component of the microbiota that can cause infections. *S. epidermidis*, is a commensal of the skin microbiota contributing to its homeostasis and protection against pathogens. In debilitated individuals, however, *S. epidermidis* are the most frequent cause of medical device-associated infections. The groups of Maria Miragaia and Ana Varela Coelho are jointly characterizing differences between *S. epidermidis* clones which differ in their virulence potential. The combined use of genomic, proteomic and metabolomic approaches suggests that each strain plays a specific role in skin ecology and has its own combination of virulence mechanisms.

A major host innate defense mechanism is the production of reactive oxygen and nitrogen species that can target DNA, lipids, and important cellular proteins in the pathogen. These toxic antimicrobial molecules can be active against extracellular or intracellular pathogens, as they can be released by phagocytes (macrophages and neutrophils) at the site of infection or delivered into the phagosome following phagocytosis of a microbe.

One way for pathogens to escape the killing activity of reactive oxygen (oxidative stress) and nitrogen (nitrosative stress) species is to produce proteins that can inactivate the toxic molecules (4). For example, by transporting peroxidases to the periplasm, bacterial pathogens can destroy membrane permeable and toxic

hydrogen peroxide. To understand the mechanism of action of these enzymes, the group of Sofia Pauleta biochemically characterized peroxidases from *Neisseria gonorrhoeae* (5), which causes a common sexually transmitted disease (gonorrhoea) affecting millions of people worldwide, and from *Escherichia coli* (6), which, depending on the strain, can be a harmless gut commensal or a deadly pathogen. Furthermore, homologs of the *E. coli* YhjA peroxidase are found in other related pathogens such as *Salmonella* or *Yersinia* (6).

The ability of organisms to cope with oxidative and nitrosative stress has been extensively studied by the group of Miguel Teixeira. For example, in collaboration with Carlos Frazão, they unraveled the crystal structure of an *E. coli* nitric oxide reductase, which is a virulence factor in pathogenic *E. coli* (7). Furthermore, the group of Miguel Teixeira biochemically characterized an oxygen reductase from *Entamoeba histolytica*, an anaerobic protozoan pathogen (8). In a final example, they collaborated with the group of Lígia Saraiva and described proteins of *Helicobacter pullorum* (an avian bacterium that causes gastroenteritis, intestinal bowel and hepatobiliary diseases in humans) enabling its resistance to nitrosative stress (7).

Among the proteins that can be damaged by oxidative and nitrosative stress are iron-sulfur proteins, which participate in essential metabolic processes. The group of Lígia Saraiva analyzed the repair of iron clusters (RIC) protein from *E. coli* (9), with homologs in pathogens such *Haemophilus influenzae*, *Salmonella*, *Yersinia*, and *Clostridioides*, illustrating that another way for pathogens to circumvent oxidative and nitrosative stress is to produce proteins that repair their iron-sulfur proteins (9).

Besides reactive oxygen and nitrogen, a very important antimicrobial molecule of the host innate system is lysozyme – an enzyme that attacks the bacterial cell wall by cleaving its main component (peptidoglycan). Lysozyme is present in secretions, such as tears or saliva, at mucosal surfaces, or within phagocytes. An immune evasion mechanism used by pathogenic bacteria is to covalently modify the peptidoglycan to render it resistant to hydrolysis by lysozyme. The groups of Rita Sobral and Hermínia de Lencastre found that peptidoglycan amidation mediated by the *Staphylococcus aureus* MurT and GatD enzymes promotes resistance to lysozyme (10). *S. aureus* is a common commensal that is also a successful and dangerous pathogen, causing different types of infections. In particular, methicillin-resistant *S. aureus* (MRSA) is a major cause of nosocomial infections. However, infections caused by community acquired MRSA have also been emerging. The

group of Rita Sobral further detailed the contribution of peptidoglycan amidation to lysosome resistance in different lineages of hospital or community acquired MRSA (11), and showed that MurT and GatD form a complex (12). The protein domains and essential amino acid residues that mediate the strong MurT-GatD interaction were deciphered (12). This structural dissection of the MurT-GatD interaction can be used in the design of novel antimicrobials.

Another property of the peptidoglycan that influences lysozyme resistance is its degree of crosslinking. The groups of Sérgio Filipe and Mariana Pinho showed that wall teichoic acids, sugars attached to the peptidoglycan, control the level of peptidoglycan cross-linking by regulating the localization of the bacterial enzyme mediating the formation of highly crosslinked peptidoglycan. Accordingly, a *S. aureus* mutant unable to synthesize wall teichoic acids had decreased levels of peptidoglycan crosslinking and of lysozyme resistance (13).

Peptidoglycan is not only a target of lysozyme but also a molecule specifically sensed by host innate immune receptors. The group of Sérgio Filipe, in collaboration with Petros Lygoxygakis from the University of Oxford, showed that *S. aureus* unable to synthesize wall teichoic acids are more easily detected by a peptidoglycan binding protein (14) – an innate immune sensor of the fruit fly *Drosophila melanogaster*, a model organism. The group of Sérgio Filipe went further to show that enzymes (autolysins) that trimmer peptidoglycan while *S. aureus* grow and divide also contribute to hide it from host immune receptors (15). Furthermore, this was also observed for *Streptoccocus pneumoniae* (15), a bacterium frequently found in the nose and throat, but which can cause severe illness in people with a weakened immune system. Overall, this showed that there are multiple factors (at least, teichoic acids and autolysins) that restrict the access of immune receptors to intact peptidoglycan on the bacterial cell wall.

Host cell-pathogen interactions: microbial protein secretion, adhesion, invasion, and intracellular growth

Many pathogens interact with host cells through the secretion of virulence proteins. Some of these proteins are directly delivered into host cells by specialized systems that function as injection devices. The injected proteins (effectors) can interfere with a wide variety of host cell processes and their combined action contributes to microbial virulence. The group of Jaime Mota has been studying effectors of the human pathogen *Chlamydia trachomatis*, which causes widespread ocular and urogenital infections that can result in blindness or sterility. *C.*

trachomatis multiplies exclusively within host cells in a membrane-bound vacuolar compartment, characterized by the presence of ~50 chlamydial proteins (named Incs) inserted in its membrane. Moreover, *C. trachomatis* directly delivers many other effectors into the host cell cytosol. The group of Jaime Mota performed a screen to identify novel chlamydial effectors, resulting in the identification of several candidates (16). Among these candidates, a novel effector was identified that at distinct times of infection localizes at the host cell Golgi and plasma membrane (17). In another line or work, the group of Jaime Mota characterized a *C. trachomatis* Inc that binds and might modulate the function of a host cell centrosomal protein (18). Overall, this contributed to a better understanding of *C. trachomatis* virulence.

Proteins secreted by microbial pathogens often mediate adherence and invasion of host cells. These processes are essential aspects of the ability of a pathogen to cause an infection. The groups of Ilda Sanches (deceased) and Alexandra Fernandes explored this idea to study the potential of *Streptococcus dysgalactiae* subsp. *dysgalactiae* (SDSD) as a human pathogen. SDSD has been considered as an exclusively bovine pathogen that induces environmental losses and severe economic repercussions in the dairy industry. However, in the last years, despite rare, human infections by SDSD have been reported and it became urgent to understand its ability to interact with human cells. To address this, these researchers reported that SDSD adheres and internalizes into different human cell lines (19). They also showed that SDSD could cause invasive infections in a zebrafish, and that in this infection model the morbidity and mortality caused by SDSD were similar to those caused by *Streptococcus pyogenes*, which is considered an important human pathogen (19). More recently, they reported that SDSD can form biofilms on different surfaces, which might be an important factor in its virulence (20). These studies established that SDSD strains can infect different hosts and therefore have a potential zoonotic capability.

The capacity to form biofilms is important to the life cycle of many bacteria and for virulence. The group of Cecília Arraiano showed that a bacterial transcription factor (BolA) is required for biofilm formation by controlling the intracellular levels of the second messenger cyclic dimeric GMP (c-di-GMP) (21). On the other hand, c-di-GMP has a negative influence in expression of the *bolA* gene, indicating a crosstalk between these two regulators of biofilm formation (21). As BolA-like proteins are widely conserved in prokaryotes these findings can be extended to several bacterial pathogens (21).

Beyond adherence and invasion of host cells, and biofilm formation, many important pathogens evolved the ability to survive and replicate within infected cells, which also often depends on secreted proteins. Such is the case of the malaria-causing parasite, *Plasmodium* spp., which in its life cycle uses liver cells for its replication, before infecting erythrocytes. For intracellular survival and replication, pathogens often subvert eukaryotic membrane trafficking pathways, such as the endosomal and autophagic pathways, which both involve compartmentalized acidification that can lead to lysosomal destruction. The groups of Duarte Barral and Miguel Seabra addressed how *Plasmodium berghei* parasites multiply within liver cells. They revealed that *P. berghei* take advantage of the autophagic (22) and endocytic pathways (23) to grow within liver cells. Interestingly, parasite growth is impaired when host endosome acidification or autophagy are inhibited (22, 23). Moreover, the intracellular compartment where parasites reside does not acidify (23). These studies indicated that the malaria parasite takes advantage of host autophagic and endocytic pathways to access nutrients while, by mechanisms that remain to be elucidated, avoids transforming its intracellular compartment into an acidic and degradative compartment.

The impact of vaccination and pollution on microbial virulence and infection

The virulence potential and transmissibility of pathogens can be inadvertently altered by human intervention, for example, because of vaccination. With this regard, the group of Raquel Sá-Leão has been focused on upper respiratory tract pathobionts (symbionts that in certain circumstances can be pathogens), including *S. pneumoniae*. The groups of Raquel Sá-Leão and Hermínia de Lencastre showed that the use of pneumococcal conjugate vaccines has led to extensive serotype replacement among Portuguese children (24). They have also found that temporal trends in pneumococcal carriage exist and can be modeled and, thus, can be used to explore scenarios for the use of novel vaccines (24). The group of Raquel Sá-Leão has also recently studied pneumococcal carriage dynamics among adults and found that, contrary to what is generally assumed, acquisition is frequent and duration of carriage is often long lasting several months (25). This suggests that some adults may act as reservoirs of pneumococci and hence, depending on the social structure of a community, the magnitude of herd effects potentially attainable through children vaccination can vary. These findings are important when designing strategies to prevent pneumococcal disease in adults.

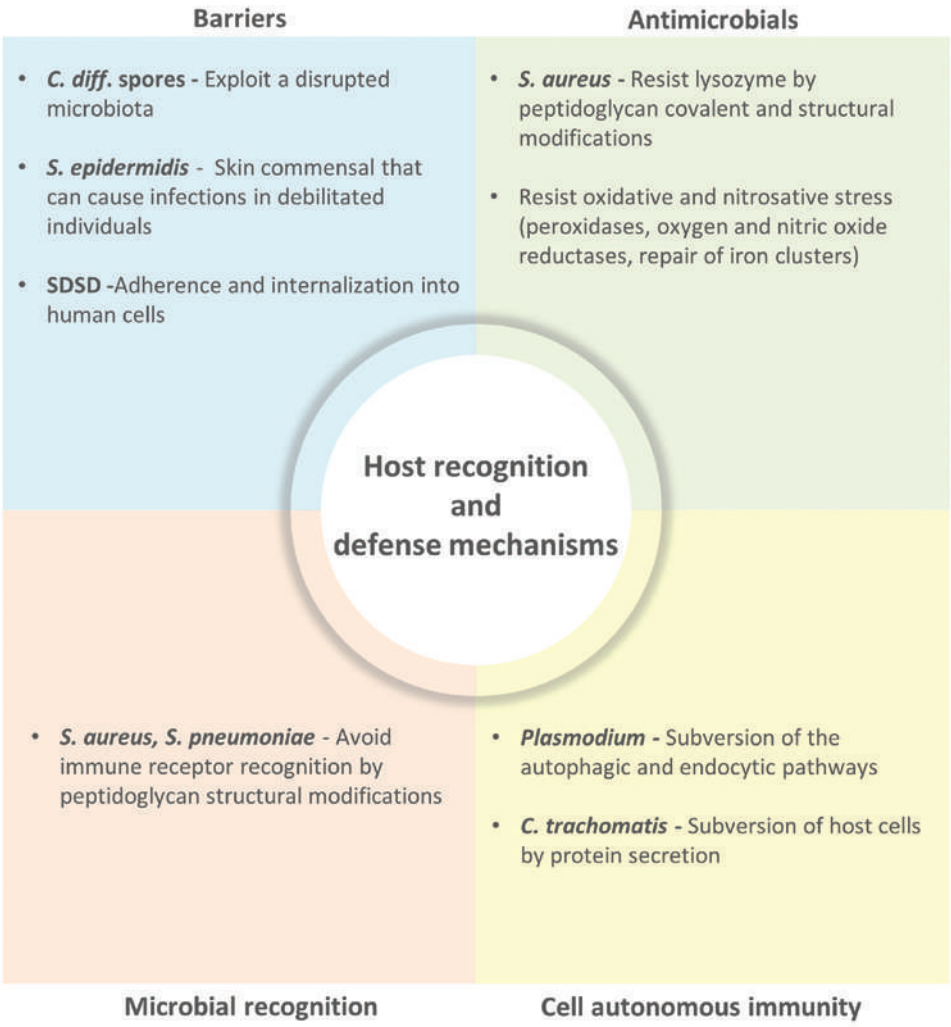

Figure 1 – Summary of representative examples of NOVA contributions to understand how microbes subvert host recognition and defense mechanisms. In addition, biofilm formation is also being studied by research groups at NOVA. See main text and Table 1 for further details.

Pollution is another form of human intervention that can have consequences for microbial ecology and virulence. The group of Cristina Silva Pereira has been studying the relationship between exposure to persistent organic pollutants with the functional biodiversity of belowground fungi, stressing alterations in virulence potential (26). Specifically, the group has shown that exposure to an archetypal

pollutant (pentachlorophenol), despite its efficient degradation and mineralization by belowground fungi, resulted in major metabolic dysregulation with patterns suggestive of increased virulence potential in fungi (26). These alterations could be a significant novel threat to animals and plant hosts.

Emergence, evolution, and dissemination of antibiotic resistance

The emergence and spread of antibiotic resistant clones of *Staphylococcus* spp., and of *S. pneumoniae* pose a public health threat worldwide. The phenomenon also presents fascinating problems of basic science, such as the evolutionary origin of resistance genes; the mechanism of antibiotic resistance and the question of what combination of determinants provides the epidemic "success" of these pathogens. The group of Hermínia de Lencastre has been actively researching in this area for over 30 years. They have characterized the molecular epidemiology of MRSA in hospitals and in the community, in Portugal and several other countries (27, 28). Other staphylococcal species, such as *S. epidermidis, S. haemolyticus, S. hominis, S. sciuri, S. saprophyticus*, were also characterized and found to be important reservoirs and key players in the evolution of β-lactam resistance in staphylococci which, they found strong evidence, occurred following widespread use of β-lactams (29). In particular, the groups of Maria Miragaia and Hermínia de Lencastre found that the gene *mecA*, which provides resistance to virtually all β-lactams in staphylococci, evolved from a native gene from the most ancestral species of this genus (30). The development of resistance involved several sequential evolutionary steps. The more impacting stage was the integration into a mobile genetic element that promoted extensive *mecA* dissemination among several staphylococcal species.

Mechanisms of antibiotic resistance

Recurrent exposure of bacteria to antibiotics has triggered selection of resistant variants that are able to survive in the presence of antibiotics. The mechanisms of antibiotic resistance are diverse depending on the pressure (antibiotic) and on the microorganism.

Efflux pumps are membrane proteins that use energy to pump molecules from the cytoplasm to the exterior of the bacterial cell. The groups of Isabel Couto and Miguel Viveiros investigate the role played by efflux systems as a first line response of bacteria to antimicrobial agents. They characterize this response at the molecular level and its relationship to the development of multidrug resistance

phenotypes. They focus on two main bacterial pathogens: *Mycobacterium tuberculosis*, the agent of tuberculosis and staphylococci, a major hospital agent. They have identified and characterized several efflux systems, including how they respond to antibiotics, how they evolve leading to the development of mutation-derived high-level resistance, and their intricate interplay (31). These studies were the basis for the development of clinical chemistry drug design and are now being extended to other pathogens (32).

The bacterial cell wall is a dynamic structure that surrounds the cell and confers protection, rigidity and shape. The cell wall is involved in the bacterial interaction with the surrounding environment. The peptidoglycan is the major cell wall component and the target of β-lactam antibiotics, such as penicillin. This class of antibiotics contains a β-lactam ring, which is recognized by proteins involved in peptidoglycan synthesis resulting in inhibition of this process. Bacteria have evolved sophisticated mechanisms to resist to β-lactam antibiotics. The group of Hermínia de Lencastre investigate the molecular mechanisms leading to β-lactam resistance in MRSA, namely through the study of the biosynthetic steps of peptidoglycan. Several approaches have been used, including the biochemical characterization of cell wall mutants, studies on gene expression regulation and protein-protein interactions (10). The group of Rita Sobral, while studying the MurT-GatD complex of *S. aureus*, uncovered a crucial role for peptidoglycan amidation in peptidoglycan turnover and biofilm formation (10, 12). Studies of the effect of composition or organization of peptidoglycan on the expression of penicillin resistance have also been investigated by the group of Sérgio Filipe (15).

Bacterial cells divide by assembling a complex machinery, called the divisome, at mid cell. The divisome includes cell division proteins, as well as proteins involved in the synthesis of the bacterial cell wall. The group of Mariana Pinho works on bacterial cell division, with a focus on understanding the complex organization of bacterial cells. They use, as a model organism, *S. aureus* with the aim of understanding the basic cell biology of this bacterium as a way to uncover the mechanisms of resistance to antibiotics and the modes of action of new antimicrobial compounds (33, 34).

Resistance to carbapenems (a type of β-lactams) has recently emerged in *C. diff*. The group of Adriano Henriques, in collaboration with Mónica Oleastro at the Portuguese National Institute of Health and Sérgio Filipe, has characterized the molecular mechanisms underlying carbapenem resistance in *C. diff*. They have shown that resistance involves the accumulation of mutations in genes coding for

peptidoglycan synthases and the cooperation between these endogenous altered synthases and an acquired enzyme, intrinsically resistant to these antibiotics (35, 36). Remarkably, the acquired synthase, termed PBP5, is a structural homologue of the MecA/PBP2a protein, a central element in methicillin resistance in *S. aureus* suggesting the adoption of a similar mechanism of resistance to carbapenems by *C. diff.* and the risk of the uncontrolled spreading of the resistant strains.

Development of novel antimicrobial drugs and novel diagnostic tools

The global problem of antimicrobial resistance demands intensive efforts to identify alternative drugs to improve treatment of resistant strains. The research of several groups from NOVA University Lisbon is directly or indirectly designed towards this goal.

The group of Lígia Saraiva has been investigating haem utilization pathways in bacteria. Although haem is essential for most living systems (e.g., necessary for haemoglobin to bind oxygen in the bloodstream), free haem is toxic. Therefore, by understanding the proteins and pathways by which different bacteria controls haem toxicity could help the design of inhibitory drugs that might have a therapeutic benefit for the treatment of infections. In particular, the group of Lígia Saraiva showed that sulfate-reducing bacteria, present in the human microbiome, synthesize haem via a novel pathway that is considered to be the original pathway from which all others are derived. In addition, they found that *S. aureus* produces heme endogenously by a third type of pathway. They uncovered, in *S. aureus*, a communication mode between heme biosynthesis and capture, that allows the bacteria to avoid intracellular toxicity derived from heme accumulation (37). Furthermore, the group is developing Carbon Monoxide Releasing Molecules (CORMs) as an alternative strategy to fight infections (38).

The group of Cecília Arraiano is dedicated to study the control of gene expression; in particular, how microbial RNA levels are regulated by transcription factors, ribonucleases that degrade and mature RNAs to maintain the right levels needed for survival, and RNA chaperones which help in these processes. They have shown that ribonucleases can act as determinant virulence factors in many pathogens, and they actively contribute to microbial resistance to stress in the environment and survival inside the host, leading to persistence and antibiotic resistance (39). Given the importance of ribonucleases in virulence of several pathogens, including SARS-CoV-2, they are prominent targets for the development of antimicrobial and antiviral drugs (39, 40).

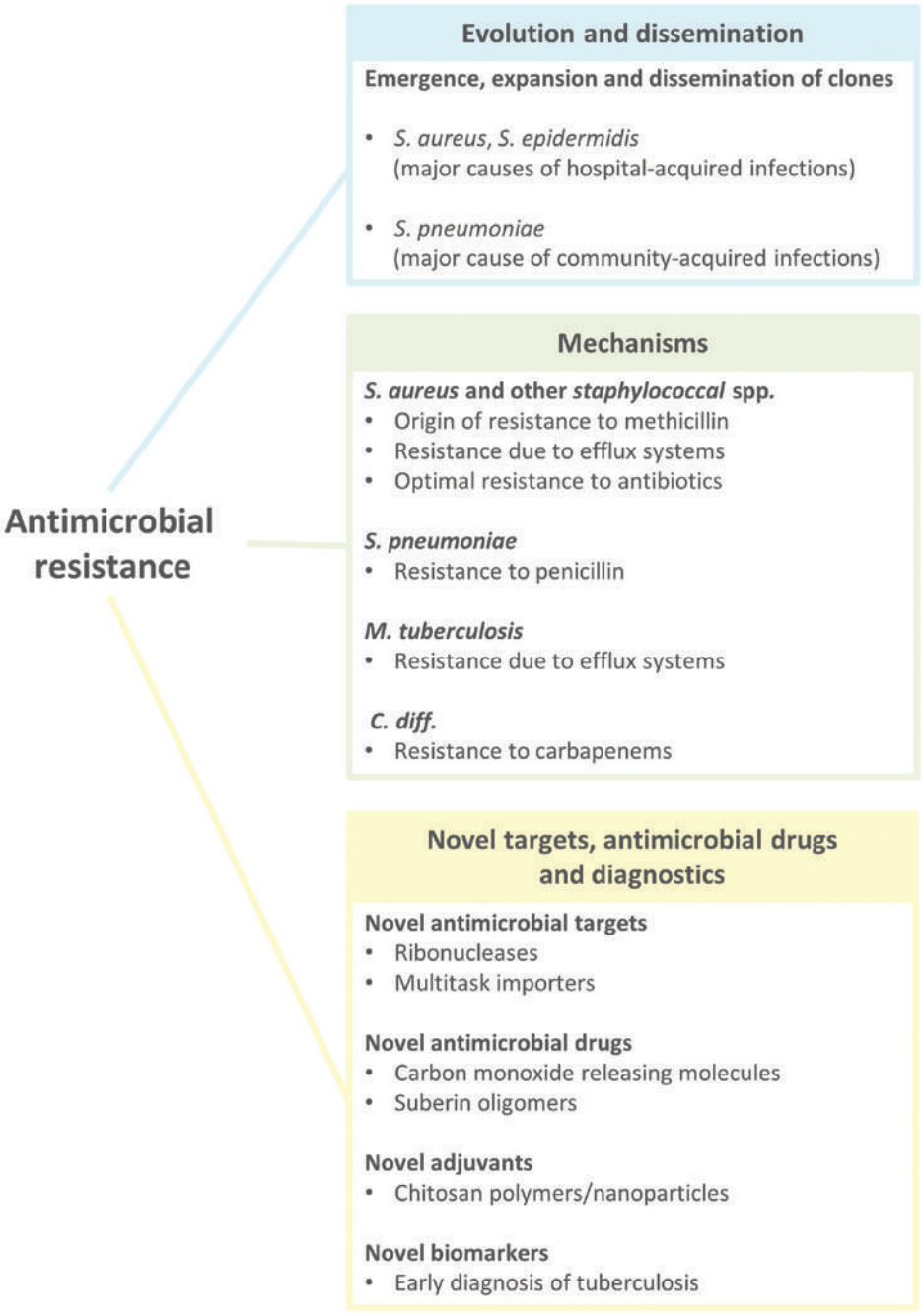

Figure 2 – Summary of representative examples of NOVA contributions to tackle the problem of antimicrobial resistance. See main text and Table 1 for further details.

The group of Isabel Sá-Nogueira studies how bacterial regulatory networks interact with other cellular components such as the metabolic system. This involves the regulation of the transport of sugars that is often mediated by ATP-binding cassette (ABC) type I importers. These importers have a modular structure that includes nucleotide-binding domains (NBDs). The group of Isabel Sá-Nogueira found a multitask NBD, able to serve as the cellular motor for multiple sugar importers (41). Further characterization on this group of multitask NBDs supported that they can be new targets for antibacterial therapy development (42).

The group of Ana Varela Coelho has investigated the mode of action of chitosan polymers/nanoparticles, promising adjuvants to enhance antibiotic effectiveness against human pathogens and in food decontamination. They found that their antibacterial activity is triggered by disassembly of the outer bacterial membrane (43).

The group of Cristina Silva Pereira has found that oligomeric structures, derived from the plant polyester suberin, display potential bactericidal activity and that killing occurs through contact. They have shown that these oligomers, when in an aqueous medium, form nano-sized aggregates – suberinsomes that kill bacteria. Their mode of action is being investigated. The group has found that killing does not result on formation of visible membrane pores nor cell lysis (44).

Early diagnostics of tuberculosis is a long-time challenge given that the etiological agent of the infection, *M. tuberculosis*, is a fastidious microorganism and often takes several days to grow *in vitro*. The group of Ana Varela Coelho has used an integrated metabolomics and proteomics biomarkers approach to analyze serum from tuberculosis patients and from controls. They identified a signature with high potential for early diagnosis of tuberculosis. Six selected metabolic biomarkers were further validated with an independent cohort. Three proteins were found to be responsible for the discrimination of controls/latent tuberculosis and patient groups (45).

IMPACT TO SCIENCE AND SOCIETY

As briefly highlighted above, research conducted at NOVA focuses on several clinically relevant pathogens (summarized in **Table 1**) with the ultimate goal of improving human health through advancing knowledge. Collectively, NOVA research in this area is uncovering the microbial mechanisms of essential cellular

processes, of survival and adaptation during infection, and upon exposure to drugs such as antibiotics. This knowledge is key to unravel strategies and targets for the development of new alternatives to prevent transmission, colonization and infection of specific pathogens while protecting the remaining microbiota.

Several studies are, in parallel, studying the origin and dissemination of antimicrobial resistant bacteria in various settings. The findings provide evidence that overuse and misuse of antibiotics in human and veterinary settings are main drivers of emergence of antibiotic resistance; the linkage between air pollution and emergence of opportunistic fungal infections is also worrisome. Together, these studies emphasize the urgent need of rethinking antimicrobial usage policies in a One Health perspective.

Local epidemiological data deriving from bacterial clonal surveillance in Portugal and elsewhere provides valuable information to Public Health Authorities to decide, for example, on the use of vaccines or adequate antibiotic stewardship programs.

The identification of novel diagnostic tools for specific pathogens can have direct implications on disease management. Similarly, the elucidation of the specific traits that increase the infection potential of a strain may help.

The plethora of research topics and strategies used to study them provide advanced training to young researchers in multiple fields of science including molecular microbiology, infection biology, biochemistry, and structural biology.

The regular engagement of NOVA researchers on outreach activities contributes to disseminate their findings to the general public, to strengthen links with the society and increase awareness of the problems associated with infections and antibiotic resistance.

FUTURE RESEARCH

The several research groups of NOVA, which address how microbes subvert their hosts and evolved to resist the strategies developed by humans to combat microbial infections, will continue to aim to advance their studies within and beyond the topics that were described. It is, therefore, expected that NOVA will continue to provide fundamental insights in these topics.

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D

SMALL MOLECULES DRUGS AND BIOPHARMACEUTICALS

Paula M. Alves
Pedro M. Matias
Ana Cecília Roque

1. INTRODUCTION

The foundations for modern pharmaceutical research began in the early 20th century with the famous postulate by the German bacteriologist Paul Ehrlich: "*Corpora non agunte nisi fixata*", i.e., a substance will not work unless it is bound. Later, British pharmacologist W. D. M. Paton delivered his "Principles of Drug Action" address to the Royal Society of Medicine in 1960, where he presented his theory of drug action based on the rate of drug-receptor combination (1). Thereafter, pharmaceutical research of NMEs (New Molecular Entities) adopted a systematic method whereby after a biological target is identified and validated, a series of high-throughput methodologies are applied, until a drug lead is obtained, with a suitable affinity and specificity to the target under consideration. However, before these molecules can be commercialized as new drugs, extensive clinical trials must be carried out, to ascertain *in vivo* efficacy, identify correct dosage and assess any dangerous secondary effects. The development cycle of a new drug can take up to 15 years and cost more than USD $1Bn. Furthermore, the success rate is low, and of the many drug leads identified, only a very small fraction (*ca.* 1%) ever becomes a marketed drug (2).

Biopharmaceuticals are therapeutic entities extracted or produced by biological sources or semisynthetic routes, and include DNA, RNA, peptides, proteins, viruses, cells or tissues. They are far more complex than small molecule drugs – as a reference, a molecule of aspirin (acetylsalicylic acid) is composed of 21 atoms and biopharmaceuticals are typically 100–1000 times larger. Peptides and proteins have long been recognized as powerful therapeutic agents. Insulin directly purified from animal pancreases is considered the first therapeutic protein, used to treat *Diabetes mellitus* in 1922. The real boom of therapeutic proteins occurred in the 1980's after the discovery of recombinant DNA technology and with the landmark of hybridoma technology in 1975. In 2020, 35% of new FDA approvals were biologics, with antibody-based therapeutics occupying the top positions with an estimated market of USD 230710 million (3). Recently, the increased entry of biosimilars (the "generics" version in biologics) and biobetters (biologics with improved performance) increased the range of available protein biopharmaceuticals (4). In addition, more than 80 peptide drugs have reached the market for a wide range of diseases and are gaining increasing interest due to their simplicity in production and simple engineering and discovery (5).

In the mid 50's, viruses, used as vaccines against infectious diseases, were the first approved biopharmaceuticals produced in large scale bioreactors (6). Since then, many advances were made in vaccine development using different protein

expression systems. Virus-like particles (VLPs), that are multiprotein structures that mimic the organization and conformation of authentic native viruses but lack the viral genome. Commonly expressed in yeast or insect cells (7), VLP's prove to be effective, safer, and cheaper alternatives for vaccination (e.g., for hepatitis B and Papilloma approved in the mid 80's and 2006). The ability to manipulate viral genomes enable the development recombinant viral vectors-based biopharmaceuticals. These can be used for vaccines, e.g., Adenovirus (8), gene therapy, e.g., AAV (9) and to modify cells for cell-based therapies, e.g. lentiviruses (10). More recently, major hopes have arisen with the breakthroughs on stem cells' potential for therapy (MSCs, PSC and iPSCs) (11) and of efficient gene editing methods (e.g., CRISPR) (12). In the last 10 years, mRNA proved to be a flexible technology, with many clinical trials ongoing and the first product authorized in 2020 to fight the COVID-19 pandemic (https://www.fda.gov/media/14441). All these advanced tools and technologies pave the way for a new classification of biopharmaceuticals in the early 21st century, the ATMPs (Advanced Therapy Medicinal Products). These are medicines for human use that are based on genes, tissues, or cells with the view to regulating, repairing, replacing, adding, or deleting a genetic sequence.

2. STATE OF THE ART

In the search for new small molecule drugs, the first step is the identification and validation of the biological target (typically a protein), ideally with known 3D structure, related with a specific human (or animal) pathogen, health condition or disease. The target is then usually expressed recombinantly and purified using chromatographic methods. Purified protein samples are tested *in vitro* against compound libraries using high throughput methods to identify potential hits or via fragment-based screening, where a "molecular fragment" is a molecule that is smaller than the probable final drug-like molecule yet still has some of the required activity towards the required target (13). Promising lead candidates are usually identified by biophysical methods, such as Surface Plasmon resonance, Biolayer Interferometry, Thermal Shift Assay, or Isothermal Titration Calorimetry and optimized using a structure-guided drug design process that almost exclusively relies on X-ray crystallography to ascertain the structural details of the protein-ligand interaction. Ideas for the growth of the ligand into its binding pocket can then be explored by medicinal chemists and relayed to the synthetic chemists to produce

new and hopefully improved ligands, to be fed back into the biophysical, biochemical, and structural pipeline.

Peptides (less than 5 kDa) and proteins represent an extremely important class of therapeutics. In terms of development, peptides and proteins with therapeutic activity can be found as a result of bio-prospection and isolation from natural sources, or discovered from *in vivo* or *in vitro* evolution approaches, namely animal immunization and display technologies, respectively. In addition, peptide and protein drugs can be *de novo* designed (14) or can be engineered to display improved biological activity (e.g., chimeric antibodies) (15). When it comes to production, peptides and proteins typically have a more complex manufacturing process in comparison with small molecule drugs. Peptides can be produced by chemical synthesis or biological expression in host cells using recombinant DNA technologies. Proteins are produced in host bacterial or mammalian cells, depending on protein expression, solubility, and post-translational modification requirements. The biological production of peptides and proteins is named as upstream processing, and the subsequent purification stages are named as downstream processing. During the last years, upstream processing has seen remarkable improvements in developing engineered cell lines with very high production yields. Downstream processing is still considered the major bottleneck in manufacturing, due to the high costs and challenging procedures to achieve high purity products – compliant with regulatory agencies – at high yield (16).

Biopharmaceuticals, in particular ATMPs, comprise a broad range of products with different characteristics, quality attributes and clinical applications. Thus, challenges for their development, manufacturing, storage, and administration are diverse. Protein-based biopharmaceuticals, e.g., highly glycosylated proteins and multiprotein complexes are already produced in large scales using robust manufacturing platforms (17), based on CHO, insect cells and yeast. Currently, the major efforts are in the development of Continuous Integrated bioprocesses (18), Process Analytical Technologies and predictive mathematical tools (19) to ensure proper post translational modifications (20) and higher volumetric productivities (21). Viral based products for gene therapy, immunotherapy and vaccines applications still pose many challenges in cell line development, host cell engineering and scalability. Ongoing efforts are the development of stable cell lines capable to produce high titers of viruses, in suspension, using scalable stirred tank bioreactors (22). Cell-based products demand a deep understanding in cell biology and exquisite analytics to depict stem cells differentiation pathways and unveil the impact of process parameters in

cell fate and functionality (11). Proteomic and other OMIC tools are key for profiling these ATMPs accelerating their translation into therapy (23), however safety concerns and process economics are still major bottlenecks (24). For mRNA-based products many challenges in formulation and stability during storage still need to be addressed (25). Regulatory agencies have now defined key mandatory attributes related to ATMPs identity, purity, sterility, and genomic integrity, however characterization of ATMPs potency is still evaluated in a case-by-case fashion.

3. RELEVANT CONTRIBUTIONS FROM NOVA

3.1. Small molecules as drugs

Earlier ITQB NOVA contributions to drug discovery and development pipelines for NMEs were made, through partnership with iBET – Instituto de Biologia Experimental e Tecnológica, in collaboration with pharma companies Hovione (26) and BIAL (27, 28). As iBET expanded cooperation with Schering, the pharmaceutical company in Berlin later acquired by Bayer, a collaboration with the Macromolecular Crystallography Unit (led by Professor Maria Arménia Carrondo) and iBET pilot plant protein production platform (headed by Eng. António Cunha and Eng. Mónica Thomaz), led to the determination of the crystal structures of the ligand-binding domain of the human androgen receptor (29) and of a mutant derived from an androgen-independent prostate cancer (30). To this date, the 2000 paper remains one of the most cited ITQB NOVA publication and it bears the singular distinction of having been accepted without any revision; a patent was filed by Schering with ITQB and IBET researchers as inventors.

These early joint efforts led to a close collaboration between the Macromolecular Crystallography Unit and the Structural Biology for Drug Discovery Unit of iBET (led by Dr. Pedro Matias and Dr. Tiago Bandeiras). In addition to common resources, the research infrastructure from each Unit is available to the other, in an open, collaborative, and synergistic environment, establishing a drug discovery pipeline from target construct design, expression, purification, soaking or co-crystallization with ligands to structure determination by X-ray crystallography. This work can be complemented by biochemical and biophysical assays using most of the methods described in the previous section, if necessary, with resort to other facilities available at ITQB NOVA and iBET. Several important results emerged from this collaboration, which also involved international pharmaceutical companies

Bayer (formerly Schering) and more recently Merck. The first crystal structure of the catalytic domain from human polo-like-1 kinase was determined and published in 2008 (31); knowledge of this 3D structure was an essential step to advance a drug discovery pipeline for this protein at Bayer, and several protein-ligand complex structures were later determined at iBET. In 2005, work began centered on two human proteins involved in several cancer pathways: RuvB-like 1 and RuvB-like 2, or RuvBL1 and RuvBL2 for short, resulting in the publication of the crystal structures of full-length RuvBL1 (32) (**Figure 1A,B**) and of a truncated form of the RuvBL1/RuvBL2 complex (33). The first structure clarified the protein fold and revealed the additional domain II to be intercalated into domain I while the second structure was revealed as a dodecamer composed of two RuvBL1/RuvBL2 heterohexamers facing each other through the preserved domain II region (**Figure 1C**). These results were in apparent contradiction with the only structural data available to date, from low-resolution Electron Microscopy, that suggested one RuvBL1 homohexamer facing a RuvBL2 homohexamer. However, the heteromeric structure for the RuvBL1/RuvBL2 complex, either in hexameric or dodecameric form was corroborated by later Electron Microscopic and X-ray crystallographic structures (34-36). After Bayer abandoned the RuvBL1/RuvBL2 project, the teams continued to work on the more challenging crystal structure of RuvBL2 that was finally published in 2018 (37) (**Figure 1E,F**).

At FCT-NOVA, there is also intense activity in marine prospection to find bioactive marine natural products as lead-like agents for drug discovery (38) or products with novel activities. For example, Pedro Costa team has contributed for the discovery of novel porphyrinoid metabolites derived from haem metabolism in worms common in the Portuguese rocky intertidal, combining microscopy and HPLC-based metabolomics (39). These pigments, far more complex and diverse in these organisms than in vertebrates have important natural roles as antioxidants, anti-UV and even as photoreceptors and biocides, which renders them of particular value for marine biotechnologists. Most importantly, the team discovered photodynamic properties in some of these pigments through bioassays aiming at determining light/light phototoxicity, which creates new prospects for their value as photosensitisers in frontier photodynamic therapies for skin disorders such as infections and cancer (40).

3.2. Peptide and Protein biopharmaceuticals

NOVA's contribution in the field of peptide and protein therapeutics has ranged from early discovery and engineering of new protein candidates to the development of

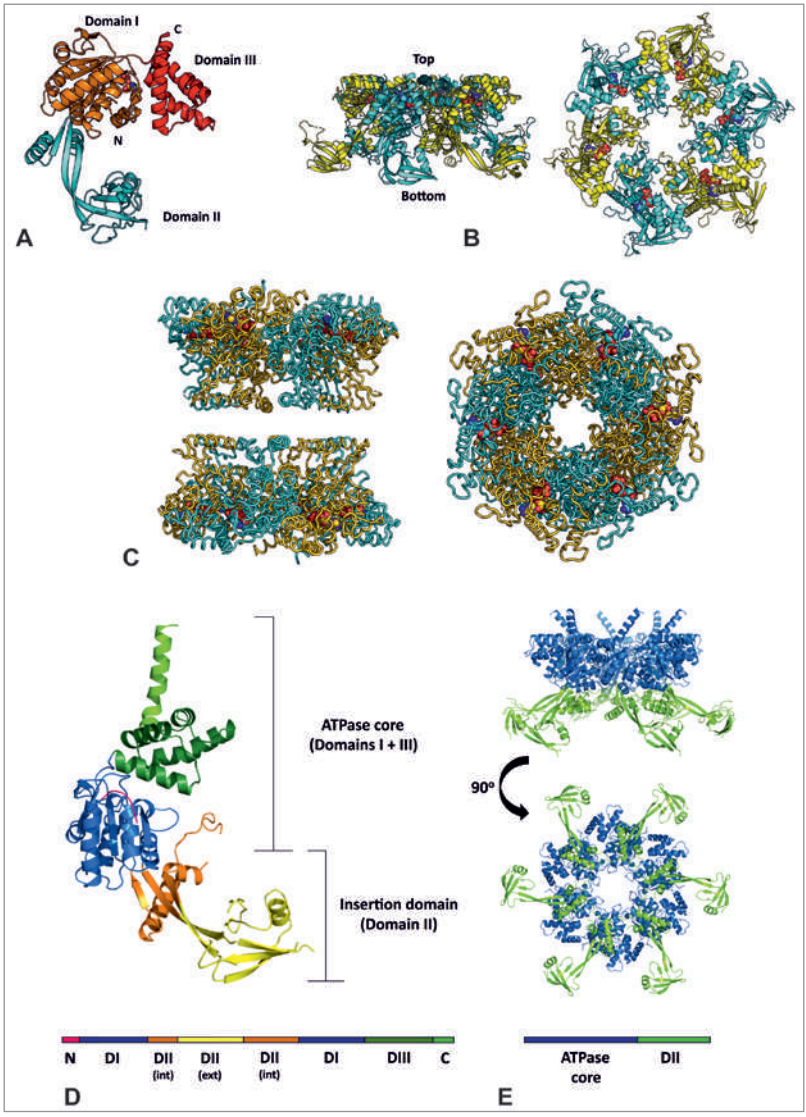

Figure 1 – RuvBL protein structures determined at ITQB NOVA. **A.** Cartoon representation of the RuvBL1 monomer, highlighting the domain structure. **B.** Cartoon representations of the side and top views of the RuvBL1 hexamer; alternating monomers are coloured yellow and cyan for clarity. **C.** Side and top views of the RuvBL1/RuvBL2 dodecamer with a truncated Domain II. RuvBL1 (gold) and RuvBL2 (cyan) monomers are shown in C^{α} tube representation. **D.** Cartoon representation of the RuvBL2 monomer, highlighting the domain structure. **E.** Cartoon diagrams of the side and top views of the RuvBL2 hexamer. Panel **C** adapted from (33) with permission. Panels **D** and **E** reprinted from (37) under the conditions of a Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

more efficient downstream processes. At FCT-NOVA the labs from Pedro Costa, Paula Videira and Cecília Roque have been the most active. The discovery of new protein toxins from marine sources has been a main focus of interest. Combining ecological traits with toxicity assessment and multiple omics (proteomics and RNASeq-based transcriptomics) to circumvent hindrances from reduced genomic resources for these organisms, Costa team discovered novel proteinaceous toxins in the secretions from a marine invertebrates (**Figure 2**)(41), which showed to be more cytotoxic to aggressive human cancer cell lines (such as A2780) than to normal cells (42, 43). These findings have important applications for the exploration of marine bioproducts in alternative anti-cancer therapies, which is an on-going line of research.

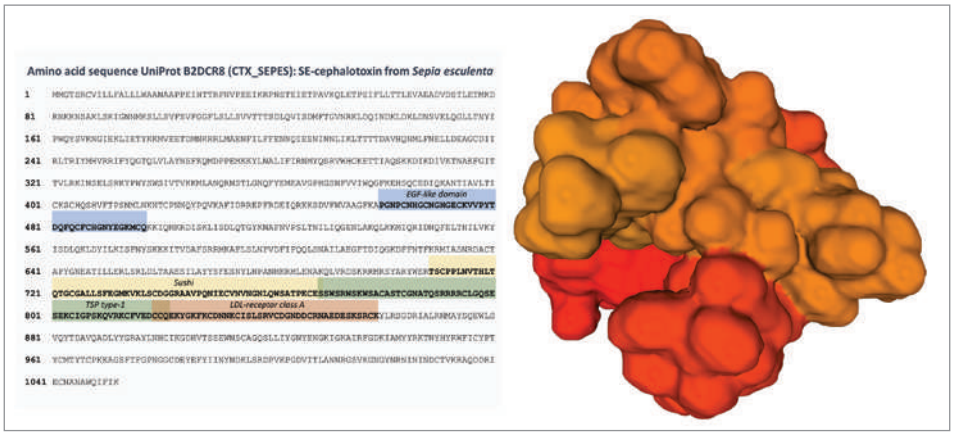

Figure 2 – Sequence and predicted 3D model of SE-cephalotoxin. Conserved domains are highlighted in the sequence, namely EGF-like, Sushi, TSP type-1, and LDL-receptor class A. Molecule was produced with Swiss-Model from the UniProt record B2DCR8 (CTX_SEPES). Glycosylation is not represented. Reprinted from (43) under the conditions of a Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

Videira's lab has been focused in understanding and translating research on human glycans, carrying out seminal research concerning the role of glycans in tumor progression and as immunomodulators. These findings paved the way to the development of innovative sialic-acid targeted potential therapies in cancer or other glycosylation diseases. Also, high-affinity anti-glycan antibodies that selectively target cancer cells and reduce tumor-burden in animal models were developed (44, 45). The observation that sialic acid modulates the potency of dendritic cells and MHC-I turnover is being explored as a mechanism of action of future therapies. In Congenital Disorders of Glycosylation (CDG), Videira's team is contributing to the acknowledgement that

the immunological response is affected and studying the mechanisms to propose new therapeutics, and ultimately improve patients' quality of life. This research is in the basis of CellmAbs company, a NOVA- spinoff developing immuno-oncology agents.

Roque's team has developed several biomimetics to protein-protein and protein-virus interactions through a combination of rational design, chemical biology and synthetic biology tools with the aim of designing minimalist versions of large receptors for important targets as phosphorylated proteins involved in cancer (46, 47). In addition, *de novo* protein design combined with *in vitro* evolution methods (phage & ribosome display), enabled the establishment of a new scaffold protein library (48) that is screened against biological targets involved in disease (49). An additional input of Roque's lab concerns with the development of alternative downstream processes for biopharmaceuticals, namely for monoclonal antibodies and virus-like particles, the later in close collaboration with iBET. FCT-NOVA has proposed several non-chromatographic approaches to monoclonal antibody purification, namely crystallization and precipitation (50, 51) as well as adsorbents fabricated using sustainable materials and clean processes (52). In addition, Roque's lab has a unique framework towards the development of low-cost and highly robust mixed-mode and affinity ligands for the affinity-based bioseparation of target biopharmaceuticals (antibodies, human serum albumin, phosphorylated peptides & proteins, tagged recombinant proteins), using rational design (53) and chemical biology tools (54) (**Figure 3**).

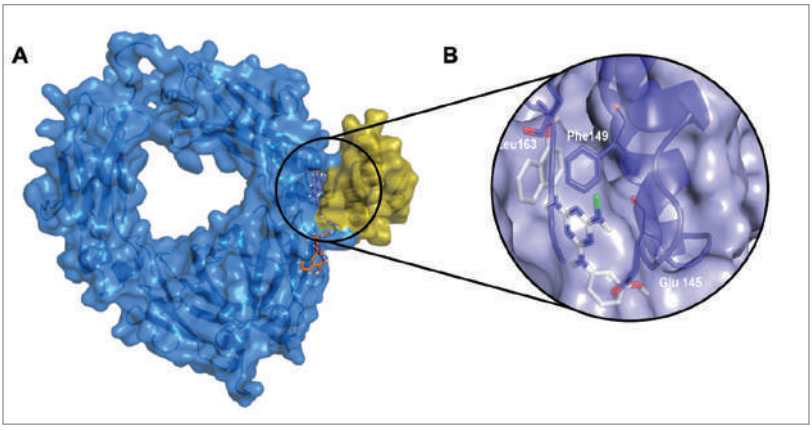

Figure 3 – How small synthetic ligands mimic biological molecular recognition events. A- proposed binding spot of a protein A-mimetic at the surface of human IgG Fc-fragment. In yellow is represented the biological protein A-ligand. B- Close-up showing the molecular structure of the proteinA-mimetic ligand and in blue the amino acid residues of the biological protein A-ligand responsible for the interaction with antibodies.

3.3. Advanced biopharmaceuticals

Advancing biopharmaceuticals at ITQB NOVA is since the early 90's, done through a strong partnership with iBET. Some examples are complex glycosylated recombinant proteins, VLPs and cell based viral vaccines, gene and cell products, and more recently stem cells for therapy and regenerative medicine and pre-clinical research.

Animal Cell Technology (ACT) was the basis for the development of these biopharmaceutical products and novel therapies. Combining knowledge and competences from scientists and engineers, several labs join large projects to cover the entire animal cell production process, from the initial genetic studies and cell line development, through the stages of process development and validation (production, purification, stability, and storage), regulatory issues, and of the development of in vitro models for screening and pre-clinical testing of efficacy and safety (**Figure 4**).

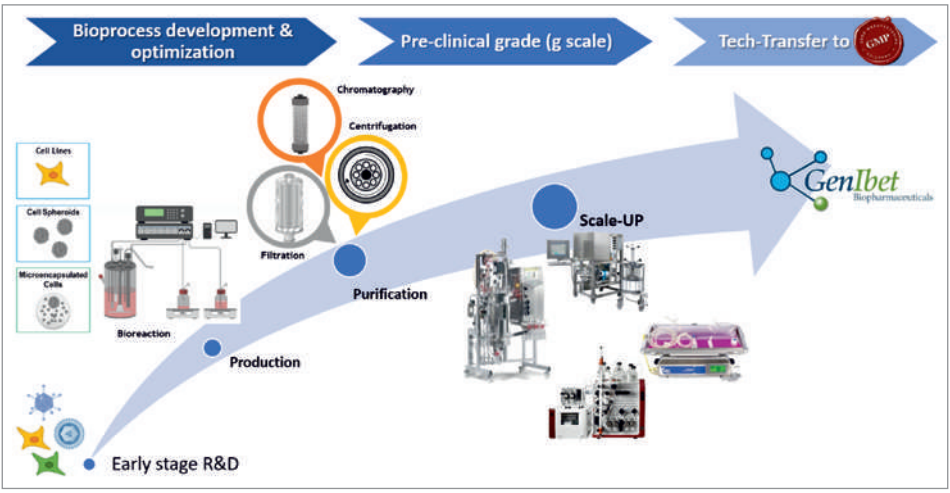

Figure 4 – Biopharmaceutical development cycle. ITQB NOVA and iBET teams can advance projects from early discovery (e.g. cell line development, vectors design), to bioprocess development (upstream and downstream) and scale up (up to 100L). Thought Genibet, iBET spin off, clinical lots of biopharmaceuticals for Phase I/II clinical are be produced since 2010.

Groundbreaking work from Manuel Carrondo's Lab advanced the field of insect cells for biopharmaceuticals production, in particular VLPs (Virus like particles). Contributing to a better understanding on virus-cell interactions, developing VLPs assembly mathematical models (55) and using metabolic and bioprocess

engineering (56) Carrondo, Alves and Roldão labs from ITQB NOVA and iBET depicted bottlenecks in virus production improving volumetric productivities and VLPs quality. Alves and Coroadinha Labs developed insect cell lines for stable production of complex proteins (57). Other major contributions from Coroadinha lab to the fields of vectorology, vaccines and gene therapy were the development of human cell lines, adapted to grow in suspension for constitutive production of retrovirus and lentivirus vectors (22, 58). At iBET Peixoto's Lab, important technologies for the development of chromatography based downstream processes (DSP) for purification of viruses were developed, recently expanded to continuous DSP processes, in collaboration with Carrondo's Lab (59).

Using bioreactor technology, Alves Lab was pioneer in the development of 3D advanced models for pre-clinical research using primary cultures, in particular brain, hepatic (60) and later derived from human stem cells (hESC and iPSC). More recently, Britos's lab has developed cell models to study breast tumor microenvironment (61) and Oliva's lab is contributing for novel skin models for melanoma. Combined with microfluidics technology (Oliva's lab) these models are used for drug design and screening to perform more mechanistic studies in disease progression. Earlier ITQB NOVA contributions to the stem cells and regenerative R&D were made through partnership with iBET. Alves and Serra Labs developed scalable and integrated bioprocesses for the expansion and differentiation of stem cells, namely MSCs, HESC and iPSCs. A recent major achievement was the Metabolic Maturation of Human Pluripotent Stem Cell-Derived Cardiomyocytes in Bioreactors (62) done in collaboration with Damian group at Harvard Medical School.

Strong competences in Glycobiology available at Costa Lab from ITQB NOVA contributed to depict structural and functional aspects of protein glycosylation with impact in health (63). Combination of analytical methods to characterize Biopharmaceuticals attributes, omics supporting bioprocess development and the design and development of (human) cell-based assays to establish potency and evaluate cytotoxicity of biopharmaceuticals and ATMS are also important competences contributing to advance this field that differentiates ITQB NOVA and iBET teams, globally.

Overall, iBET and ITQB teams have been creating opportunities for innovation in biopharmaceuticals development through generation of knowledge ranging from molecular biosciences to technology & engineering, streamlining the discovery process of new chemical and new biological therapeutic entities, from molecules to manufacturing.

4. IMPACTS TO SCIENCE AND SOCIETY

The impact of NOVA in Science and Society regarding research and development of new small molecular drugs, as well as more complex biopharmaceuticals, is significant at the different levels of the discovery, development and production pipeline summarily described above. In addition to facilities for protein production using bacterial, yeast and animal cell expression systems, coupled to advanced downstream processing capabilities, numerous methods for biophysical characterization of proteins as well as ligand-protein or protein-protein interactions are available, that include UV-Visible, Raman, Nuclear Magnetic and Electron Paramagnetic Resonance Spectroscopies, Circular Dichroism, Dynamic Light Scattering, Advanced Mass Spectrometry, Surface Plasmon Resonance, Biolayer Interferometry, Thermal Shift Assay and Isothermal Titration Calorimetry. Furthermore, X-ray crystallography is well-established, with instruments for automated set-up of crystallization screenings, optimization of crystal hits and in-house X-ray diffraction data collection, complemented with routine access to European Synchrotron Radiation Facilities, and more recently also to Cryoelectron Microscopes in European Centres.

As part of NOVA strategy to increase impact in Science, several FCT-NOVA, ITQB NOVA and iBET members have leading roles in internationally recognized organizations that promote science and discoveries within the field of (bio)pharmaceuticals, while establishing important bridges between academia and industry. Examples include the European Society of Animal Cell Technology (https://esact.org), the European Federation of Biotechnology (http://www.efbiotechnology.org) and the International Society for Molecular Recognition (http://ismr.org).

NOVA has also been extremely active in raising society awareness around the topics related with small molecules drugs and biopharmaceuticals. The FCT-NOVA, ITQB NOVA and iBET teams are involved in several outreach activities to the public, namely open days (e.g., annual EXPO-FCT and Open ITQB days), and multi-organization events (e.g., European Researchers Night). FCT-NOVA created the CDG&Allies-PPAIN, an international patient-centric network, with more than 100 professionals and patient associations dedicated to rare diseases of glycosylation. Under this umbrella, several international Glycosciences courses as e-learning and Workshops dedicated to society including families were organised, and 2 clinical guidelines written.

The involvement of NOVA in training the next generation is also remarkable. There are several undergraduate, master and PhD-level courses offered by

FCT-NOVA and ITQB NOVA in covering the field of (bio)pharmaceuticals, namely BSc courses (Cell and Molecular Biology; Biochemistry; Chemical and Biological Engineering), MSc courses (Biotechnology; Biochemistry for Health, Chemical and Biological Engineering) and PhD courses (Biotechnology; Biochemistry; Chemical and Biochemical Engineering and Molecular Biosciences). Training actions also involve the participation in COST Actions (e.g., "European Transdisciplinary Networking Platform for Marine Biotechnology" – COST Action CA18238) and Marie Curie training Networks BRAINVECTORS (Gene Therapy) and the ITN STACCATO: European Industrial Doctorate for enhancing upstream biopharmaceutical manufacturing process development.

A highlight must be given to a recently developed ELISA serologic test for COVID. In a joint effort several teams from ITQB NOVA, iBET and NOVA Medical School participated in the Serology4COVID consortium that, together with IGC and IMM combined their unique skills to implement and improve a methodology described by Florian Krammer (64) in a truly translational project. In just two weeks the iBET and ITQB NOVA were able to develop a bioprocess to produce, purify and characterize high quality SARS-COV-2 antigens (65) that were delivered to NOVA MS, IGC and IMM, where an ELISA serologic test was developed, and later used in several public health epidemiological studies: NOVA University, University of Lisbon, and Healthcare workers study at CHLO and Hospital Fernando Fonseca. To allow for a broader public health implementation and market access, efforts were made to find a licensee that could take the technology to regulatory approval and commercialization. This has been achieved and the test has been recently licensed to the Portuguese pharmaceutical group Medinfar and will enter the market in 2021.

5. ON-GOING RESEARCH AT NOVA

The fruitful collaboration between ITQB NOVA and iBET on RuvBL1 and RuvBL2 continues to this day, with two more ongoing PhD projects. A recent collaboration with French colleagues already resulted in two publications (66, 67). The focus of the work moved to the biophysical and structural characterization of cellular complexes involving these two proteins, mainly by CryoEM, and is laying the foundations to a future drug discovery campaign. A joint PhD project between Merck (Darmstadt, Germany), ITQB NOVA and iBET started in 2017, targeting the human protein Cyclophilin D. Cyclophilins are peptidyl-prolyl isomerases, catalyzing

the cis-trans interconversion of proline in proteins during folding (68, 69) (**Figure 5**). Cyclophilin D (CypD) is the mitochondrial isoform of the enzyme and a key regulator of the mitochondrial permeability transition pore. Mitochondrial dysfunction has been implicated in multiple sclerosis and cardiovascular disease (70-72). An earlier Merck drug discovery campaign targeting CypD was unsuccessful due to a limited number of protein-ligand crystal structures, but recent developments in crystallization methods rekindled the interest by Merck to revisit this project in an academic setting.

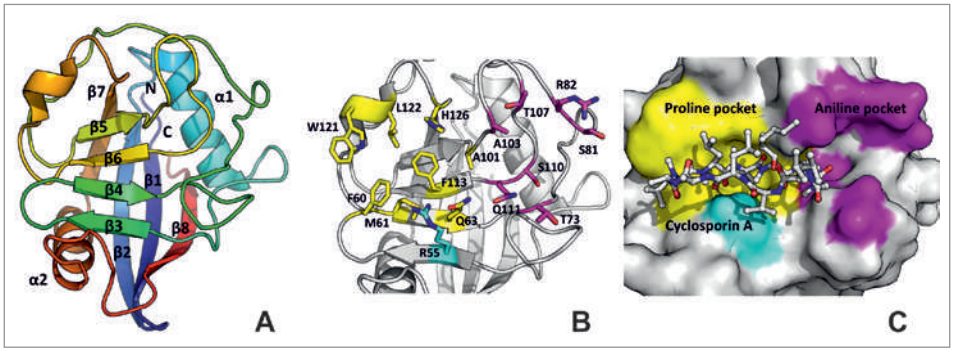

Figure 5 – **A**. Cartoon representation of the CypD 3D structure (PDB 2Z6W) rainbow-colored (blue to red) from N- to C- terminal showing the numbering scheme of the secondary structure elements. This fold is common to all cyclophilins: an 8-strand antiparallel β-sheet and 2 α-helices that pack against the sheet. **B** and **C**. Detail views of the active site of CypD in PDB 2Z6W. In **A**, the active site (proline pocket) of CypD includes the catalytic arginine Arg55 (cyan carbons), and a highly conserved combination of hydrophobic, polar, and aromatic residues (yellow carbons). A second nearby (aniline) pocket has its base defined by the main-chain atoms of a loop between strands β5 and β6 (magenta carbons). CypD is drawn in cartoon and the labeled residues are drawn as sticks. In B, a representation of the molecular surface of CypD in 2Z6W is shown, and the Cyclosporin A ligand is drawn in ball-and-stick representation, with light grey carbon atoms. Other atomic colors in **B** and **C** are red for oxygen, blue for nitrogen and gold for sulfur. All views are drawn in the same orientation.

FCT-NOVA teams are continuously engaged in ocean bioprospecting. Marine bioprospecting can replace the risky, expensive, and time-consuming design of synthetic bioreactives by exploring the outcomes of millions of years of natural selection and evolution. Taking advantage of 'omics' methods, which can yield up to thousands of molecules in single runs, Costa team will continue exploring novel bioproducts, secondary metabolites or proteins, from marine invertebrates. In terms of protein engineering, Videira's team will carry out research and development

of specific glycan-targeted therapies, namely for cancer in collaboration with CellmAbs, and CDGs through CDG&Allies-PPAIN and the EJP-RD consortium. This work also includes patient engagement, education and empowerment for active participation in all steps of drug development and care. Roque's lab will also further exploit the protein engineered scaffold library to provide early drug discovery services to several partners, namely an on-going industrial partnership. FCT-NOVA and iBET's team are also currently collaborating in an European consortia to develop sustainable downstream processes for biopharmaceuticals.

ITQB NOVA and IBET teams are continuously investing in the development of tools and technologies for complex biopharmaceuticals discovery and manufacturing, namely of complex proteins, vaccines, viral vectors for gene therapy and stem cells. Alves's Lab is focused in understanding the impact of biological relevant phenomena and bioprocess parameters in product quality attributes, Coroadinha's Lab develops and improves host cell lines for manufacturing of ATMPs, in particular gene therapy products, and Carrondo's Engineering Processes Lab integrates upstream and downstream processes, aided by physico- and biomathematical tools. The development of tools for pre-clinical research is the main focus of Brito's lab that contributes to advance 3D *in vitro* models enabling studies of cellular microenvironment in disease progression and biopharmaceuticals therapeutic response. Oliva's Lab is committed to develop 3D *in vitro* human skin models for physiology and cell interaction studies, to screen pharmacological molecules and develop of nanostructured lipid carriers for transdermal drug delivery of therapeutic molecules. Strong capacity in analytical method development to characterize Biopharmaceuticals attributes exists, of major relevance the studies on going in Costa's lab with structure and function of glycosylation, in human cell proteins, with implications in biomarker discovery. All these teams collaborate closely with major international pharmaceutical & biotech companies, therefore some of their researchers are directed to more fundamental research, while others work in the scope of established R&D contracts with industry.

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CHAPTER II

MATERIALS, DEVICES & REGENERATIVE MEDICINE

A

ENGINEERING WITH CELLS AND BIOMATERIALS: TISSUES AND ORGANS IN THE MAKING

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INTRODUCTION

Failure or loss of a tissue or organ is one of the most demanding situations that Medicine has to deal with. Current treatments involve surgical reconstructions, the use of prosthesis or extracorporeal devices, like a hemodialysis instrument, or tissue and organ transplantation. While these methods may represent life-saving procedures, or at the very least provide a significant improvement of the quality of life of patients, the complexity, risks, shortcomings and availability problems, in particular those associated with reconstructions and allografting, ask for better solutions. These will probably come from a recent area of Biomedical Engineering called Tissue Engineering, a multidisciplinary field that uses the principles of the life sciences and the methods of engineering in the development of biological substitutes to improve, restore or replace the function of damaged or missing tissues or organs.

Tissue Engineering (TE) combines, as basic building blocks, cells and biomaterials, under the adequate regulators of cellular metabolism, to elaborate new therapies aimed at the regeneration of all tissues, organs and systems of the human body, be it articular cartilage, the nervous system, skin lost to burns, bone defects or the treatment of more serious conditions such as those affecting organs like the kidneys, heart, liver or lungs.

Tissue Engineering has greatly benefited from technologies like additive manufacturing, bioprinting, nanotechnology, stem cells (including induced pluripotent), bioreactors for cell culture under stimulation, and so forth. Increased knowledge and improved technology will enable Tissue Engineering to not only create customized tissues or organs for Regenerative Medicine but also for disease modelling, testing of new medications and the discovery of new drugs and also provide more reliable methods that contribute to reducing the number of animals used in the cosmetics industry.

Biomaterials are biocompatible and biodegradable materials of both natural and synthetic origin that are used to produce scaffolds for TE. Scaffolds are three-dimensional porous structures designed to support cell adhesion and all cellular processes. In 2016, the U.S. Food and Drug Administration approved MACI (Matrix-assisted Autologous Chondrocyte Implantation), the first product that applies the process of TE to grow autologous chondrocytes on porcine collagen scaffolds that are implanted over the area where the defective or damaged cartilage was removed for the repair of symptomatic, full-thickness cartilage defects of the knee in adult

patients (1). Collagen, a ubiquitous protein, is one among the many biomaterials used in TE. Research in the field of biomaterials currently focuses on the development of intelligent materials, biomaterials that are designed to modulate their physical, chemical and mechanical properties in response to changes in the local physiological environment or external stimuli.

Several techniques have been employed to produce scaffolds starting from raw biomaterials. These techniques can be classified into foam forming, textile, additive manufacturing and hydrogel production techniques. Among the foam formation techniques, lyophilization stands out. This is a simple process that allows the incorporation of polymers, ceramics and sensitive bioactive molecules in a porous structure with high porosity and interconnected pores. Textile (spinning) techniques are also widely explored in ET. The most popular is electrospinning, a relatively simple and versatile that uses an electric field to stretch a polymeric jet in order to reduce its cross section and obtain fibers whose diameter is typically in the range of 100 manometers to 1 micrometer. Polymeric or molten solutions can be processed, with various additives, from metallic nanoparticles to ceramic powders and even cells. The most interesting feature of the structures obtained through this technique is the fact that they mimic the organization of fibrous Extra-Cellular Matrix (ECM) proteins in connective tissues.

Additive manufacturing is the name of a set of production techniques that have become quite popular in recent years due to the simplicity of some of their implementations, in particular fused deposition modelling (commonly known as 3D printing). The most used systems are the extrusion systems, which use filaments or pastes, and allow combining biomaterials, cells and bioactive molecules. Additive manufacturing allows the production of complex 3D constructs that mimic many ECM features, are anatomically correct and human-scale. The greatest disadvantage of these techniques lies in the resolution of the printing process and the difficulty in reproducing the entire micro-architecture of the tissues and organs, such as the network of capillary blood vessels. A significant advance in this area was recently reported in the journal Science. The authors produced collagen hydrogels to develop components of the human heart from the scale of capillaries to that of the complete organ (2). The resolution reached, of 20 micrometers, and the porous microstructure, allowed a rapid cellular infiltration and vascularization. The mechanical resistance of the structure makes it possible to manufacture and perfuse a multi-scale vascular network and tricuspid valves. Using anatomical information obtained by computed micro-tomography, the authors printed hearts that accurately reproduced

the patient's specific anatomical structure. Ventricles printed with human cardiomyocytes showed synchronized contractions, directional propagation of the action potential and wall thickening of up to 14% during peak systole.

Human tissues contain more than 200 types of specialized cells. Still, Integra®, a dermal regeneration template that consists of a porous matrix of collagen and chondroitin-6-sulfate, two macromolecules naturally present in the dermis, and a thin silicone sheet, manages to promote dermis regeneration in patients with deep and extensive burns relying only on the freeze-dried structure to support the scaffold's invasion by cells migrating from the wound bed (3). In the case of organs, their complexity and variety of cells present in their constitution probably requires the application of cells in conjunction with a biomaterial to achieve regeneration. The cells used can be autologous or allogeneic, adult, progenitor or multipotent or pluripotent stem. Adult autologous cells can be obtained from a small biopsy, extracted and expanded in the laboratory. An example is bladder enlargement, where smooth muscle and urothelium can be easily isolated from native tissue, expanded in culture and used to create new tissue. Other cells, such as those in the kidneys, hepatocytes, insulin-producing cells, cardiomyocytes and neurons, are much more challenging. In these cases, the existence of alternative sources can advance the engineering of these organs and significantly improve the treatment of associated diseases. An especially interesting type of cells are stem cells because of their ability to self-renew and to give rise to particular classes of differentiated cells. Three main sources of stem cells are currently under investigation: embryonic stem cells (ES), which are derived from discarded human embryos, and embryonic germ cells (EG); induced pluripotent stem cells (iPSC) obtained by genetic reprogramming of somatic cells; stem cells from adult autologous or allogeneic tissue (from fetal, neonatal, pediatric or adult tissue).

The first therapy involving cells derived from ES cells to be approved for clinical trials was aimed at treating spinal cord injuries and involved OPC1 cells, progenitors of oligodendrocytes (4). These trials require large investments and the company that started them in 2009, Geron Corporation, from the United States, suspended them. Even so, the safety of these cells was demonstrated in phase I clinical trials in 5 patients. The tests were then resumed by Lineage Cell Therapeutics, also from the USA. The tests are currently in phase I / IIa. In November 2019, the company reported positive results from the ongoing study: the safety profile remained excellent and 96% of patients achieved a robust motor recovery in the upper extremities.

IPSCs represent a direct way to ensure the immunological compatibility of ET products because the recipient also serves as a donor. IPSC cells are obtained

by reprogramming mature somatic cells to a pluripotent state. This reprogramming was achieved for the first time in 2006 by the introduction into fibroblasts of genes that encode four specific transcription factors. IPSCs were obtained for the first time in the laboratory of Shinya Yamanaka (Kyoto, Japan) who, together with Sir John Gurdon, received the Nobel Prize in Medicine or Physiology in 2012 "for the discovery that mature cells can be reprogrammed to become pluripotent".

A particular safety concern is that undifferentiated ES and iPS cells form teratomas in vivo. Recent developments in the field of cell reprogramming prevent the pluripotent state through direct trans-differentiation between cell lines. Several studies have reported that fibroblasts or other adult cells can potentially be reprogrammed directly into various types of specialized adult cells, such as neural progenitors, cardiomyocytes, endothelial cells and hepatocytes. If this technology is proven safe and effective, a solution for sourcing specialized autologous cells might have been found.

SKIN TISSUE ENGINEERING

Physical trauma and burns resulting from thermal, electrical or chemical action are two of the most common reasons for acute skin wounds. These wounds may become chronic due to conditions that slow or stop the healing process, like poor blood supply to the wound caused by pressure or circulation problems, or a weak immune system. Both types of wound may result in significant cutaneous tissue loss that needs specialized care to evolve and heal. While the epidermal layer possesses full regenerative capacity and reforms through division and migration of basal cells from the wound edges and any remaining skin appendages, thereby closing the wound, the dermis lacks such regenerative capacity. When the dermis suffers substantial damage, wound healing is characterized by extensive scarring and contraction, leading to unpleasant aesthetic outcome and functional loss, in particular when joints are involved. Clinical treatment of deep partial and full thickness wounds is therefore clinically demanding.

A large number of biologic and synthetic skin substitutes were developed and commercialized during the last 40 years. In spite of the improvements these tissue-engineered constructs brought to the treatment of chronic and acute wounds, autologous split-thickness skin grafting is still used in the treatment of extensively damaged skin, an indication of the failure of the engineered substitutes to promote

skin regeneration in a cost-effective and surgeon and patient friendly way. The composite substitutes, bilayered constructs that incorporate both dermal and epidermal layers and cells (5), are the most complete substitutes available. However, most, like Apligraf, Orcel, Karoskin and others, make use of allogeneic cells that have a finite lifetime on the host. An FDA-approved construct that uses autologous keratinocytes and fibroblasts seeded on a collagen sponge and cultured in vitro for 2 to 3 weeks before implantation is Permaderm (6). Cells are obtained from skin biopsies but must be expanded in vitro, with all the costs, delays and risks this implies, besides requiring multiple surgical procedures. Research towards more complex and better performing substitutes has been reported in the literature. Despite the promising results reported, shortcomings of these substitutes include scaffold contraction, fast degradation, poor mechanical properties, several weeks of in vitro maturation, various surgical interventions, and the lack of antimicrobial agents (7).

In 2009, the Tissue Engineering Group of the School of Science and Technology of Nova University of Lisbon started a project aimed at researching and developing an integrated solution for the treatment of deep partial and full thickness burn wounds. The concept developed – the Skin2 biosynthetic skin substitute – integrates the scientific knowledge and technological developments reported in the literature and has the potential to fulfill most of the requirements of an ideal skin substitute. The Skin2 substitute obeys several fundamental design rules:

- its structure and composition should promote the concurrent regeneration of both dermis and epidermis,
- it should be possible to apply to the wound bed on the same day the patient is admitted to the burn centre and not require further surgical interventions,
- only biocompatible and biodegradable materials of non-mammalian origin may enter its composition,
- it should only use autologous cells without requiring in vitro expansion or differentiation,
- an antimicrobial agent should be incorporated to avoid the risk of infection.

The goal is to provide the surgeon with a complete therapeutic solution, quick and easy to apply, effective in promoting skin regeneration, at a reasonable cost.

Research is underway (8)(9)(10)(11). Figure 1 shows the result of an *in vitro* test on extracellular matrix production by human fibroblasts. *In vivo* results have shown this approach to be effective in promoting healing of full thickness skin excisions

in the Wistar rat. This confirms the initial expectations on the performance of the Skin2 substitute.

The project is being carried out by researchers belonging to several research centres of the Nova University of Lisbon: CenimatIi3N, UCIBio and CEDOC, all evaluated as Excellent by international evaluation panels. Furthermore, the project has the participation of: Dr. José Carlos Parreira, Head of the Plastic and Reconstructive Surgery Service of Hospital Garcia de Orta, Almada; Prof Lucie Bacakova of the Department of Biomaterials and Tissue Engineering, Institute of Physiology of the Czech Academy of Sciences, Prague, Czech Republic; Prof Dimitrios I. Zeugolis, director of the Regenerative, Modular & Developmental Engineering Laboratory (REMODEL) of the National University of Ireland, Galway, Ireland.

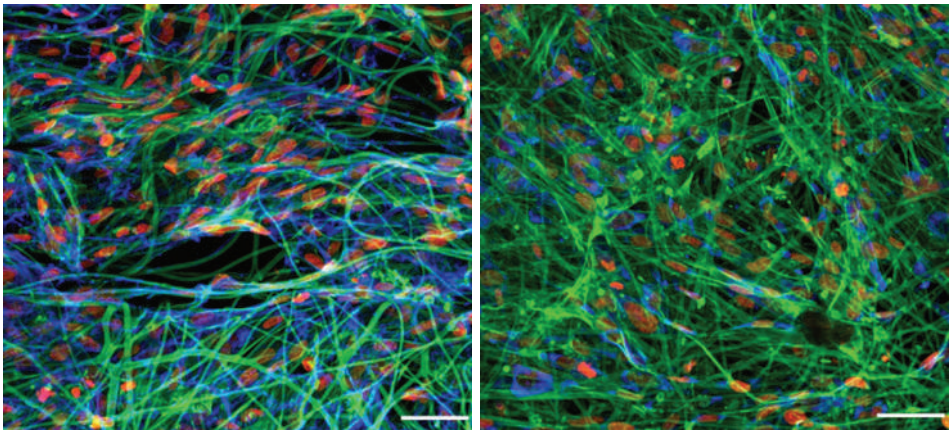

Figure 1 – Human fibroblasts growing on a chitosan/gelatin/polycaprolactone nanofibrous scaffold produce collagen I (left) and fibronectin (right) on day 7 of culture as expected in an in vivo environment. Collagen and fibronectin are stained in blue, DNA in red. Scale bars = 50 μm. Reprinted from (10) with permission from Elsevier B.V.

NERVE CONDUITS FOR NEURAL REGENERATION

Nerve regeneration presents big challenges. In severed peripheral nerves, axonal regeneration is observed only in small-sized lesions. Gaps are closed by suturing of the nerve stumps or bridged with autografts, allografts or artificial nerve conduits (such as the nerve repair devices Neurotube, Neurolac, NeuraGen, NeuroMatrix, Neuroflex and SaluBridge), thereby surpassing limited availability, donor

site morbidity and pain and immune reaction. However, delayed axonal regeneration through large gaps leads to impaired or absent repair and functional recovery (12). Among Central Nervous System lesions, spinal cord injuries (SCI), mainly caused by accidents, are a serious limiting condition for many young people worldwide. Although the spinal cord reveals potential to regenerate through a nerve bridge (13), the innate immune response triggers a secondary injury that amplifies damage. Clearance of debris doesn't occur and a cystic cavity surrounded by a glial scar is formed preventing regeneration. Due to the complexity of a SCI, its treatment likely requires combinatory strategies (14). Tissue engineering scaffolds with their potential to deliver drugs, neurotrophic factors and cells are a key component of such a strategy. The scaffold should provide mechanical support for cells, include topographical or biological cues to direct axonal growth and also prevent the ingrowth of scar tissue. It should be mechanically soft to integrate well with the host tissue and have a conductive nature in order to allow electrical stimulation of cells (15). Vascularization and control of immune response are believed to be key factors for a successful regeneration. Transplantation of various types of cells is being explored.

Due to their versatility, both natural and synthetic polymers have been investigated to fabricate tubular scaffolds to assist neuronal regeneration. Scaffolds comprising polymeric fibres produced by the electrospinning technique (16) can be tailored with regenerative stimuli to neural cells (14). Fibre alignment gives an important contribution for neural regeneration by guiding axonal growth. Electrical stimulation of neurons has also been shown to influence the rate and orientation of neurite outgrowth in vitro and to induce guided axonal regeneration in vivo. ES was also recognized to influence proliferation and differentiation of various cell types (15). Conductive polymers, a class of attractive polymers envisioning direct ES through scaffolds, present practical problems: inadequate physico-chemical properties in view of material-cell interactions, inability to degrade that may lead to chronic inflammation and poor solubility and processability (17).

Macrophages may acquire pro-inflammatory (M1) or anti-inflammatory/regulatory (M2) phenotypes, depending on stimuli received from the environment. In comparison with a normal healing, after a SCI, populations of M2 macrophages reduce too early while M1 remains for a long period after the injury. Along with microglia, blood monocyte-derived macrophages (BMM) participate in SC immunologic response. Some authors associated the anomalous SC healing with insufficient M2 phenotype of BMM (18). The ability of a material to polarize macrophage towards M2 phenotype opens an opportunity to promote regeneration.

NEUral Regeneration with conducTivE Scaffolds (NEURiTES) is an ongoing project at the Nova University of Lisbon since 2018. Its main goal is to optimize the electrical conductivity of composite scaffolds of aligned electrospun fibres comprising a conducting polymer (CP) and a biodegradable polymer (BP), envisioning the promotion of neural regeneration. The project explores new routes for the synthesis of the CP in order to produce small particles that may lead to a conducting network interspersed in the BP electrospun fibres. A new gelatin-based polyurethane designed in our lab (19) is one of the BPs included in the production of the scaffolds, since we have previously verified that scaffolds of this material support neural stem cells and their neuronal differentiation (see Figure 2).

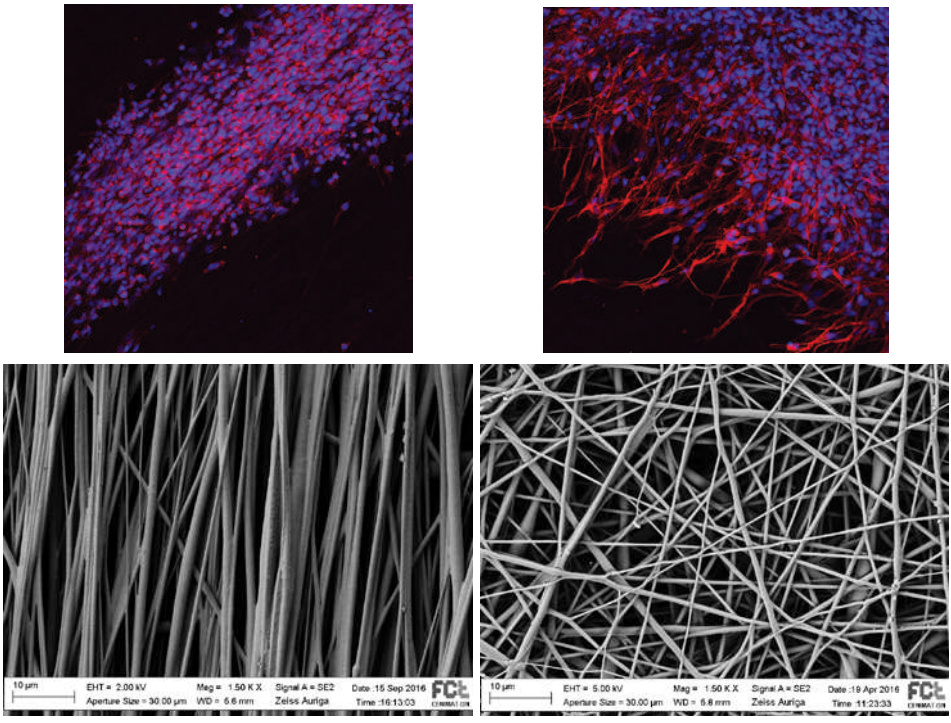

Figure 2 – Fluorescence images of human neural stem cells (coloured images) 3 weeks after being seeded on electrospun fibrous scaffolds (grey scale, scanning electron microscopy images, displayed as insets). The scaffolds are made from a new gelatin-based polyurethane designed in our lab (19) and cell nuclei are stained in blue. On the left, fibres are aligned and a neuronal filament protein (NF70) is stained in red. On the right, fibres are randomly oriented and a microtubule-associated protein (MAP2) is stained in red. Both, NF70 and MAP2 proteins, reveal the neuronal differentiation of the neural stem cells supported by the scaffolds.

To anticipate benefits of these innovative conducting scaffolds in neural tissue regeneration, studies of electrical stimulation (ES) are performed with human cells: neurons, stem cells both neural and mesenchymal and macrophages. Particular attention is given to the impact of ES on polarization of macrophages into phenotype subsets and of neural stem cell into the neuronal phenotype, since macrophage polarization controls the inflammation process and, consequently, the remodelling outcome and the success of transplantation of stem cells to the lesion requires cell fate control. These studies will elucidate on possible strategies to promote neuronal regeneration through ES.

NEURiTES' research team comprises researchers belonging to the research centres CenimatIi3N and UCIBio of the Nova University of Lisbon and has the participation of S. Kubinova from the Department of Biomaterials and Biophysical Methods at the Institute of Experimental Medicine Academy of Sciences of the Czech Republic.

BIOMATERIALS FOR HARD TISSUE ENGINEERING

Since the 1960s, ceramics have been widely used in orthopedics and dentistry when bones and teeth cannot heal without external intervention. In spite of the natural regenerative ability of bone, in some situations (like massive bone loss, which leads to critical sized defects, invasion of bone volume by soft tissue or the repair of congenital defects), bone does not regenerate by itself. Calcium phosphates (or apatites) are one of the choices for bone repair, such as in coatings of implants (20) or in non-load bearing scaffolds as bone fillers (21). The apatites have a high similarity with the natural hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$), the main inorganic component of hard tissue.

It is known, since the late XIX century, that mechanical stress is important in the remodeling processes of bone (22). Also, since the 1950s, Fukada and Yasuda showed that bone is piezoelectric and mechanical charges generate electrical cues for cells involved in bone remodeling (23,24). Bioceramics that have electrically charged surfaces exhibit enhanced bioactivity (formation of an apatite layer) and in vivo testing shows faster osteointegration (connection between living bone and the surface of a load-bearing artificial implant). Synthetic hydroxyapatite has the ability to be electrically charged (25) and can also be obtained in a piezoelectric phase (26), which, after electrical polarization, can be used to develop bone substitutes with surface charges.

PolarBone – New synthetic bone substitutes based on electrically polarized calcium phosphates (2014-15) was a QREN project involving the Biomaterials Group from NOVA School of Science and Technology, Universidade de Aveiro and Altakitin (now BioCeramed), a Portuguese company in the healthcare area. Altakitin's products – hydroxyapatite and biphasic calcium phosphates (hydroxyapatite and β-tricalcium phosphate composite) – were successfully charged and showed increased bioactivity (Figure 3). Furthermore, in vitro tests using osteoblasts also showed better cell adhesion and proliferation on surfaces with electrical charges.

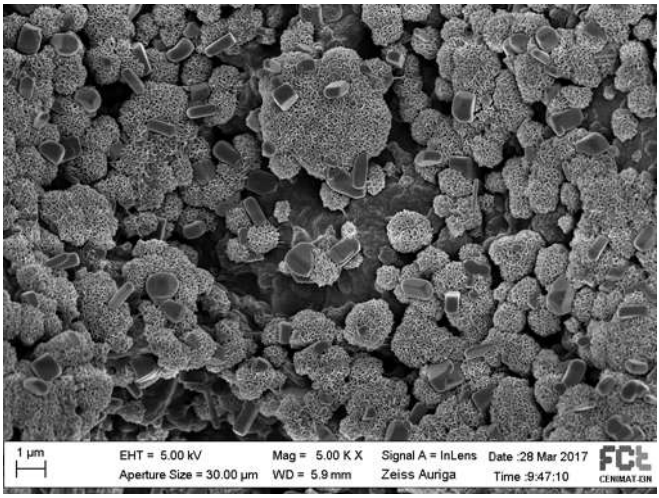

Figure 3 – Scanning Electron Microscopy (SEM) photo of bioactivity assays of a polarized scaffold of chitosan and hydroxyapatite. After electrical polarization, the scaffold was immersed in a simulated body fluid (SBF) for 3 days. The apatite crystals deposited during the test are observed displaying the typical flower-like shape. Reprinted with permission from Ribeiro E. (2017). Melhoria das características eletroativas de matrizes porosas à base de hidroxiapatite e quitosano destinadas à regeneração óssea. MSc. Thesis in Biomedical Engineering. Nova School of Science and Technology, Universidade Nova de Lisboa.

In the framework of PT2020, another project DentalBlast Development of antibacterial coatings based on bioglass for dental implants (2016-2019) of i3N-Cenimat Biomaterials Group and with the participation of UCIBio, also a NOVA FCT research centre, two Portuguese companies, Ceramed and Hospital Veterinário de São Bento and the Universidade de Aveiro. The project allied electrical polarization with antibacterial properties of calcium phosphates and bioglass composites to be used as coatings on dental implants. The materials were further doped with ions with

antibacterial activity (e.g., silver and zinc) and were used has coatings. The coating was made using CoBlast (a recent coating technique (27)) in titanium alloy screws that were successfully used in dental implants in dogs. The CoBlast technique was developed in Ireland to overcome the problems of high temperature coating by plasma spray, which is the most used commercial technique for ceramic coating of implants.

Carrying further the work developed in DentalBlast, the project ORAiDEA – Development of multifunctional dental implants (2020-), which adds to the micro-structured coatings obtained by CoBlast nanostructured coatings done by Physical Vapor Deposition, started recently. In this project, beside the Biomaterials Group and UCIBio, three Portuguese companies are involved: Prifer, Ceramed and Hospital Veterinário de São Bento. Besides NOVA, three more Portuguese universities integrate the research team of ORAiDEA: Universidade de Aveiro, Universidade do Minho and Universidade de Coimbra. The project intends to develop new multifunctional dental implants with better bioactivity and antibacterial properties enhanced by electrical polarization to reduce peri-implant infections.

In recent years, the Biomaterials Group started to work with biocompatible and bioactive piezoelectrics, such as the lead-free ceramic barium titanate and polyvinylidene fluoride (PVDF), a polymer. The piezoelectric biocompatible materials can be combined with calcium phosphates to further enhance the osteointegration by electrical polarization (28). These materials can also be used either as 3D ceramic scaffolds or in the production of polymeric membranes by electrospinning to mimic the hard tissue microenvironment and improve the cell response using electrical cues.

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B

MEDICAL DEVICES FOR MONITORING, DIAGNOSIS AND REHABILITATION

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1. INTRODUCTION

Human physiology can be better understood if one has access, through measurements, to certain biophysical quantities. Medical devices, throughout history, enabled the assessment and monitoring of human body functions, and allowed for disease diagnosis and prevention. They opened the possibility to design novel clinical interventions, in a more suitable and timely manner. In many situations, such devices have also contributed to the aforementioned intervention. Overall, technology has revolutionised the way we approach Health Care in general, and Medical Practices in particular.

Currently, medical devices are paramount in each of the four main vectors of modern medicine: prevention, diagnosis, treatment and, transversal to those, rehabilitation. That involvement acts both at the hardware and software levels.

The Biomedical Engineering group of the Laboratory for Instrumentation, Biomedical Engineering and Radiation Physics (LIBPhys) and the Department of Physics of NOVA School of Science and Technology has elected, as their main focus, the aforementioned biophysics measurements, their analysis and clinical support systems. Throughout the development of such transfer of technology, from basic research to the applications, one has always kept into consideration requirements of usability and security of both the patients and the data collected.

The complete process of development has been sought in very close collaborations between the academic research, and a large network of clinical partners, companies and patients themselves.

The goals of the biomedical engineering group has always been to study several biomedical systems, understand various pathologies and develop possible interventions to solve or mitigate them. In the current chapter we will illustrate the research done at NOVA School of Science and Technology, in the context of the development of medical devices. We grouped the examples shown in three main topics: monitoring effects of postural deviations, development of instrumentation, and the human computer interaction. All of those answer growing societal concerns, and have a strong impact therein, as they target the subject's health, quality of life and wellbeing.

2. MONITORING EFFECTS OF POSTURAL DEVIATIONS

Shoulder Pain – The level of pain reported by patients is one of the most important markers for the diagnosis of various pathologies. In clinical practice, it is

usual to assess such pain via an analogue pain scale, being this solution considered subjective and often inaccurate. Thus, it is clearly relevant to develop an objective and precise method for pain evaluation. With this purpose, our group is studying the possibility to use electrophysiological signals to estimate the pain quantitatively.

Electrodermal signal analysis (EDA), which measures the skin conductivity changes, and electrocardiographic data, which gives information about the heart rate (HR), both have shown promising results regarding the quantification of the autonomic nervous system's sympathetic response to pain.

Considering the high prevalence of orthopedic injuries and their strong impact on daily activities, we studied the pain associated with this kind of disease. Particularly, taking into account that jobs that require repetitive movements, including the use of the computer mouse, can lead to severe shoulder injuries, our study was carried out on 21 patients from Hospital Curry Cabral, who were part of the Occupational Therapy department's care in the area of Physical Medicine and Rehabilitation. All participants followed an experimental protocol consisting in the measurement of electrodermal and cardiac signals, and the filling of an analogue pain scale, based on the individual report. This data was recorded during the performance of two different movements: a simple movement of shoulder flexion with elbow extension and a more complex one consisting of internal rotation of the shoulder with elbow flexion. These two movements were chosen in order to cause two very different levels of pain.

Preliminary results [1], presented in Figure 1 indicate that the greater the pain experienced by subjects, the greater the amplitude of the electrodermal signal and heart rate, especially for highest levels of pain.

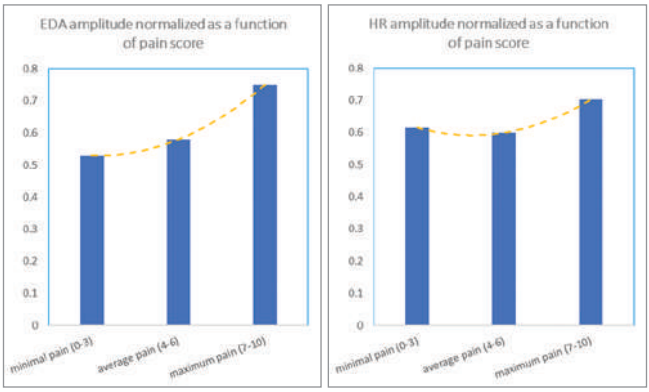

Figure 1 – Normalized mean amplitude of the electrodermal signal and heart rate of all participants as a function of the grouped pain score.

These outcomes indeed encourage us to continue with this research line, extending the study to other types of pathologies, namely the ones that are associated with neuropathic pain. We believe that establishing a clear relationship between pain and physiological signals will have an important impact in the intervention methodology and in the diagnosis process, allowing a better and more personalized health care.

Vertebral Metrics – Back pain is an important problem in modern society, with a huge impact on the quality of life of each person. About 80% of people experience back pain at some point in their lives and, from those, 80 to 90% are caused by mechanical changes in the spine [2]. In view of the number of people affected, it was relevant to develop an instrument to evaluate the spinal column in a standing position. The resulting device, VM, patented national and internationally [3], is a non-invasive system designed to identify the spatial position of the spinous processes, from the first cervical to the first sacral vertebrae. It evaluates the curvatures and lateral deviations of the spinal column, resulting in a quantitative diagnostic of postural deviation [4].

VM requires an initial marking of each spinous process, to be done by a specialized technician, through palpatory anatomy [5]. The ability to estimate correctly the coordinates of the spinal processes was validated with an optical system of stereophotogrammetry with ten infrared cameras [6].

Although initially completely mechanical, as shown on the left-hand frame of Figure 2, VM has since evolved to an optical one, as displayed in the right hand frame.

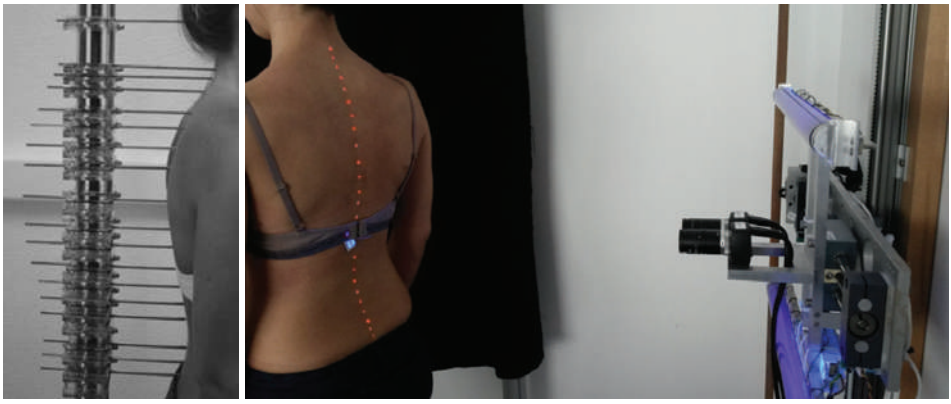

Figure 2 – Illustration of two working models for vertebral metrics. On the left, the original, mechanical version [6]. On the right, the third version of the device, with double cameras, mounted on a motorised vertical support [7].

This version uses UV light and a specific fluorescent dye to identify the 3D position of each vertebra. as well as a developed software capable of distinguishing prominent marks in the skin. After the location estimation processes, the spatial coordinates of all vertebrae are used to build a 3D model of the spine. A resolution better than 1 mm in each direction is provided by the stereo vision system [7].

VM allows for a global assessment of the spine and represents an innovation in the field of screening, with potential use for prevention and diagnosis, since it can be applied several consecutive times without affecting the measured subject. Moreover, VM costs a fraction of existing techniques, is easy to transport and presents few logistic requirements [7]. Therefore, it can be easily used in different contexts, such as in ambulatory, being it public or private (health centers / clinics) and in hospitals. Its areas of application include orthopedics, neurosurgery, and rehabilitation. In addition, it is expected to be of very high impact as a general screening device for back pain [7].

VM was already applied to study the biomechanical changes in the spine of pregnant women that occur throughout pregnancy, as well as in patients diagnosed with ankylosing spondylitis. The clinical trials have confirmed the potential of VM to evaluate biomechanical changes in the spine. That research was performed in collaboration with NOVA Medical School, Maternity Hospital Dr. Alfredo da Costa, Regional Health Administration of Lisbon and Tejo Valley and Hospital Egas Moniz–Centro Hospitalar de Lisboa Ocidental.

3. INSTRUMENTATION

Physioplux – Biofeedback is a process by which information about the performance of a particular part of a subject's body is given to him/her, in order to self-regularize its function.

Biofeedback can be based on a set of biosignals, such as an electromyogram (EMG) electroencephalogram, skin conductance, heart rate or posture [8]. It may be used, for instance, in rehabilitation, to improve one's health, exercise performance, or to promote positive physiological changes. Those accomplishments are achieved through a process of relearning, after several treatment sessions.

EMG is the most used electrophysiological signal, for the design of biofeedback tools. The key idea is the introduction of the biofeedback mechanism within the retraining process of movement patterns of a patient. This process has been

applied to several conditions that go from urinary incontinence to gait functionality relearning and shoulder impingement treatment.

The need for monitoring the muscular function, through EMG collected at the surface of the skin, as well as the use of such electrophysiological signals to build reliable biofeedback, in a user-friendly technology, led to the creation of Physioplux.

Physioplux is a Class I medical device, with CE and FDA approval, designed by PLUX, a Portuguese medical device company, devoted to the analysis of clinical biosignals, with a strong research involvement with NOVA University Lisbon. This partnership is particularly improved via an academic of the Physics Department, founder of the company, and via several Phd students and biomedical engineering alumni collaborating with PLUX. The process of certification and clinical data collection, supporting the applicability of Physioplux in practice, was partly done in collaboration with the NOVA University Lisbon

In Figure 3-(left) we see the complete medical device kit, with the biosignal collection unit: the Physioplux device and the tablet, where the biofeedback signal constructed from 4 muscles of the body is being presented, and a pair of wearable EMG sensors, which can be used in home treatment, connected to a mobile phone.

Physioplux has been used in several clinical situations and research projects. As an example, one of the conditions treated with Physioplux is shoulder impingement (with a lifetime prevalence of near 60%). In this condition, lower trapezius, upper trapezius serratus anterior, and lateral deltoid are monitored and presented to the patient in a tablet interface with easy-to-follow graphs (see Figure 3-(right)).

Figure 3 – Physioplux medical device. On the left, we can see the EMG collection unit and a tablet presenting the biofeedback information. On the right, an example of a therapy session for shoulder impingement treatment. [used with permission].

Within Physioplux, the acquisition protocol starts with the measurement of the sequence of muscle contractions, to verify if the control of several movements is correct or should be retrained. A second step measures a reference value for muscular activity, called maximum voluntary contraction, which is collected under standardised movements, with the help of a clinician. With such a calibration procedure done, a set of control tasks is then performed, and awareness of the internal mechanics that generate muscular activity is transmitted to the user, as biofeedback. The final step is a training session, where the clinician defines thresholds of muscular activity that should be sustained or reduced, under several exercises.

The aforementioned example of biofeedback has proven to reduce the treatment time in 50% [9].

The possibility to continue the treatment at home is also part of Physioplux, by adding two wearable sensors that can connect to the patient's mobile phone. With a set of prescribed exercises, the treatment can be executed at home, monitoring only two muscles, and following a set of simpler goals than its clinical version, which has the continuous participation of the physiotherapist. The usage and the achievements are reported to the clinician, and can be evaluated in face-to-face sessions, enabling a better follow up of the complete treatment.

This illustrative example should be viewed as one of the countless applications that Physioplux allows, rendering the processing of rehabilitation easier, more effective and intuitive.

Eye Tracker – In a complex and multi-sensory environment, it is important to understand where one puts one's focus of attention. If we take, as a proxy for such an environment, the viewing of a movie, one may even be tempted to state that an audience may watch the same movie, while viewing totally different ones. To truly understand what visual stimuli are effectively processed by someone, it is crucial to identify which portions of the image are indeed attended. In addition to the aforementioned applications, eye trackers have been employed in areas such as gaming, through human-machine interactions, the analysis of customer impact of publicity, neuromarketing, clinical evaluation of strabismus and several types of degenerative diseases.

At LIBPhys, a portable eye tracker was proposed, rooted in mobile phone technology and a cardboard box support, designed by Google for virtual reality applications [10]. The device is highly portable, and very low cost, assuming that

most people have their own mobile phones. In spite of its affordability, the accuracy and precision attained values of 1.06-1.51° and 0.6-0.86°, respectively. Those are reasonably close to the ones presented by more expensive commercial devices, with values of 0.5-1.0° and < 0.3°, at a fraction of their cost. Figure 4 displays one example of the use of such an eye tracking device, showing clearly how certain important features stand out from the background, as they contain more relevant structure and information.

Figure 4 – Example of an eye tracking outcome. On the left are displayed pupil estimates for three different directions of gaze. On the right, superimposed to the image, several locations of visual attention. Adapted from [10].

We have also extended the useability of the aforementioned device to estimate pupil dimensions [11]. Pupilometers, together with the heart rate variation (through ECG) and sweat (through EDA) are often used to assess the integrity of the autonomic nervous system. Significant pupil dilation occurs via the increase in sympathetic nervous activity.

After validating both the eye tracker and the pupilometer, in controlled environments, we used the eye tracker device to study, together with researchers from the Faculty of Psychology of the University of Lisbon, differences in decoding facial emotional expressions, confronting subjects with and without formal musical education [12]. We found that the group of individuals with musical training displayed a frequency and duration of fixations in the eye region higher than the values presented for the group without musical training. There was also a less dispersed and less erratic scan path for the group with musical training.

We are currently also studying the nystagmus, a condition associated with involuntary eye movements. This work is being carried out in collaboration with the Department of Ophthalmology of the Hospital Lusíadas, in Lisbon.

We used the pupilometer, together with ECG and EDA recordings, to study the autonomous nervous system's reaction to the cold pressor test, *ie.,* the insertion of one hand in melting ice water, in a collaboration with NOVA Medical School and the USF – São Marcos, in Sintra [13]. Although very preliminary, the results of that study were in line with what was expected, showing clear pupil dilation as a result of the hand insertion in cold water.

EDXRF – The concentration of trace elements (Mn, Fe, Cu, Zn, Se, Co, Mo, I) in the human body ranges only from tenths to hundreds of μg/g, but they play crucial roles in the normal functioning of the organism, by participating in many essential processes, like the activation, inhibition, and promotion of enzymatic reactions. Recent works pointed out that the excess or deficiency of these elements may lead to the development of pathologies, including cancer.

For example, copper and zinc are cofactors of the superoxide dismutase enzyme that, if not regulated, causes cell damage; iron is responsible for the formation of reactive oxygen species that trigger oxidative stress and, consequently, cell damage [14].

On that account, it is relevant to study trace element contents in different tissues, both normal and cancerous, in order to establish possible correlations between trace elements and factors like age, sex or cancer stage, leading to a better understanding of carcinogenesis.

The Energy Dispersive X-ray Fluorescence (EDXRF) technique is a suitable option for that kind of analyses, since it is non-destructive (i.e., samples are unaltered for further analyses/treatments), sensitivity at ppm levels and with high detection limits, the LIBPhys team developed a system to map the distribution of elements in large sampling areas, achieving high detection efficiency using the EDXRF technique. This system, [15] has been applied in human healthy and cancer tissue samples provided by the Serviço de Anatomia Patológica, Instituto Português de Oncologia de Lisboa Francisco Gentil with promising results.

This work is being carried out in collaboration with the NOVA Medical School, Instituto Português de Oncologia de Lisboa Francisco Gentil, and the University of Aveiro.

4. HUMAN COMPUTER INTERACTION

Rehabilitation through visual biofeedback – Rehabilitation can be performed with conventional procedures or by applying modern technology. The traditional ways are predominantly static and based on "paper and pencil" tasks. The methods used to evaluate and treat are, normally, subjective, not personalized and do not promote systematic monitorization. Our team has developed several innovative tools to circumvent those limitations. The main focus of these tools is to promote a standardized assessment, real-time monitoring and personalized intervention, according to the needs of each patient. In the following, we present two examples of such tools – RehaVisual and RehaBrain. Both were developed in close collaboration with hospitals.

RehabVisual has the objective to promote a personalized and adaptable stimulation of visuomotor skills, in patients with perceptive dysfunctions. In addition, it provides a systematic report of the performance, to be delivered to clinicians (therapists and physicians). Although developed to be applied to children up to 18 months old, it has been adapted to cope also with adults with stroke.

The RehabVisual platform has two sections: a database, that records all the clinical information of the patient, and a set of intervention protocols, with visual stimuli (Figure 5) of different shapes, colors and movements, according to the clinical needs of each subject. The database also allows for the recording of patient's personal data and the various clinical assessments made throughout treatment. Thus, it is divided into patient chart; assessment of ophthalmological parameters; behavioural evaluation; visual functional evaluation; records of intervention sessions [16]. The intervention protocol was based on combinations of the following set of parameters: shapes; dimensions; colours; contrast; movements; presentation distance. The goal was to allow for a proper stimulation of visuomotor skills; have adequate incentives for the needs of each participant; take into account her/his cognitive development levels; allow for an increasing level of complexity of the stimuli.

Figure 5 – Example of one stimulus [14].

The platform has three user profiles with different access functionalities: physician, occupational therapist and patients/caregivers [17]. Regarding the interaction between the patient and the stimulus, and in line with what was presented earlier in this chapter, the direction of gaze was assessed, exploiting the front camera of the laptop in which the stimuli were presented [18].

The RehabVisual was developed by NOVA School of Science and Technology, in collaboration with Hospital Dona Estefânia – Centro Hospitalar Universitário Lisboa Central (CHULC) and tested with children with developmental delay. Currently, the tool is being adapted to stroke patients, in collaboration with the Hospital Curry Cabral (CHULC).

Concerning RehabBrain, it is a platform that aims to provide a systematic evaluation and a personalized monitorization in real time using gamification. As in RehabVisual, RehabBrain, has also two sections: a database to record all personal information of the patient; and an intervention protocol, which uses games with different levels of difficulty (fig 6).

Figure 6 – Example of one level of difficulty in the RehabBrain intervention protocol. The goal is to follow a required task, passing with the mouse over specific elements in the screen.

This project was developed in co-creation between NOVA School of Science and Technology and Hospital Curry Cabral. We established the functionalities that the platform should offer: enable better organization and management of patient records; a more careful and complete monitoring of the rehabilitation process; include a set of simple games, with increasing levels of difficulty, and related to basic daily

activities, which allow for a proper assessment of patient's cognitive abilities such as attention, memory, understanding and visual, temporal and spatial perception; allow for the evaluation of the patient's progress, throughout therapy sessions.

RehabVIsual and RehabBrain are versatile and diversified tools, to be applied during the rehabilitation process. In addition, it allows to bring the treatment out from the clinical environment and into home, while following the patient's evolution, in real time.

Mindfulness – Modern society's growing demand in immediate, innovative and overwhelming outcomes has led to a stressing and agitated lifestyle, with a concomitant increase of mental illnesses. This situation constitutes a serious public health concern, with the rise of medical drug consumption and with their associated high economic costs. In this context, Mindfulness emerges as a possible approach to prevent and respond to these high levels of stress and anxiety, and their negative consequences.

Although there has been relevant research about the effects of the Mindfulness in the improvement of the quality of life, it is necessary to deepen neuroscientific knowledge about physiological changes related to the practice of this kind of meditation.

In the last years, we were involved in a longitudinal study to assess the effects of mindfulness meditation training in electrophysiological signals, such as electrodermal activity, heart rate and electroencephalography. In the study, 30 subjects attended a Mindfulness Based Stress Reduction course and were evaluated in 4 sessions: in the beginning, in the middle and at the end of the course, and 2 months after the course.

We observed a wide agreement between the self-reported results, acquired by surveys, and the physiological data. In both, there are clear indications that the practice of Mindfulness meditation, in a continuous manner, helps in reducing levels of stress, anxiety, and depression [19, 20]. Hence, Mindfulness meditation can be seen as an effective method to improve the mental health of the individuals, promoting functional changes occurring both at the autonomic and the central nervous systems.

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C

NANOBIOTECHNOLOGY TOOLS FOR DIAGNOSIS AND THERAPY

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Pedro V. Baptista

INTRODUCTION

In nature, there are a multitude of complex molecular processes responsible for the many essential tasks of a living cell and of any complex organism. At the core of these molecular pathways lies the comprehension of how the information accumulated in the Genome of a cell is used to convey the instructions for the complex operations that constitute the basis of life. Any disruption to this intricate yet disciplined and efficient interaction between millions of small molecules triggers an anomalous response that is at the basis of disease. In fact, as a result of the fast-evolving technological advances, the molecular complexity of diseases has been uncovered, which correlates to the dynamic changes to the genome and the way the genes are expressed. Modulations of genome expression initiates and controls several alterations to cell processes and a cascade of molecular events that results in a disease phenotype. The stepwise evolution from an initial molecular event drives disease progression, eventually leading to tissue malfunction and organ failure. The primary molecular event is often associated to may flawed gene expression that result in abnormal proteins, epigenetic modification and/or sporadic genomic mutations emerging throughout the life span of the organism. Each one of these molecular occurrences may be analyzed and are often considered biomarkers of disease and suitable for molecular diagnostics and as a valuable molecular target for precision therapeutics. For example, a therapeutic strategy may be focused on silencing the expression of the mutated gene expression to prevent incorrect protein production that alters cell function. However, we are only starting to understand the molecular inception of disease and the search for effective tools to tackle these molecular alterations must follow towards the development of successful therapeutics.

This natural role models have made researchers dream of being able to design and implement similarly small and functional molecular devices. The rapid development in the fields of molecular biology, biochemistry, supramolecular and polymer chemistry as well as the enormous expansion of physical measurement methods and nanotechnological manufacturing processes have made this dream seem a bit more realistic. The biologically inspired nanotechnology, which uses biological molecules as building material and operational tool for retrieving valuable molecular information to modulate cell and molecular response, is characterized by a high degree of interdisciplinarity and is known as nanobiotechnology. The research is primarily motivated by the general development towards the miniaturization of components and their functionalization with particular impact in biotechnology

and medicine. Nanobiotechnology is a very young, interdisciplinary that forms an interface between research on biological and non-biological systems and aims at their technical use in various areas.

Many of these nanomaterials-based systems have been used for diagnostics and for conveying therapeutics with improved efficacy, since their size is a good match to that of proteins, DNA or RNA, thus capable to improve the distribution of therapeutic and diagnostic elements within the organism. What is more, the resourcefulness of these nanobiotechnological platforms have allowed for the possibility to convey Diagnostics and Therapy in one single platform – nanotheranostics, which has been revolutionizing the way we tackle disease. In fact, enhancing the performance of theranostics systems should provide for improved and more personalized clinical intervention, and where inventive multifunctional nanocarriers may provide improved diagnostics and biomonitorization coupled to molecular therapeutics through selective and precision gene therapy[1-5].

Due to the high degree of interdisciplinarity, nanobiotechnology interfaces the biological disciplines of biology, biochemistry and biophysics tackling processes that are required for the production of applicable biotechnological components, such as isolation of functional biomolecules, genetic engineering processes for the construction, analysis and interaction with DNA/RNA molecules towards genome and gene characterization and intervention. In fact, one of the biomedical applications where nanomaterials are currently vastly explored is gene therapy. Gene therapy is the treatment of disease by the delivery of genetic material. The later provides new genetic instructions to the patient target cells in order to treat or cure the disease. Originally envisioned as a treatment solely for inherited disorders, gene therapy is now being applied to acquired conditions, as cancer, cardiovascular, neurodegenerative or infectious diseases.

In 1989 the first clinical protocol to insert a foreign gene into the immune cells of persons with cancer was approved and, in 1990 W. French Anderson and colleagues performed the first approved gene therapy procedure on a four-year-old girl born with severe combined immunodeficiency (SCID)[6,7]. Over 30 years have now passed since these first gene therapy trials. The advancements in the field concerning safety, improvements in transfer efficiency and delivery have finally resulted in substantial clinical progress. Several gene and gene-modified cell-based therapies are already approved drugs. The small size of nanoparticles from 10 to 200 nm, in the range of protein molecules, facilitates their interaction with biomolecules both in the cell surface and inside the cell to deliver genetic material such

as DNA or RNA[8]. The small size can also offer potential of targeted gene delivery, allowing the penetration in tissues at the proper site, like solid tumors, with a high level of specificity[9]. Such vectorization platforms are not only capable to enhance vectorization of DNA and RNA into cells and tissues, but also to provide theranostics capability by just small variation to core material, size and shape.

The far-reaching framework in Life Sciences for Health at NOVA has provided for the nurturing for state-of-the-art research in gene therapy, which has been focused on the development of vectorization systems relying on viral vectors and on concepts for molecular theranostics based on noble metal nanoparticles.

VIRAL VECTORS FOR GENE THERAPY

Virus delivery vectors are one among the many nanoscale systems that are being developed as drug, protein and genetic delivery materials. Viral vectors are widely used in gene therapy for the delivery of genetic material either in the form or DNA or RNA[10]. The use of recombinant viruses in therapy has long been practiced belonging to the class of viral-based treatments known as virotherapies. Being obligatory intracellular agents, viruses have evolved to efficiently deliver their genetic material into the cells. The current accumulated knowledge on viruses allows to develop numerous promising viral vector-based strategies to use in gene therapy treatments.

Viral vectors have two main components: (1) the protein capsid and/or envelope that encapsidates and protects the genetic material and defines the vector's tissue or cell tropism and (2) the transgene vector cassette containing of the genetic material of interest which promote the desired effect under the control of regulatory elements. Four viral vector platforms have gained wide use in gene therapy and regulatory approval. These are adenoviral vectors (AdV), adeno-associated vectors (AAV), retroviral vectors (RV) and lentiviral vectors (LV)[11]. Despite the significant experience in the clinic gathered with these vectors they still present several challenges. These are specific to each viral vector but common to all are the difficulties in developing efficient manufacture bioprocesses providing high quality material at the high doses required in the clinic[12-16].

ITQB NOVA has been contributing to gene therapy research and development in viral vectors since the late 90's through partnership with iBET – Instituto de Biologia Experimental e Tecnológica and holding collaborations with diverse international academic industrial partners. Namely through European Commission

networks as CliniGene European Network for the advancement of Clinical Gene Transfer & Therapy and Brainvectors for Brain Gene Transfer Gene Therapy. This research combines areas of applied virology, molecular biology, cell culture and bioprocess engineering ongoing at the labs of Paula Alves, Manuel Carrondo e Ana Sofia Coroadinha (Animal Cell Technology Unit (ACT)).

The ACT team at ITQB NOVA and iBET labs engineered a pioneering cell line using advanced systems of targeted integration enabling the establishment of flexible master retroviral vector cell lines where, transgene vector and the envelopes can be exchanged by Cre or Flp recombinases **(Figure 1a)**[17-18]. This work was performed within the scope of a European consortium aiming to treat epidermolysis bullosa rare disorder. A patent was filed protecting the use of this innovative type of cell lines for viral vector production within the consortium. This research was followed with advancements in the understanding of virus-cell interactions[19]. It was discovered 8 essential metabolic pathways for enveloped virus replication, through functional genomics which enabled to further understand the serum dependence of these systems[20]. Through rational cell genetic manipulation, it was possible to improve the quality and yields of retroviral vectors[21].

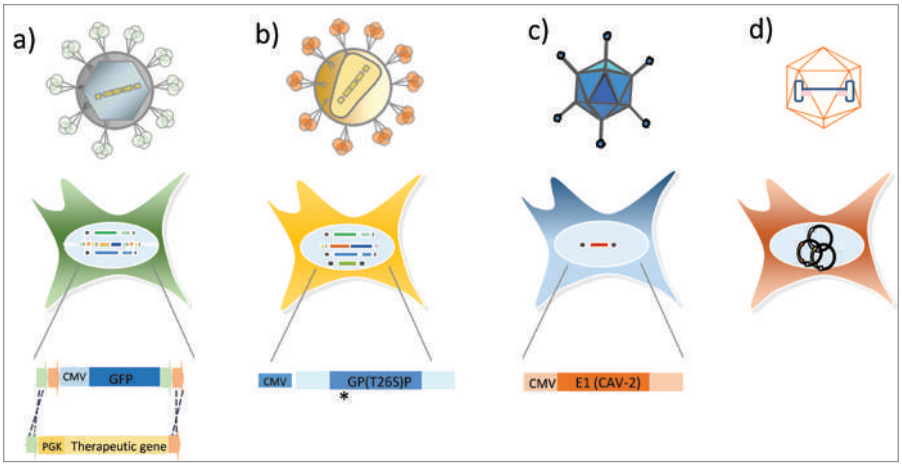

Figure 1 – Representative image of technologies developed to deliver genetic material in gene therapy. For the generation of these complex bioparticles animal cells are modified to generate: a) retroviral particles (represented is the use of a pioneering system of recombinase mediated cassette to swap the delivered genetic material according to the therapeutic purpose); b) lentiviral particles (represented are the ones developed with a less active mutated protease (T26S)); c) canine adenoviral particles (represented are E1 deleted vectors requiring the use of E1 trasncomplementing cell lines enabling vector propagation) and; d) adeno-associated particles (represented are vectors generated by transient transfection).

On the adenovirus vector field ACT team contributed with research on the use of both human and non-human adenovirus. While human adenovirus poses the issue of pre-existing immunity in the patients, and thus more used now as vaccines or as oncolytic vectors, non-human adenoviral vectors may provide more specific tropisms. However, the later pose the challenge of developing suitable non-human expression systems. Canine adenoviruses (CAV) permissive cell substrates were studied and a GMP compliant process was developed using an MDCK cell line[22,23]. Through collaboration with Genibet, a spin-off company of iBET, a master GMP cell bank of MDCK transcomplementing cells **(Figure 1b)** was generated. The CAV viral vectors were used in pre-clinical animal models by international collaborators to further elucidate Mucopolysaccharidosis type VII and Parkinson diseases.

Currently lentiviral vectors are one of the most used vectors in clinical trials particularly for the treatment of rare diseases affecting hematopoietic cells and in immunotherapies requiring modification of T or NK cells. Lentiviral vectors are possible the most difficult viral vector to be manufactured due to its short half-life, instability and inherent cytotoxicity. At ACT at ITQB NOVA and iBET a major breakthrough was achieved by reducing the cytotoxicity of the viral protease and the engineering of novel non-toxic viral envelopes **(Figure 1c)**[24,25]. This allowed to generate one of the few existing constitutive lentiviral producer cell lines which may unblock the use of efficient bioreaction operating modes. A patent was filled on the novel engineered envelopes.

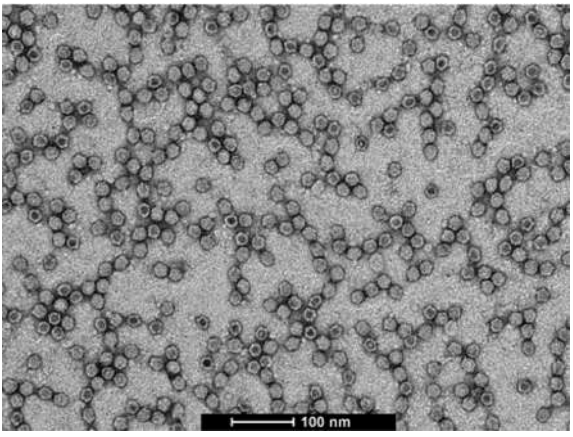

Figure 2 – Transmission electron microscopy of purified particles of AAV. AAV vector particles carrying the therapeutic gene for Duchenne Muscular Dystrophy generated for pre-clinical studies in the scope of an academic collaborative project. The AAV vectors were produced at ITQB-NOVA and iBET labs using HEK 293T cells using a transient production systems. The particles were purified by affinity chromatography.

ACT is also highly active in research and development of novel expression systems to generate AAV gene therapy particles **(Figure 1d and Figure 2)** in collaborative and synergistic approach with academic and industrial partners[26].

METAL NANOPARTICLES FOR MOLECULAR NANOTHERANOSTICS

Gene modulation holds great promise for innovative treatment of diseases and towards personalized medicine. Gene modulation/silencing strategies have received renewed drive through nanotechnology-based systems for the delivery of molecular cargo to the cells. The use of nanocarriers for gene therapy has crucial advantages, including; i) bypass biological barriers that enable to use lower doses of drugs and doing so by the direct targeting of cancer cells while spearing healthy surrounding tissue, ii) increase the aqueous solubility and bioavailability of traditional chemotherapeutic agents, iii) can bear sufficient loads of drugs, nucleic acids, and protein-based anticancer therapies (multifunctional nanoparticles), iv) have increased circulation times compared to conventional therapeutic agents, v) can respond to stimuli, etc. A quick survey of the existing literature highlights the overabundance of conceivable systems, but only a few concepts have made the whole drug development pathway and are presently under investigation in early-stage clinical trials[27-30].

Precise molecular therapeutics is pivotal when dealing with cancer cells, namely in what concerns the capability to silence activated oncogenes and/or modulate tumor-suppressor genes. For this enterprise, precise and selective tools for RNA interference may be considered, such as antisense oligonucleotides, short hairpin RNA (shRNA), microRNA (miRNA) and small interfering RNA (siRNA), whose efficacy relies on the use of adequate vectors capable of delivering the functional moieties to the desired target sites with high selectivity. In recent years, RNAi approaches have profited from the development of bionanotechnological concepts for the delivery of the molecular actuator to cells, relying on the use of new nanomaterials capable to directly target the distinctive characteristics of cells and tissues. Among the plethora of nanovectorization strategies, those based on nanoparticles have been successfully used as delivery vehicles of therapeutic nucleic acids. Of particular interest are gold nanoparticles (AuNPs), which can be easily produced via simple chemical derivatization of a gold salt to attain the desirable size range, and whose easiness of surface functionalization

with a multitude of active moieties have made them ideal nanomaterials for the development of nanotheranostics concepts. These AuNPs may be easily functionalized with a wide range of biomolecules for the simultaneous delivery of the (molecular) cargo and active targeting moieties that enhance the accumulation at selected sites (e.g. cancer-, inflamed- sites). Additionally, in terms of vectorization capability, the use of such metal nanoparticles provides for considerable protection against enzymatic degradation allowing for increased circulating half-life[29,30].

Gold nanoparticles (AuNPs) exhibit an augmented light absorption and scattering associated to their localized surface plasmon resonance (LSPR), which has been instrumental for several biomedical applications, including gene therapy. What is more, these optical properties of AuNPs make them formidable imaging agents. Multifunctional AuNPs have, thus, been designed and used for combined therapy modalities and multimodal imaging schemes (diagnostics)[31]. Pioneering work lead by the Nanomedicine Group at FCT-NOVA lead by Pedro V Baptista has put forward a nanotheranostics concept for conveying gene silencing capability based on the combination of antisense oligonucleotides labeled with fluorescence and the quenching of fluorescence associated to the LSPR – gold nanobeacons[32]. This nanobiotechnology system relies on a stem-loop ssDNA oligonucleotide functionalized with a fluorophore at one end and bonded to a AuNP at the other. Because the selected fluorophore's emission overlaps with that of the AuNP LSPR, the later act as quencher of fluorescence when the fluorophore is in close vicinity to the nanoparticles' surface. In the native form, the antisense oligonucleotide adopts the closed stem-loop configuration, bringing the fluorophore to close proximity of the AuNP, resulting in fluorescence quenching; intracellular recognition of the target gene mRNA triggers the opening of the loop structure leading to the emission of fluorescence, thus providing the signal for the specific silencing of the gene and subsequent mRNA degradation. These Au-nanobeacons constitute a remarkable example of nanotheranostics platform, where it is possible to couple fluorescence signal to the silencing effect, i.e. gene therapy and biomonitorization of therapeutic action (**Figure 3**). In fact, this Au-nanobeacon strategy allows assessing the efficacy of the specific silencing of the gene, because the fluorescence signal is proportional to the quantity of mRNA targets being silenced, thus providing data on extent of gene expression suppression without invasive or disruptive approaches. In fact, it is possible to fine tune the fluorophore emission to the desired wavelength by simply adjusting the size of the core nanoparticle,

whose tissue localization may also be followed by additional imaging techniques, such as surface-enhanced Raman spectroscopy (SERS), computed tomography, two-photon fluorescence and magnetic resonance imaging.

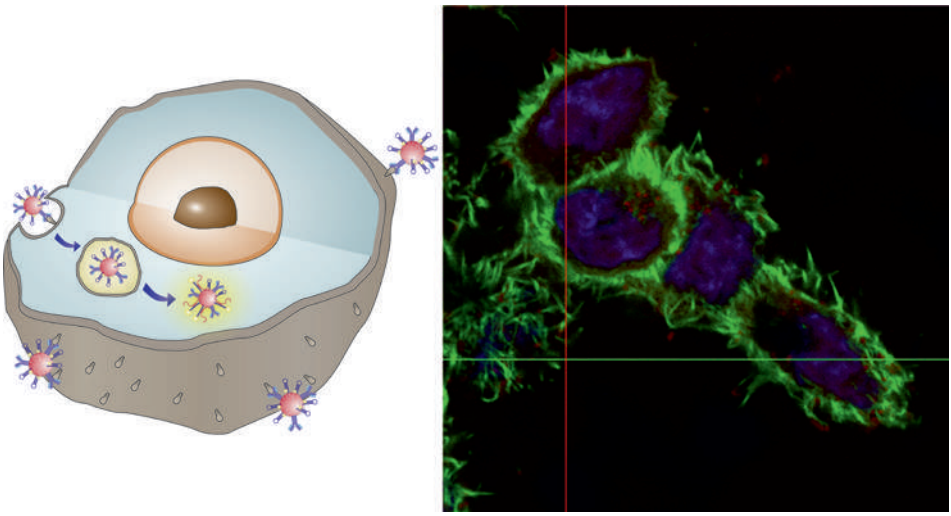

Figure 3 – Au-nanobeacons: theranostics tool for gene silencing. Schematic representation of Au-nanobeacons entering the cell and illuminating upon target recognition. HCT cell line challenged with Au-nanobeacons that recognize the target mRNA emitting light from the red fluorophore (blue-nucleus, green cytoskeleton).

In cancer development, tumor growth depends on the continuous supply of oxygen and nutrients to support the unrestrained proliferation of the malignant cells. Therefore, tumors trigger the growth of new blood vessels – neoangiogenesis – pivotal for supplying the ever-growing need for sustenance. Among the several participants in this process, the vascular endothelial growth factor (VEGF) is critical for the angiogenic trigger, resulting the establishment of new blood vessels to feed the cancer. Again, gold nanoparticles hold great promise to tackle this stage in tumor development since they are capable to effectively deliver therapeutic agents selectively to tumors via the enhanced permeability and retention effect, which results in the passive accumulation of nanoparticle conjugates, coupled to localized plasmon photothermal therapy. Indeed, spherical AuNPs show tremendous photothermal efficiency upon laser irradiation at their LSPR wavelength, allowing for successful use in hyperthermia approaches[33]. At FCT-NOVA, we used spherical AuNPs conjugated to anti-angiogenic peptides towards the optimization of valuable photothermal agents that are proficient to block the angiogenic pathway *in vivo*

via down regulation of the VEGF dependent pathway. In fact, we used a laser for the selective ablation of the new vessels surrounding the tumor, while simultaneously blocking VEGF response and impeding neo-vascularization. The combination of chemo- and phototherapy should allow for improved efficacy of current anti-angiogenic therapeutics[34].

Other approaches at CENIMAT, FCT-NOVA have focused on superparamagnetic iron oxide nanoparticles (SPIONs) for nanotheranostics. These nanoparticles' potential as superior contrast agent for MRI have allowed for optimal detection of liver ischemia-reperfusion injuries[35]. These SPIONs have also been used for the design of smart materials, whose therapeutic action may be triggered by an external stimulus that can be spatiotemporally controlled. Such magneto-responsive devices can be used for a multitude of biomedical applications with particular focus on cancer theranostics. At FCT-NOVA, magnetic nanoparticles were introduced into hybrid microgels as an implantable scaffold suitable for magnetic hyperthermia strategies aimed at the destruction of cancer cells[36,37].

CONCLUSIONS

The pioneering work on nanobiotechnology approaches has only been possible due to the interdisciplinary nature of NOVA's research on new materials and their use biomedicine. This context has allowed for the development of several new concepts in cancer nanotheranostics.

Some of the research work in nanobiotechnology has already spun off to the society as one NOVA SPIN-OFF, which aims to recognize start-ups created within the innovation and entrepreneurship ecosystem of NOVA University – Nano4 Global is a young start-up launched in 2015 to market innovative nanotechnology based molecular assays, relying on the use of gold nanoparticles towards decentralized molecular diagnostics – from genetics lab to point of need (www.nano4global.com).

The research on viral vector particles is multidisciplinary combining diverse areas of virology, cell biology, and technology. In this context ITQB NOVA and iBET research on viral vectors ranges from vector and cell line development and engineering to upstream and downstream bioprocess understanding and development. While on the earlier years it was focused on adenoviral and retroviral vectors, the main used vectors in late 90's and early 2000's, it was extended to lentiviral and AAV vectors, currently the most used gene therapy vehicles. ITQB-NOVA

laboratories are continuing the research and development in the area of gene therapy viral vectors held with iBET and collaborating with international institutes. Ongoing research on lentiviral vectors aims at further understanding vector cytotoxicity and means to circumvent it but also bridging vector engineering with target cell transduction i.e. studying ways to improve genetic material delivery. Work on AAV engineering is ongoing towards the improvement of vector cargo payload which is currently limiting their broader use. PhD projects are ongoing on the above mentioned topics as well as on bioprocess control, upstream and downstream engineering, and essential to enable translation of the novel vectors to the clinic. On-going industrial partnerships were established in these areas leveraged by the extensive research and development knowledge gathered over the last 25 years.

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BIOSENSORS AND DIAGNOSTIC TESTS

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INTRODUCTION

In the field of analytical chemistry, environment monitoring and clinical diagnostic, the biosensors have provided an enormous contribution since their invention. Biosensors combine a transducer with a biorecognition element that interact with an analyte present in a sample (chemical element, molecule, protein, DNA, bacteria, virus, cell, etc.), and transform that biochemical event into a measurable signal (Figure 1). The sensing takes place as either a binding reaction or a biocatalytical event. These interactions produce a measurable change in a solution property, which the transducer converts into a quantifiable signal. The first biosensor was presented by Clark [1] in 1962 as an "enzyme electrode" for monitoring glucose in blood. The Clark-type electrode was based on the incorporation of a glucose oxidase enclosed within a Teflon membrane at the end of an amperometric electrode for pO_2, measuring the electron current induced by the oxidative reaction of the glucose in the sample.

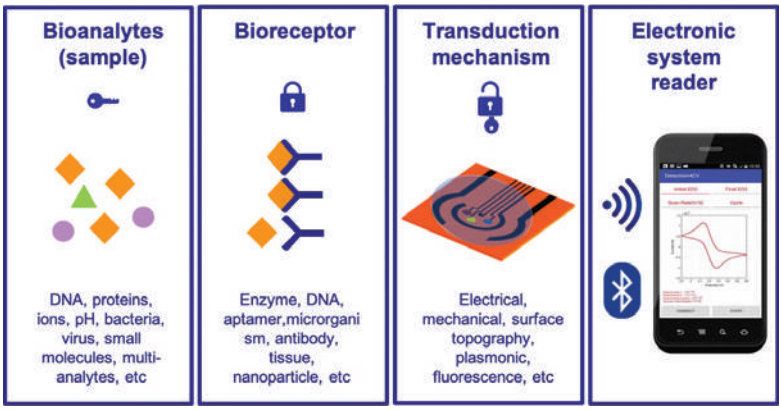

Figure 1 – Schematic representation of a generic biosensor components. The bioanalytes in the sample, the bioreceptor layer (e.g. enzyme, DNA, aptamer, antibody, microorganism, tissue, etc.) directly attached to a transducer mechanism that transform the physico-chemical interaction of the bioreceptor with the bioanalyte present in the sample in a measurable electronic signal.

In the following six decades after the first invention, an increasingly interest for new biosensor and applications has been evidenced in the scientific community. The development of alternative transducers, new bioreceptors and improved immobilization techniques for higher selectivity and sensitivity has been

continuously increasing, as several end-user and time-saving analytical methods for the detection of multiple analytes (e.g., food, clinical, and environmental analytes) have been described, in about 7000 scientific papers published in 2020 (statistics of Web of Science).

The biosensor field is a multidisciplinary area of research, involving biology chemistry, physics, materials, and can adopt varied setups and configurations, depending on the target analyte, the bioreceptor to be used, the interface sample/transducer, the transduction principle applied and the test format for the specific application (clinic diagnostic, bioprocess monitoring, biosafety and environmental surveillance, etc.).

The medical field is one of the most attractive area for the biosensors, as can provide devices for rapid, reusable and accurate diagnostic of physiological parameters and the demand for cost efficient platforms is the key success and acceptance in a competitive market.

Microfluidic devices provide the possibility of performing sophisticated analysis within chips that have a hand hold size, allow fast sample analysis, high throughput, portability, and reduced reagent use, all of which are associated with a decreased cost per analysis. These devices can also be designed for multi-parallel operation, making the system more reliable as several control assays can be performed simultaneously with multiple samples. This makes of microfluidics an essential component of modern lab-on-a-chip platforms for point-of-care (POC) and point-of-need (PON) analysis. Typically, microfluidics allows to transport, mix, separate, heat, and make reactions with fluids.

Traditionally microfluidic flows are constrained inside closed channels with tens of micrometers made of silicon or polymers like polydimethylsiloxane (PDMS). Due to the reduced scale, surface forces induced by capillary forces, surface roughness and chemical interactions with construction materials dominate. However, inside a microfluidic channel the flow is considered essentially laminar, which critically limits the mixing efficiency, meaning that mixing at the micro-scale must be artificially enhanced. Accordingly, two basic principles are exploited to enhance mixing at the microscale: active or passive. Active mixers depend on an external energy source to achieve mixing, whereas passive mixers solely rely on fluid pumping energy and use special channel designs to restructure the flow, reduce the diffusion length and maximize the contact surface area between fluids. In contrast with continuous microfluidics, droplet-based microfluidics allow the manipulation of discrete volumes of fluids in immiscible phases. Microdroplets allow the handling

of reduced volumes (nanoliters to femtoliters) of fluids providing better mixing, encapsulation, sorting, sensing and high throughput.

Portability and process automation are also extremely important in the design of new lab-on-a-chip systems. Digital Microfluidics (DMF) is a relatively recent technology, which allows the individual control of discrete picoliter to microliter droplets in integrated systems, just by applying a series of potentials to electrode arrays and without the need of any external devices such as pumps or valves. DMF devices, based on electrowetting on dielectrics effect, start to be disseminate in the beginning of 2000s, by two American groups, namely the Fair group at Duke University [2] and the Kim group at University of California [3]. Since the above-mentioned studies, several advances have been made regarding DMF. In a typical configuration, a DMF device are composed of a rigid or flexible substrate (bottom plate) where a thin film electrode pattern (actuation electrodes) is deposited. On the top of the electrode layer a thin dielectric layer (few microns) follow by a hydrophobic layer (few nanometers) are added. Sample droplets above the final hydrophobic surface may be surrounded by air, or by other fluid, and the overall system can be covered by a top plate to improve droplet actuation and prevent contamination.

Droplet movement are promoted by applying electric signals to each electrode, which in turn results in forces applied to the droplets on the top. Each electrode can be independently controlled by a dedicated interface with a computer. This way, all droplet movement can be automated, meaning that even when dealing with complex reactions protocols, it is possible to simply schedule the various routes and droplet movements (e.g. dispensing, moving, splitting and droplet merging) necessary to complete all protocol steps.

DMF has several potential applications in the fields of biotechnology, namely in microfluidic immunoassays, chemical microreactors, proteomics, diagnostics and DNA amplification and detection [3]. DNA amplification is particularly relevant in many lab-on-a-chip systems as a fundamental step towards DNA detection/identification. DMF systems allow easy sample delivering and mixing and integration with DNA related application such as extraction and purification, nucleic acid sequencing or real-time hybridization monitorization [4,5].

In 2003 the World Health Organization (WHO) has established guidelines for the development of diagnosis for use in economically disadvantaged regions, known under the acronym ASSURED (Affordable, Sensitive, Specific, User-friendly, Rapid and Robust, Equipment-free and Delivered to those in need) [6]. More recently

in 2019, this concept was redefined to REASSURED by adding: R – Real-time connectivity and E – Ease of specimen collection and environmental friendliness), due to: i) the rapid and new advances in digital technology and mobile health and ii) to provide real-time quality control for testing and treatment and overcoming the difficulties in specimen collection and/or processing, which currently limits scaling-up of diagnostics in resource-limited areas [7]. Following these demands paper-based biosensors are an emerging and new class of devices that fulfill these needs [8]. Besides that, paper presents several advantages like: abundance in nature, biodegradability, easy fabrication and manipulation of the architecture, easy microfluidics induced by capillary forces (a "zero" device that do not need any pumping or energy supply), versatile chemical functionalization capabilities, high thermal stability, high mechanical strength and easy integration of various nanomaterials which makes these devices quite attractive while looking for cost efficient and green/sustainable alternative production technologies. Moreover, these tests do not require complex instrumentation and are easy to perform outside sophisticated laboratories like the ones in urban locations where trained technologists are available.

In 2007, a landmark study by Whitesides group at Harvard University introduced a new paper based microfluidic device as an inexpensive and sustainable platform that potentially meet all the ASSURED requirements [9]. The devices are based on the definition of microchannels on hydrophilic paper via the patterning of walls of hydrophobic polymers, photoresist or wax, allowing multiple detection zones for different target compounds by deposition of reagents on the paper surface (e.g. enzymes, antibodies). Usually the designation is microfluidic paper analytical device (μPAD) [10].

This chapter provides updates on the latest developments done in biosensors and diagnostic tests at ITQB and CENIMAT | i3N at Nova University Lisbon.

BIOSENSOR DEVELOPMENT FOR DIAGNOSTICS

The group of Abel Oliva of ITQB-NOVA started to develop biosensors in the '90, mainly for diagnostic applications. The first biosensor produced was based on optical identification of antibodies in blood samples for diagnosis of African swine fever virus [11]. Further immunosensor comprise the development for diagnosis of veterinary disease [12] like *Brucella spp* [13], *Anaplasma spp* [14] and *Babesia spp* [15].

The evolution of the biosensor development in the group was directed to reducing size of detection chambers and reagents volume, driving force for exploring the incorporation of microfluidic. The integration of microfluidic in a biosensor setup allows the better manipulation, reproducibility, control and reduction of sample volume and reagents waste in each assay. In addition, the manipulation of single cells in designed narrow channels (up to 100 µm) layouts is possible. In an alternative strategy for cell handling, chips have been developed by microfabrication that included electrodes (Figure 2).

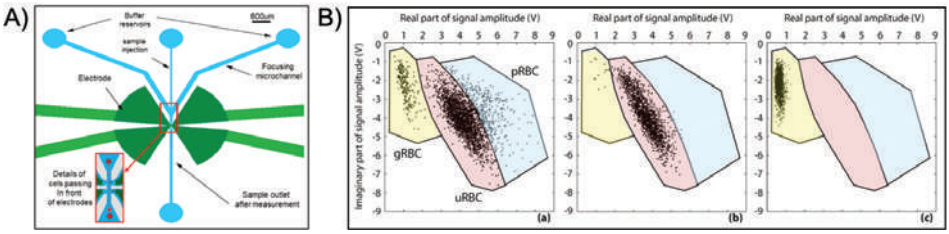

Figure 2 – A) Chip layout with the electrodes and the central microfluidic channel, where the cells are guided and measured. B) Scatter-plots from impedance spectroscopy measurements display the dielectric changes in *Babesia bovis* infected cells. Scatterplots of (a) a *Babesia bovis* infected sample of bovine erythrocytes, (b) a control of uninfected bovine erythrocytes and (c) a control of uninfected erythrocyte ghosts. The areas for uninfected RBCs (uRBC, red), parasitized RBCs (pRBC, blue) and ghost RBCs (gRBC, yellow) are marked in the three scattergrams. Adapted from [15] and [16].

Alternative physico-chemical phenomena were also used to measure biological parameters, like impedimetric properties impedance spectroscopy is a powerful tool for label-free analysis and characterization of living cells. In this work, we achieved the detection of *Babesia bovis* infected red blood cells using impedance spectroscopy on a microfabricated flow cytometer. The cellular modifications caused by the intracellular parasite result in a shift in impedance, which can be dielectrically measured. Thus, a rapid cell-by-cell detection with microliter amounts of reagents was possible. Unlike other diagnostic tests, this method does not depend on extensive sample pre-treatment or expensive chemicals and equipments. This strategy of impedimetric evaluation of cells allows the identification of infected erythrocytes in a non-destructive assay (label free), as in the microsystem developed for measuring parasitaemia in *in vitro* cultivated *Babesia bovis,* a major pathogen in cattle [15,16].

A further development of microchips with integrated electrodes made possibly the particle sorting based on the opposition of dielectrophoretic forces, generated by an array of electrode chambers located in both sidewalls of a main flow channel. Particles with different dielectric response perceive different force magnitudes and are continuously focused to different streamlines in the flow channel. We relate the particles dielectric response to their output position in the downstream channel. This way was demonstrated the performance of the device by separating a mixed yeast cell population into pure fractions of viable and nonviable cells. Finally, we use the device to enrich red blood infected cells, and simultaneously confirm the hypothesis that infection with *Babesia bovis* causes significant changes in the dielectric response of red blood cells [17,18].

An alternative method for identification of cell properties was explored by the use of the measurement of optical characteristic of cells, as the refractometric index of the external membrane of e.g. erythrocytes can evidence the physiological status as in the case of infected cells (like intra-erythrocyte infections of *Babesia spp* or *Plasmodium spp*). A chip that integrates a microchannel for cell conduction and a sided placed fibre optic allowed the capture of an image of the passing through cells, performing the spectroscopic and refractometric analysis in real time [19].

The integration of the previous developed approaches in one chip was a challenge that has been successfully produced by the collaboration with the groups of ITQB-Nova and CENIMAT | i3N. The chip included the development of a microscale setup for simultaneous near infra-red (NIR) interferometry and absorption or fluorescence spectroscopy based on an epoxy negative photoresist microfluidic platform with embedded fibre optic sensors and electrodes. The electrodes allowed the measuring of impedimetric properties of the cells, in real time, during passing-through the microchannel in front of the electrodes. Several strategies were evaluated in order to simultaneously detect refractive index and absorption properties of cells [20]. The approach involved the use of pressure driven delivery of the cells. Top placed reservoirs for the cells and diluting solutions conducted to the PDMS inlet microchannel in the bottom. The manipulation of the pressure made by the operator controlled the movement of the cells through the microchannel inside the chip. The movement inside the chip was observed in real time by an inverted microscope that controlled the alignment of the cells in the central region, between the fibre optic and the electrodes, during measurement (Figure 3).

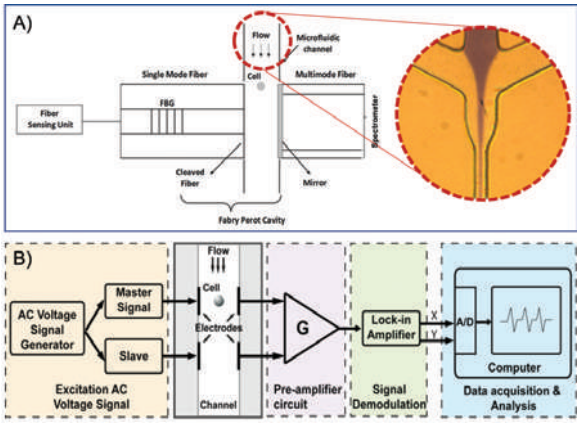

Figure 3 – Schematic diagram of the microfluidic platform of the hybrid chip: A) top view of the setup for the simultaneous measurement of the refractive index, absorption and fluorescence by the two axial aligned fiber optics side-by-side the microfluidic channel and detail of the hydrodynamic focusing profile in the central channel; B) schematic diagram of the complete microfluidic single cell impedance analysis system, including instrumentation. Adapted from [20].

MICROFLUIDIC DEVICES FOR DIAGNOSTIC TESTS

Here we present the work that has been developed at CENIMAT | i3N in collaboration with UCBIO in the last years, concerning the development of microfluidic devices for DNA detection.

Nanodiagnostics, i.e. the use of nanotechnology platforms for nanosensing applications based on the amazing optical properties of gold nanoparticles [21] have revolutionised the molecular field of analysis. The use of thiol-ss DNA functionalised gold nanoparticles (gold nanoprobes, Au-nanoprobes) for DNA detection has paved the way for simple yet sensitive and specific molecular diagnostic strategies. Amongst these, a detection scheme based on the differential colorimetric behaviour of Au-nanoprobes after salt induced aggregation mediated by the presence of a complementary target sequence has been widely explored: the presence of a complementary target prevents aggregation and the solution retains its original red colour; while absence of this specific sequence results in aggregation and the solution turns blue [22]. This colorimetric detection scheme was successfully applied to the molecular identification of *Mycobacterium tuberculosis*, the main aetiological agent of human tuberculosis that affects more than 8.3 million people worldwide

[23]. This molecular detection strategy has also been successfully integrated in to an easy-to-operate, inexpensive and reliable optoelectronic platform using green and red-light sources and thin p–i–n silicon [24,25] or TiO_2-based ink-jet printed photodetectors [26] as well as with paper-based microfluidics [27].

In order to reduce the reagent's volume needed for detection and to increase sensitivity, a bio-microfluidic platform was developed following the same colorimetric Au-nanoprobe detection method and as first step towards integrated lab-on-chip device for the POC use. For that, we defined the optical path length along a microchannel, and used optical fibres to transport signal from a light source to the microchannel and afterwards to a photodetector. The optical fibres were self-aligned with each other and with the detection channel by using insertion grooves defined in the microfluidic chip (Figure 4A). The microfluidic chip with the optical fibres could be disposed after each measurement to avoid risk of cross contamination, while a fixed setup was constituted by green and red-light emitting diodes, a photodiode and an electrical circuit to acquire the signal. The chip was fabricated in PDMS, a silicon rubber, chosen due to its low price, biocompatibility, good optical properties and the ability to reproduce features on the micrometer scale with very high fidelity by replica moulding, based on an epoxy-based negative photoresist. This microfluidic platform was used for the detection of *Mycobacterium tuberculosis*. We were capable of performing a specific identification of DNA using only 3 µl of solution with a concentration of 30 ng/µl [28], which represented a 20-fold reduction of volume when compared to the state of the art [25].

To further improve the sensitivity of this system, self-aligned planar microlenses were incorporated in the chip to properly collimate light into the detection channel and then to the output fibre core taking advantage of the refractive index differences between air and PDMS. (Figure 4B) These 2D micro-optical components had the advantage of not requiring any additional steps in the chip fabrication process. To focus the light on the channel content (width: 100 µm) a bi-concave air microlenses was used, while for the output a pair of lenses was used: biconcave and bi-convex air lenses. Comparing with the previous state-of-the art, the optimized setup with the air lenses provided 160% higher discrimination of the AuNPs colour. The incorporated lenses not only increased the light intensity that reached the output fibre, but also increase the interaction of the light with the fluid, (Figure 4C, D). Therefore, the incorporation of microlenses allowed the interaction of all the available fluid with the propagating light, yielding a much higher discrimination between signals obtained from the solutions of different colours.

This optimized microfluidic platform resulted in a sensitive and accurate sensor for single nucleotide polymorphism detection operating using very small volumes of solutions. We were able detect DNA using 10× lower solution volume and a target DNA concentration below the limit of the detection attained with a conventional microplate reader (i.e., 15 ng/mL). The signal-to-losses ratio reaching the output optical fibre was increased 6× and the colorimetric discrimination, between positive and negative results was improved by 34% [29].

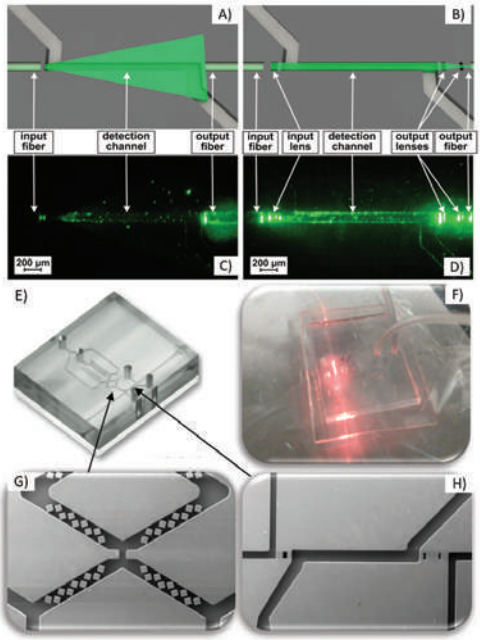

Figure 4 – A) 3D schema of light propagation in a microfluidic chip without lenses and B) with 2D air microlenses. C) Top view microscopic images of the microfluidic channel when illuminated by a green light from the input optical fibre, without lenses and D) with 2D air microlenses. E) 3D schema of the microfluidic chip with embedded micromixer, which includes: infusion section; mixing section and optical detection section. F) Picture of the working device with a red light coupled for measurement. G) Detailed Scanning Electron Microscopy (SEM) picture of the mixing region, designed to perform efficient passive mixing. H) Detailed scanning electron microscopy picture of the detection region. Focusing lenses are observed between microchannel and the optical fibres groove. Adapted from [29,31].

The next step of this setup optimization implied that the mixing of reagents should be performed in the microfluidic chip to reduce the risk of sample contamination. Therefore, passive microfluidic mixers were fabricated and optimized to allow

mixing the Au-nanoprobe plus DNA solution with the salt solution inside the chip (Figure 4G). The developed mixer allowed efficient fluid mixing for low fluxes using a channel much shorter (2.5 mm) than most planar passive micromixers. The developed device enabled efficient mixing (> 80%) with a low pressure drop within a very short channel at low flows, making it suitable for the envisaged mixing application [30].

Incorporating the optimized micromixer, a multifunctional chip was developed and tested using gold nanoprobes to perform RNA optical detection inside the microfluidic chip without the need of molecular amplification steps (Figure 4 E,F,G,H). As a proof-of-concept, this device was used for the rapid detection of chronic myeloid leukemia (CML), a hematooncological disease that would benefit from early-stage diagnostics and screening tests. The chip passively mixed target RNA from samples, gold nanoprobes and saline solution to infer a result from their final colorimetric properties. An optical fibre network like the used in the previous works was employed to evaluate the transmitted light signals inside the chip. Trials provided accurate output results within 3 min, yielding signal-to-noise ratios up to 9 dB. When compared to previous state-of-art screening techniques of CML, these results were, at a microscale, at least 10× faster than the reported detection methods for CML [31].

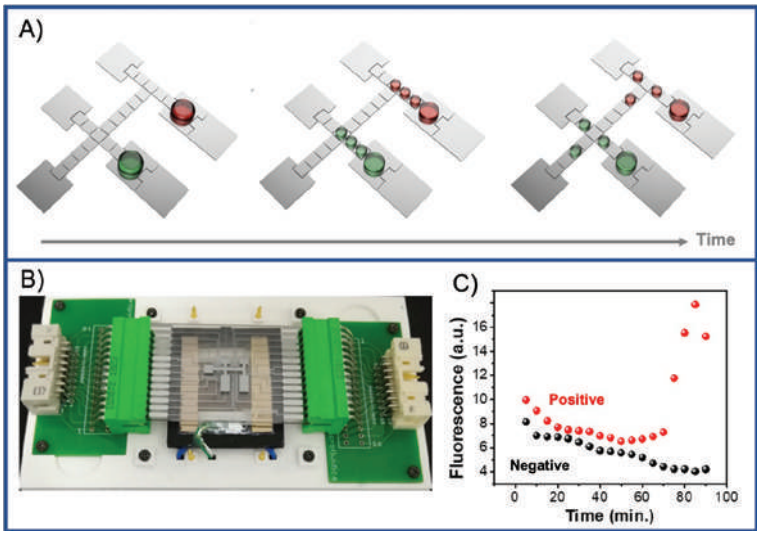

Figure 5 – A) Scheme of droplet dispensing from small reservoirs and moving along the electrodes in a DMF device; B) DMF Lab-on-a-chip device done at CENIMAT | i3N with the support and electric connections to the electronic interface; C) Obtained results from the fluorescent detection related with DNA concentration for a positive and negative sample. Adapted from [32,33].

At CENIMAT | i3N, in partnership with UCIBIO, we are developing DMF devices that allow us to perform DNA amplification reactions for the diagnosis of cancer, identifying a distinct sequence related to the growth of uncontrolled cells associated with several types of human cancers [32,33]. These DMF platforms include isothermal DNA amplification such as Loop-mediated isothermal amplification (LAMP) which presents high robustness, specificity and sensibility. LAMP eliminates the need for thermal cycling which allows for lower energy consumption and simpler devices. Also, we combine DMF lab-on-a-chip devices with standard fluorescence microscopy for isothermal real-time DNA amplification monitoring and quantification in real-time as it is indicated in Figure 5.

LAB-ON-PAPER TECHNOLOGY APPLIED TO RAPID DIAGNOSTIC TESTS

At CENIMAT | i3N and based on our activity on paper for electronics and biosensor applications, we start by applying these technology to diabetes [34,35], since this represents a major healthcare concern worldwide, which affects more than 422 million people, and the management of diabetes is of critical importance as it enables the diabetics to have normal lifestyle by avoiding costly and lethal diabetic complications.

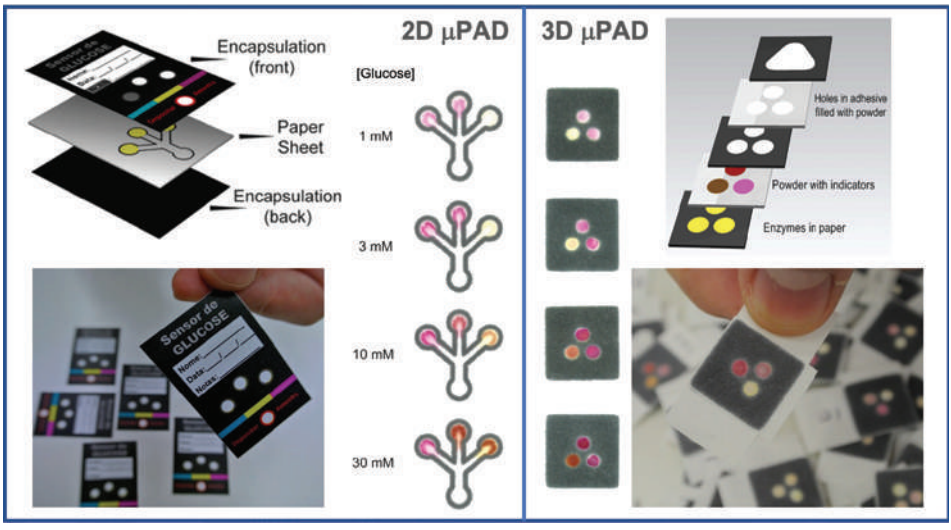

Figure 6 – Enzymatic colorimetric lab-on-paper device formats done at i3N | CENIMAT for glucose detection and quantification: 2D µPAD and 3D µPAD. Adapted from [34].

A 2D μPAD and 3D μPAD have been developed for colorimetric enzymatic detection and quantification of glucose [34]. Briefly, the action of glucose oxidase decomposes glucose into H_2O_2 that is then utilized by a second enzyme, peroxidase. Peroxidase is responsible for the oxidation of colorimetric indicators generating a visible colour change. The colorimetric response is proportional to the initial amount of glucose in the sample. The fabrication of these devices is illustrated in Figure 6. By comparing 2D and 3D μPAD the colouration is highly homogeneous, covering all the surface of the paper reaction zones in 3D sensors, which is a major advantage in comparison to the 2D lateral flow sensors, where some carryover of the coloured products usually occurs. The analysis is thus facilitated and is much more accurate. The glucose biosensor was able to determine the glucose concentration between 0.01 and 40 mM, for both layouts.

The current methods for detection and quantification of glucose available commercially or using paper-based devices are majority based on decomposition of glucose by the action of glucose oxidase or enzyme mimicking materials, producing hydrogen peroxide, followed by a catalytic reaction with H_2O_2 and the use of peroxidase to oxidize colorimetric indicators or produce detection potentials, resulting in signals that are proportional to glucose concentration in the sample. However, all the above glucose detection methods are indirect or require enzymatic reactions. In this setting, enzymes present several shortcomings, mainly related to purification and subsequent applications and immobilization in sensing platforms, associated with inherent stability issues related to the chemical environment surrounding the electrocatalytic reactions, mainly temperature, humidity, and pH, that jeopardize their high selectivity. Furthermore, the necessary use of bienzymatic systems lead to stringent environmental conditions for optimal performance, influence shelf life, and call for rigid storage requirements of platforms. Thus, non-enzymatic sensing approaches have been proposed as a beneficial alternative to replace enzymes and the necessary enzymatic bioactivity by more reliable, reproducible, and simple materials, showing great promise for high performance sensor development [36]. Following these approach we report, for the first time at CENIMAT | i3N, the development of a novel, rapid, disposable, inexpensive, enzyme-free, and colorimetric paper-based platform for glucose sensing in the physiologically relevant range (1.25–20 mM) [35]. Regarding the glucose detection method, it is based on the synthesis of gold nanoparticles by reduction of a gold salt in the presence of NaOH, required for hydrolysis of gold complex ions, in which glucose is the reducing agent [35]. When the concentration of glucose is altered, differently sized AuNPs are produced: lower

glucose concentrations correspond to larger nanoparticles, whereas higher glucose concentrations are associated to smaller nanoparticles [37]. On the paper platform, it results on the display of a red colour, that is more intense and varies its hue, when glucose concentration increases. The adaptation of this synthesis into the developed paper platform was tested and calibrated using different standard solutions with physiological concentrations of glucose. The response of the colorimetric signals obtained with this paper-based platform showed a linear behaviour until 20 mM, required for glycemic control in diabetes. The developed colorimetric sensor revealed a detection limit of 0.65 mM, depending on calibration metric and sensitivity of 0.013 AU/mM for a linear sensitivity range from 1.25 to 20 mM, with high specificity for the determination of glucose in complex standards with other common reducing interferents and human serum. Figure 7 shows a schematic of the detection mechanism as well as some of the obtained results for glucose non-enzymatic biosensors.

With further development of such colorimetric, non-enzymatic methods, sampling and sample processing in paper substrate, fully functional paper-based analytical devices can be developed and combined with digital, smartphone-based tools for automatic colour signal analysis, resulting in systems that can compete with existing glucose detection systems, with elevated sustainability.

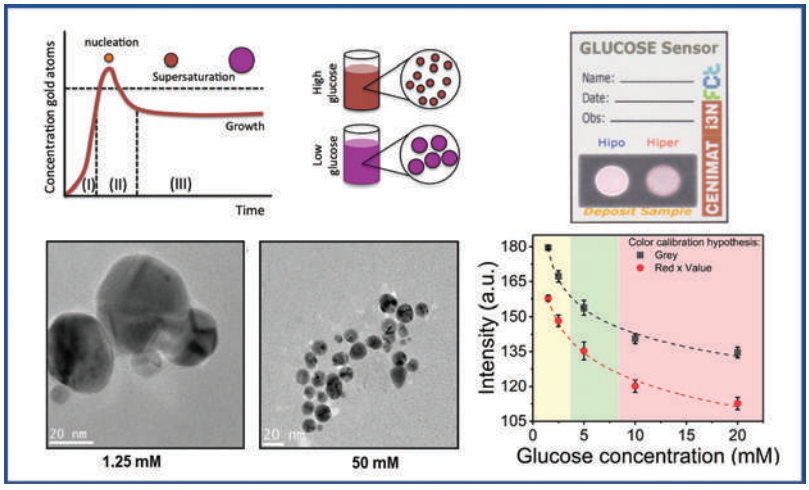

Figure 7 – Schematic of the detection mechanism as well as some of the obtained results for glucose non enzymatic lab-on-paper biosensors. Adapted from [35].

To further potentiate these approaches, gold nanoparticle-based assays were also applied to microfluidic devices, so that more complex functions could

be automatized and multiparametric measurements could be carried in a single device. By targeting specific biomarkers related to different conditions, more robust clinical decision can be derived from these diagnostic assays. An example of such paper-based devices was developed to target important biomarkers in diabetes diagnosis and prognosis, namely glucose, uric acid and cholesterol using a multiplexing biosensor [38]. By the same in-situ synthesis approach for glucose sensing, applied to a paper microfluidics platform or by immobilization of functionalized gold nanoparticles with affinity towards the analytes, colorimetric signals correlated with analyte levels give semi-quantitative information, in a sample-in-answer-out fashion, with a simpler approach for paper-based assay performance.

CONCLUSIONS AND FUTURE PERSPECTIVES

In this chapter, we presented some of the biosensors and diagnostic tests developed at Nova University of Lisbon based on microfluidic technologies. Most of the representative examples described here have been used in conventional, digital and paper based microfluidic systems, but it is believed that the latest technologies in chemistry, biology and materials science also can expand the field of application towards drug discovery, environmental monitoring and assessment, agricultural monitoring, food safety, security, and defense, just to mention a few.

Biosensors represent a powerful tool for analysis and when combined with microfluidics facilitate the fabrication of platforms with a wide variety of architectures that can be executed for several laboratory processes using small sample and reagent volumes at lower energy demands. Besides that, POC devices could exploit the capabilities presented for multi-analysis and real-time detection. Additionally, portability, automation, low cost, high throughput, and mass production are the trends for future developments, which will promote the popularization and commercialization of microfluidics-based biosensing.

Among microfluidics-based biosensors, wearable sensors are a field to pay particular attention in the future, especially in the development of sampling methods, properties of adhesive materials, and flexible electronic devices to turn these platforms smart. Self-powered biosensors are the future trend of development, the power of which can be supplied by human body movement. It is predicted that the number of microfluidic sensors implanted in the human body will increase rapidly, where real-time information can be sensed and reported.

Moreover, real-time transmission of information obtained from sensors to smart devices or data centers is also of particular interest, which can provide more precise information.

We are facing now the pandemic COVID-19 which is another evidence of the need of robust diagnostics tests for a rapid detection of various COVID-19 related biomarkers. It can be anticipated that microfluidic-based biosensors will remain a hot topic of investigations because of the ever-increasing demands in various applications ranging from industry to biomedical detection with enhanced sensitivity, improved stability, and miniaturized structure.

ACKNOWLEDGEMENTS

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CHAPTER III

NORMAL LIVING

A

DEVELOPMENT AND AGING

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NOVA has having a relevant collaboration in different areas related with normal development and healthy aging.

Research groups from Obstetrics, Pediatrics, Physiology, Immunology, Rheumatology and Psychiatry dedicate clinical, teaching, training and research time to promote these areas of knowledge.

RELEVANT CONTRIBUTIONS

The Immunology laboratory of NOVA Medical School has a continuous collaboration with Pediatric centers regarding patient evaluation in the context of Primary Immunodeficiency and Inborn Errors of Immunity (Figure 1), and this synergy has already contributed to the description of new mutations and entities in this field, allied to our major goal which is improved patient care[1-7].

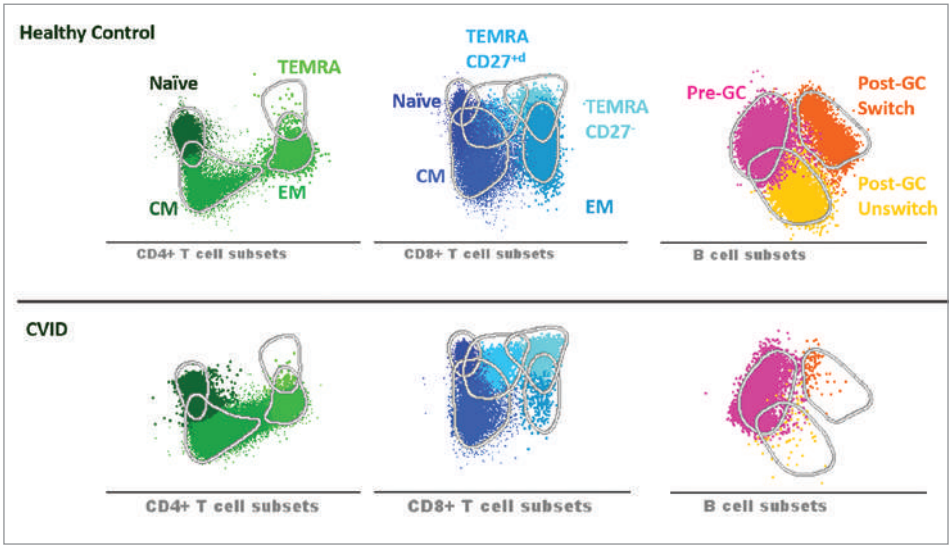

Figure 1 – Comparative Diagrams of CD4 T-cell, CD8 T-cell and B-cell subsets in a Healthy Control and a patient with Common Variable Immunodeficiency (CVID). Within T cells, the characterization included Naive, Central Memory (CM), Effector Memory (EM) and Effector Terminally Differentiated (TEMRA) CD27- and CD27+d cells. B cells were characterized in pre-germinal centre (GC), and unswitched and switched post-germinal centre. The standardized EurowFlow™ Primary Immunodeficiency Tube (PIDOT) strategy was used in a BD FACS Canto II cytometer, and all analysis were performed with Infinicyt™ software.

Type 1 diabetes is also an immune-mediated disease, despite much is still to unveil regarding the effective disease triggering mechanisms and the distinct stages of disease onset. Our works in pediatric type 1 diabetic patients, allowed us to delineate a longitudinal picture of disease progression, reporting dynamic changes of circulating key innate and acquired immune cell types along disease onset, honeymoon period and final establishment. These data may enable disease staging and patient stratification, essential for individualized treatment approaches[8].

Still addressing pediatric diseases, we collaborated with teams developing models to improve the detection of treatment-requiring retinopathy of prematurity, a condition that can be potentially blinding in premature infants[9].

In fact, our researchers have also important works in fields like Ophthalmology but also ENT (ear, nose, and throat), both covering pathologies prone to complicate the quality of life in elder patients. We have studied the immune background of non-infectious uveitis (NIU), a potentially blinding disease, frequently idiopathic or associated with a systemic disease. In these patients, we reported imbalances in immune profiles, in both peripheral blood and vitreous humor, reinforcing the vital role of cytokines in uveitis pathogenesis[10-11]. Infectious uveitis is also an important condition, in which we identified, on the other hand, elevated intraocular levels of inflammatory and regulatory cytokines, particularly in viral-associated uveitis. These insights in different types of uveitis my not only help diagnostic approaches, but also identify the potential of immune mediators as therapeutic targets.

Tinnitus is associated with various conditions such as presbycusis, infectious, autoimmune and many other diseases, often related to hearing loss, as well. Our studies in this field showed fluctuations in inflammatory markers along the hearing loss process, reinforcing the idea that inflammatory mechanisms are involved in hearing loss pathogenesis but also in tinnitus, despite different features may be present in both scenarios[12].

EpiDoC is an epidemiology and outcomes research unit, with a main focus in Rheumatology clinical research but also in life styles, other non-communicable chronic diseases, and their predictors. Established in 2016, it is located at NOVA Medical School1[3], and it's integrated in the Comprehensive Health Research Center (CHRC)[14], a clinical and public health research unit, scored Excellent by Fundação Ciencia e Tecnologia (FCT) 2019's evaluation.

EpiDoC unit aiming at providing scientific information of excellence, gathering health and diseases issues and covering clinical, social, economic and population aspects.

EpiReumaPt (EpiDoC 1)[15,16], a large epidemiological study of rheumatic diseases, collected information of a national representative sample of 10,661 participants in Mainland and Islands, retrieving the prevalence of the most relevant rheumatic diseases as well as demographic, socio-economics and clinical data associated with them. This study was performed in a join collaboration of Portuguese Society of Rheumatology, NOVA University, National Directorate of Health (DGS) and Universidade Catolica Portuguesa and was conducted between 2011-2013. Blood samples, DXA and x-Rays imaging were also collected. Two new waves were performed in 2013-2015 (EpiDoC 2) and 2015-2016 (Epidoc 3), evaluating the same participants, and building up the EpiDoC Cohort study[17](Figure 2). In 2021, ten years after the first evaluation, we are evaluating for the last time those participants (EpiDoC 4).

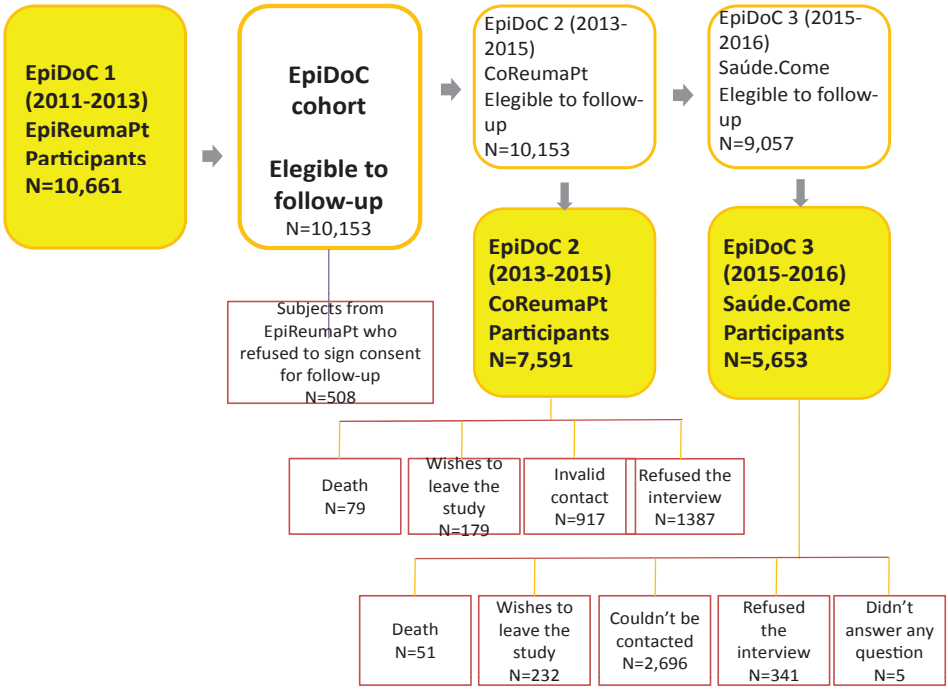

Figure 2 – EpiDoC cohort description. National representative sample of Portuguese adults. Evaluations done in 2011-2013 (EpiDoC 1 / EpiReumaPt study), 2013-2015 (EpiDoc 2) and 2015-2016 (EpiDoC 3). Currently in 2021, EpiDoC 4 is ongoing, interviewing the same subjects, ten years after the first evaluation.

Importantly, all data are available for researchers by submitting research projects to the scientific committee (http://cedoc.unl.pt/epidoc-unit/), to advance

clinical research taking advantage of the support of a multidisciplinary team involving different medical specialties, nutritionists, psychologists, statisticians and biomedical sciences' researchers.

EpiDoC Unit team has expertise in surveys, chronic disease registries, observational studies and management of large databases including a wide range of data: demographic, life habits, clinical, pharmacological, socio-economic, labor, quality of life (QoL), imaging, genetic and laboratorial data. Training and mentoring of master, doctoral and post-doctoral students are also a Unit's major activity.

EpiDoC is also dedicated to help patients and vulnerable population groups to improve their health. We are committed on promoting educational and training strategies using information and communications technology (ICT, internet, apps and smart TV tools). The Unit has also experience in conducting interventional studies using these new ICTs[18] (Figure 3). With this, we expect to contribute to patient empowerment, to reduce inequities in health and to provide evidence for public health policies.

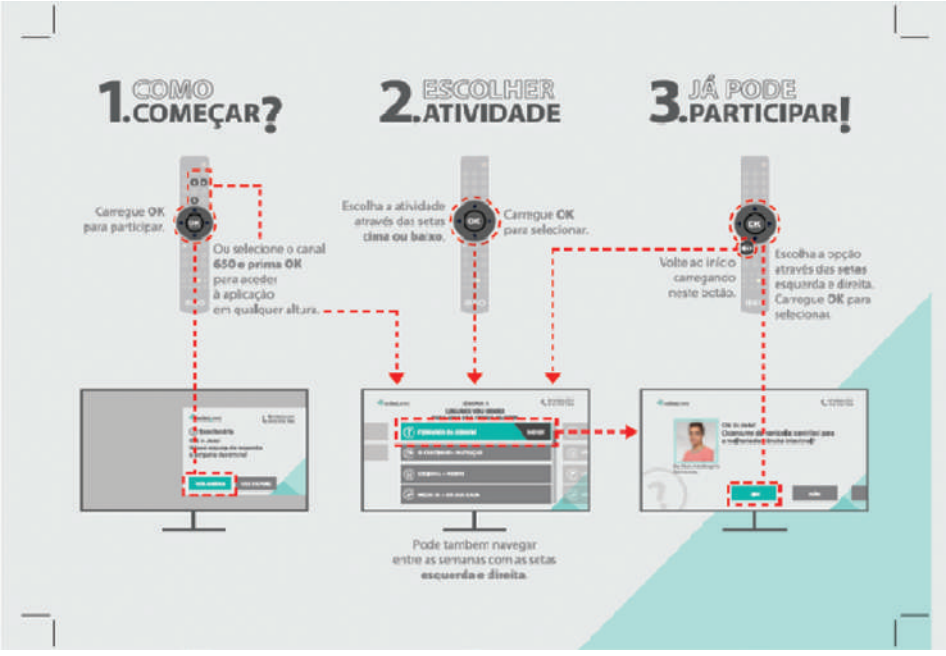

Figure 3 – Interactive TV program to educate and motivate older individuals with food insecurity, to improve lifestyles (saude.come, funded by EEA grants). A dedicated TV channel was broadcasted by NOS, Meo and Vodafone to the participants included in the study.

Within the field of psychiatry and mental health, links between late-life depression and neurocognitive disorders have been highlighted. For instance, there is now evidence supporting depression and bipolar disorders as risk factors for dementia[19].

NOVA Medical School has also been active in the field of old age psychiatric epidemiology: evidence on the prevalence of old age neuropsychiatric disorders in Portugal was recently made available, in collaboration with the 10/66 Dementia Research Group. Community prevalence rates were 3.7% (95% CI 2.8-5.0) for DSM-IV dementia, and 9.2% (95% CI 7.8–10.9) for 10/66 dementia, mirroring a potential underestimation of dementia prevalence using DSM-IV[20]. Regarding late-life depression, prevalence rates were 4.4 (95% CI 3.5-5.6) for ICD-10 depression and 13.0 (95% CI 11.2-15.0) for sub-syndromal depression[21]. These results are also important in terms of calculating the impact of dementia prevention strategies[22].

Other important topics in dementia were addressed by European multicenter studies in which NOVA Medical School participated. For instance, the evaluation of quality of life poses specific challenges in people with cognitive impairments[23,24] and specific measures for informal caregiver populations are being validated[25]. Access to community services is hindered by a series of factors[26], especially so in countries such as Portugal[27], and the imbalance between met and unmet needs may be striking[28].

Furthermore, primary care has a crucial role regarding disease prevention and health promotion in old age people[29], including brief needs assessments using the SPICE acronym (Senses, Physical ability, Incontinence, Cognition, and Emotional distress)[30] and task-sharing in dementia[31-33]. Finally, physical activity may be important in the prevention of cognitive impairment. We collaborated in a Portuguese multicenter trial, led by our colleagues of the Faculty of Medicine (University of Lisbon), aiming at evaluating the impact of moderate physical activity on cognition, quality of life, motor, and functional status in people with vascular cognitive impairment, and to explore the acceptability of physical activity in this population[34].

ONGOING RESEARCH

Some examples of other ongoing projects are listed below:

In Reproductive Immunology

- Evaluation of the uterine immune profile in women with reproductive failures, such as recurrent pregnancy loss or implantation failures, in an

attempt to identify particular features and/or potential biomarkers, to further develop specific therapies.
- Assessment of the evolution of B-cells and humoral functions along pregnancy, to further explore the impact of pregnancy in immunization processes and eventual alterations regarding particular immune conditions such as atopic diseases.

In Pediatrics:
- Explore the distinctive features of patients with different Primary Immunodeficiencies and Inborn Errors of Immunity, aiming to characterize new entities and contribute to more precise diagnostics and better therapeutic monitoring.
- Assessment of humoral and cellular immune responses in children during acute COVID-19 infection and one, three and six months after, approaching also possible differences in immune responses between mild, moderate, and severe infections.
- Continuous characterization of the immune profile of children with atopic dermatitis to identify markers that may help evaluate the effectiveness of future immunomodulatory therapies, aiming also for a better understanding of the disease's immunopathological mechanisms.

In Internal Medicine:
- Characterization of the impact of acute pancreatitis in immune cells and molecular biomarkers, to further correlate the eventual alterations with disease severity and outcome. The result of this study may have implications for future therapeutic interventions, in order to improve the prognosis of acute pancreatitis.
- Characterization of the evolution of circulating immune cells and mediators, following a remote ischemic conditioning (RIC) protocol in healthy humans, to identify characteristic variations that could bring new insights of RIC mechanisms, a protocol known to protect tissues against longer periods of ischemia.
- Molecular and cellular characterization of the peripheral sympathetic nervous system from different locations (cervical, thoracic and lumbar), and respective adipose tissue of healthy donors, seeking to identify unique features of each territory that may later be explored pharmacologically in the context of obesity.

In Epidemiology, Rheumatology and New Technologies:

- Currently, as mentioned above, we are implementing the EpiDoC 4 study.
- We are also carrying out the project "B-active: a training protocol for patients with paediatric rheumatic diseases", which aims to evaluate the motor competence of Portuguese children with rheumatic diseases, and to develop a training protocol that can be easily practiced by these children.
- In Child and Adolescent Rheumatology Department (CARD) of EpiDoC we are also developing an app in which patients with juvenile rheumatic diseases have suggestions for healthy style programs, videos and stress management strategies. It is also our aim to develop training actions in schools, in order to increase the awareness of juvenile rheumatic diseases in the school community.
- Other ongoing projects include the development of new technologies (internet, apps, smart TV) as tools to improve health and to monitor disease. We are designing and applying online platforms, apps and TV programs to collect objective measures and clinical monitoring refining individual follow-up of chronic disease patients. These tools are also applied as educational and motivation tools as well as in interventional studies namely to promote life healthy habits in elderly people. The collaborations with national and international partners as well as the participation in several networks and international projects are a key advantage.

Specific Projects:

- Develop, test and apply innovative forms of "measure" health condition in patients with rheumatic and other chronic diseases through new technologies such as internet, apps, smart TV, using patient oriented strategies;
- Identify health access and health consumption inequalities among patients with rheumatic diseases and citizens in general;
- Identify predictors of morbidity and mortality among rheumatic and other non-communicable chronic diseases;
- Study the influence of clinical and socio-demographic and life style characteristics (nutrition, physical activity) in health outcomes in the Portuguese population;
- Determine the burden of different rheumatic and other non-communicable chronic diseases on the functional capability, work status, social and economic outcomes;

- Monitor the effectiveness and safety of rheumatic and other chronic diseases' treatments in a "real-world" setting;
- Provide community services in the prevention, detection and treatment of rheumatic and other chronic diseases and in the education of the population;
- Characterize SARS COV2 exposure among EpiDoC participants and in a longitudinal study of previously infected people;
- Different projects where we are using artificial intelligence and new ICTs to study frail elderly, disorders associated to work, therapy adherence and COVID features (cough patterns recognition).

CONCLUSIONS

Aiming to increase our understanding of different physiological and pathological conditions and their unrevealed immune background and interactions, we have distinct collaborations ongoing with clinicians and researchers from diverse hospitals and institutions.

Our ultimate goal is to provide tools and support to students, fellows, researchers and health professionals, to develop, boost and elevate clinical research and public health research and, in the end, to better serve the clinical care of patients and health policies.

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B

NUTRITION

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INTRODUCTION

The current pandemic has drawn attention to the vital importance of nutrition. Indeed, the environment-health dichotomy has never been so apparent in terms of the impact of human activities on the environment as well as the environmental impact on our health. Over the last fifty years we have become increasingly aware of the negative effects of lifestyle on a healthy ageing.

Our research team has been focusing on how environmental factors, particularly diet, influence health. To that end, we have studied the molecular mechanisms involved in metabolic diseases.

This chapter describes the principal findings from the research on three topics: phenolic compounds and metabolic diseases; gut microbiota and health; endocrine disruptors and metabolic dysfunction.

In the last year, COVID-19 has been the focus of our research, both in evaluation studies of intestinal microbiota (Moreira-Rosário, et al., 2021) and the role of vitamin D metabolism (Freitas, et al., 2021) in the severity of the disease. Preliminary findings in the pre-publication phase demonstrate the relevance of the studies and they will now be presented in more detail throughout this chapter.

POLYPHENOLS AND HEALTH

Polyphenolic compounds are ubiquitously found in the plant kingdom. Products of plant secondary metabolism, they are responsible for plant survival face to environmental threats. Apart from their physiological roles in plants, flavonoids are important components in the human diet, although they are considered as non-nutrients. Several epidemiological studies provide support for the association between consumption of fruit, vegetables and certain beverages rich in polyphenols, such as tea and red wine, and health promoting effects (Hollman & Katan, 1999a, 1999b; Williamson, Kay, & Crozier, 2018).

The research group has dedicated attention to the study of dietary phytochemical, as polyphenols, and their antioxidant activity, establishing structure-activity relationships. Several works were conducted in this regard, with different sources of phytochemicals, demonstrating the antioxidant potential of these compounds (Faria, et al., 2005) and the modulation of transmembrane transport of different substrates by phenolic compounds (Faria, et al., 2006; Faria,

et al., 2009; Faria, et al., 2010; Lemos, et al., 2012; Monteiro, et al., 2005). A big advance was made perceiving that besides the phosphorylation and dephosphorylation phenomena responsible for transport protein regulation (Calhau, et al., 2002), the cell oxidative environment was also able to modulate transporters (Faria, et al., 2006).

Phenolic bioavailability has been a critical issue to the understanding the biological effect of phytochemicals. Making use of cellular models of intestinal epithelia, it was demonstrated that anthocyanins (a flavonoid) can permeate Caco-2 cell monolayers (Faria, et al., 2009) and the involvement of glucose transporter GLUT2 was suggested for the intestinal transport of anthocyanins (Faria, et al., 2009).

Considering the neuroprotective effect of anthocyanins and their metabolites, their transport at the blood–brain barrier (BBB) absorption was characterized (Faria, et al., 2014; Faria, et al., 2010; Faria, et al., 2011) and anthocyanin metabolites showed higher transport efficiency than the native anthocyanin without no further biotransformation of the metabolites (Faria, et al., 2014). Anthocyanins and their metabolites crossed this BBB cell model in a lipophilicity-dependent way (Faria, et al., 2014). In addition, the interference of these compounds in substrate transport in BBB was also shown (Meireles, et al., 2013; Meireles, et al., 2016).

In line with this, the neuroactivity of these compounds was explored in an animal model. A positive impact of anthocyanin consumption was shown (Meireles, et al., 2016), with decrease of inflammatory parameters, particularly, at brain level. The chronic consumption of anthocyanins considerably impact the neuroinflammatory status of the animals, and it was suggested the modulation of fractalkine (Marques, et al., 2016; Meireles, et al., 2015; Meireles, et al., 2016) in this process, that was confirmed in a cellular model.

Anthocyanin bioavailability was investigated in a study where human volunteers consumed a blackberry puree with or without ethanol (Marques, et al., 2016), and several anthocyanin phase II metabolites (methylated, glucuronidated and sulphated) were identified, both in plasma and urine samples. The plasma concentration of anthocyanin metabolites was about ten times higher than the parent anthocyanin's level (even without reaching the C*max* of anthocyanin metabolites during the time of the protocol), highlighting the crucial role of metabolites when studying phenolic compounds.

Microbiota plays an important role in human metabolism and dietary anthocyanins that are not absorbed in the upper GI level and their metabolites

(methylated, sulphated or glucuronidated forms) excreted in the bile and/or from the enterohepatic circulation reach the microbiota. Anthocyanins are subjected to metabolism by microbiota and are themselves or their metabolites microbiota modulators (Faria, et al., 2014), being able to change the growth levels of specific bacteria. For this reason, it can also be considered that anthocyanins have a prebiotic effect.

Anthocyanins and their metabolites are able to cross the blood-brain barrier (Faria, et al., 2014). Nonetheless, to be neuroprotective, anthocyanins do not necessarily need to reach the brain. By changing the gut microbiota and acting on the gut-brain axis, anthocyanins may exert a biological activity even without being absorbed. Alterations in gut microbial composition afforded by anthocyanins were shown to result in changes in the levels of tryptophan and kynurenic acid which has been implicated in CNS inflammation, excitation and behavior (Figure 1) (Marques, 2018). Besides, the tryptophan/kynurenine ratio has been implicated in neuropsychiatry conditions such as depression and anxiety (Kennedy, et al., 2017). Therefore, by decreasing this ratio, anthocyanins might constitute a new promising strategy for the treatment of neuropsychiatry disorders.

From the evidence gathered over the years regarding phenolic compounds it is very clear that one can not misprize metabolites that are the effectors of the perceived biological effect. On this regard, metabolomic approaches that include proteomic and lipidomic will be essential to understand the functional activity of phytochemicals, either by their metabolites direct effect or by the modulation of transcription, enzyme activity, signaling pathways.

THE GUT MICROBIOTA AND METABOLIC DISEASE

The resistance to ecological stress and the ability to recover from a stress-related perturbation (resilience) are characteristics of a healthy microbiome that empowers its maintenance throughout life (Backhed, et al., 2012). When the microbial ecosystem is perturbed to an extent that exceeds its resistance and resilience capabilities, alterations in its composition and function may occur (dysbiosis) (Levy, et al., 2017). The expansion of pathobionts (commensal microorganisms that can cause pathology under uncontrolled proliferation), the loss of beneficial bacteria and the loss of diversity (microbial richness) are common characteristics of a dysbiotic state (Levy, et al., 2017). These features

have detrimental consequences for the host (may initiate obesity and metabolic diseases) and can be caused by environmental factors such as dietary factors.

Gut microbiota is probably indispensable for obesity development, as germ-free animals are resistant to HF diet-induced obesity (Backhed, et al., 2007). On the other hand, it is well established that a disrupted microbiome, either from HF diet-induced obesity rodents, obese individuals (that usually have a diet rich in fat, sugar and food additives) or from *ob/ob* animals (that continuously have a sense of starvation and exhibit hyperphagia), initiates obesity after transplantation to germ-free animals (Ridaura, et al., 2013; Turnbaugh, et al., 2008; Turnbaugh, et al., 2006). Therefore, it is important to characterize these diet-induced changes on the gut microbiota composition since they might be responsible for the onset of obesity and metabolic disorders.

Wistar and Sprague-Dawley are the main Rat strains used as HF diet-induced obesity models (Hariri & Thibault, 2010; Panchal & Brown, 2011; Tschop & Heiman, 2001, 2002). Nevertheless, the effects of HF diet upon the gut microbiota of these animals were not previously characterized. Our group studied the Wistar and Sprague-Dawley rats in parallel to determine the most appropriate model where the effect of dietary compounds could be investigated. Our results showed that both strains can be used as models of HF diet-induced obesity, although Wistar rats seemed to be particularly predisposed to HF diet-induced obesity and metabolic disorders (Marques, et al., 2016). The gut microbiota composition of the two Rat strains differed in some of the bacterial genera analyzed which can explain different metabolic responses to HF diet (Marques, et al., 2016). As previously reported, host genetics may influence the gut microbiota composition which may predict, in turn, the effects of dietary interventions on host metabolic parameters (Kreznar, et al., 2017; Parks, et al., 2013; Zeevi, et al., 2015).

The intestinal epithelium is a highly regulated physical barrier that secretes several compounds such as mucus and antimicrobial peptides which, together, act as front lines of defense protecting the host against bacterial translocation (Peterson & Artis, 2014). Recently, it has been hypothesized that gut barrier dysfunction in obesity leads to the passage of microbial components into circulation, which drives systemic inflammation (Hamilton, et al., 2015). Moreover, HF diets can compromise gut mucosal integrity and, therefore, contribute to metabolic endotoxemia (Everard, et al., 2013; Lam, et al., 2012; Martinez-Medina, et al., 2014; Moreira, et al., 2012; Wang, et al., 2014). Our group evaluated the intestinal fatty-acid binding protein (I-FABP) and glucagon-like peptide-2 (GLP-2) as putative biomarkers of intestinal

permeability in the context of HF-diet induced obesity (Lau, et al., 2016). Although I-FABP ended up not being a good biomarker of intestinal permeability, GLP-2 was surprisingly increased in the plasma of HF diet fed animals and were positively correlated with systemic inflammatory markers (Murakami, et al., 2016).

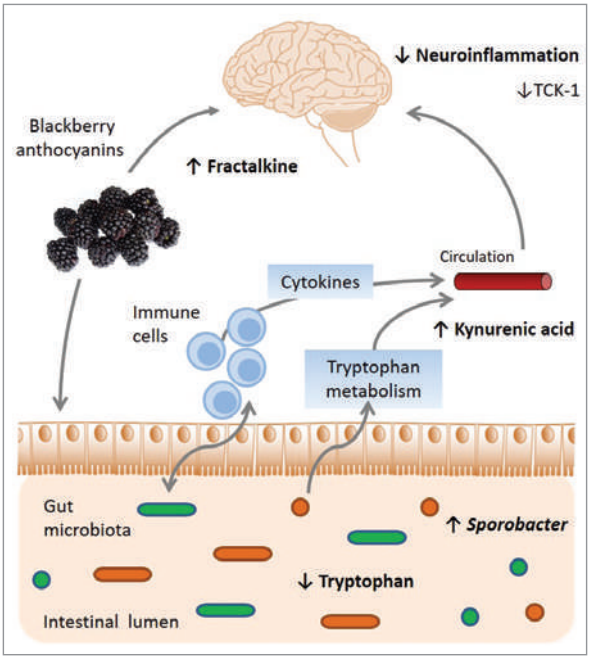

Figure 1 – Mechanisms behind the neuroprotective effects of anthocyanins, in the context of HF-diet induced obesity. Anthocyanins act directly in the brain increasing the expression of fractalkine, a chemokine extremely important in the crosstalk between neurons and microglia during synaptic plasticity (Meireles, et al., 2015). On the other hand, anthocyanins modulate the gut microbiota composition, increasing the bacterial genus *Sporobacter* and alter host tryptophan metabolism. The amount of tryptophan available is decreased by anthocyanins to undergo the kynurenine pathway and originate kynurenic acid, a metabolite whose neuroprotective actions were recently identified (Salimi Elizei, Poormasjedi-Meibod, Wang, Kheirandish, & Ghahary, 2017; Wu, et al., 2010). Through these routes, anthocyanins counteract the HF-diet induced neuroinflammation and may attenuate the neurological complications of obesity as well as neurodegenerative diseases. Anthocyanins might constitute, therefore, a new class of psychobiotics.

Our research in preclinical models have prompted the study of gut microbiota modulation by dietary interventions in obese subjects with metabolic diseases, as

a tentative to find novel therapeutic strategies for these health problems. Recently, we demonstrated that a Mediterranean diet is effective in diabetes control through gut microbiota modulation, i.e. the Mediterranean diet alters the gut microbiota and these alterations are reflected in the glycemic control of these patients (Ismael, et al., 2021). These results show that the gut microbiome modulation is necessary to observe a metabolic improvement that can be considered clinically relevant. In this regard, YourBiome a spin-off from Universidade NOVA de Lisboa founded by our group aims to developed novel gut microbiome-based strategies for obesity and metabolic disorders. By transplanting the microbiome from healthy donors to those with metabolic disease (faecal microbiota transplantation) we expected to correct patients dysbiosis and improve the success of dietary interventions.

In addition to dietary factors, only a few studies have evaluated the effect of probiotic supplementation on the gut microbiota composition and gut permeability (Ferolla, et al., 2016; Gobel, et al., 2012; Stenman, et al., 2016). Modulation of the gut microbiota, gut barrier reinforcement, stimulation of glucagon-like peptide 1 (GLP1) secretion are among the most probable mechanisms of action of these probiotics strains, as demonstrated in animal studies (Alard, et al., 2016; Park, et al., 2013; Rather, et al., 2014; Yadav, et al., 2013).

To sustain the claim that direct manipulation of gut microbiota with probiotics may contribute to weight loss, more RCTs are necessary. Additional trials that include a larger number of participants are needed to draw conclusions about the role of probiotics in weight-loss. In this regard, we will conduct a study on obese subjects undergoing bariatric surgery to evaluate if a probiotics can improve the success of a bariatric surgery.

ENDOCRINE DISRUPTORS AND METABOLIC DYSFUNCTION

The WHO has recognized the global threat of non-communicable diseases (NCDs), including the rise in obesity prevalence but also of its co-morbidities, namely with the increase of cardiovascular disease risk, that contribute to the overall increase in public health expenses of obesity treatment (Faria, et al., 2013; Ng, et al., 2014). Yet, it is puzzling why some people manifest different degrees of comorbidities or have more difficulty in losing weight than others (Bluher, 2010). Understanding the myriad of factors contributing to obesity and metabolic dysfunction, especially in vulnerable populations, is essential for curbing its decade-long

expansion. Recently, increasing data suggests that environmental and behavioural influences are "fuelling" the epidemic, namely the exposure to environmental chemicals with endocrine disrupting activity (Heindel, 2003; Heindel, Newbold, & Schug, 2015). Undeniably, even very small concentrations of these endocrine disrupting chemicals (EDCs) have the capacity to induce diverse and severe health damages through numerous potential mechanisms (Merrill, et al., 2020; Teixeira & Pestana, 2020). The "environmental obesogen" hypothesis associates EDCs to the disruption of energy homeostasis.

Since the beginning of this field, our group has been following an integrative and multidisciplinary research approach to this problematic. Fostering collaborations with national and international experts, we resort to a highly transversal research approach that enables the evaluation of the human external/internal exposure to chemical pollutants and its health effects. Our collaboration with Requimte/LAQV has been an important cornerstone for the EDCs analysis and development of new detection methods (Correia-Sá, et al., 2012; Correia-Sá, et al., 2018; Fernandes, et al., 2012; Sousa, et al., 2020). We focus on the evaluation of EDCs biological effects, assessing the environmental exposure impact on human health (e.g. general population, children, obese, diabetics, cancer patients), but also exploring how exposures interact with our unique characteristics and impact on human health, resorting to experimental and mechanistic approaches (*in vivo*/*in vitro* models)(Monteiro, et al., 2008; Monteiro, Azevedo, & Calhau, 2006; Monteiro, et al., 2006; Monteiro, et al., 2007; Monteiro, et al., 2009; Norberto, et al., 2017; Pestana, et al., 2015; Pestana, et al., 2017; Rodríguez-Alcalá, et al., 2015).

Our attention was drawn to a specific group of EDCs that include numerous compounds with common features. Some are plastics [e.g., bisphenol A (BPA), phthalates], solvents [e.g., polychlorinated biphenyls (PCBs), polybrominated biphenyls (PBBs), dioxins], pesticides and fungicides [e.g., organotins, methoxychlor, chlorpyrifos, dichlorodiphenytrichloroethane (DDT), vinclozolin], personal care products (e.g., triclosan), pharmaceutical agents (e.g., thiazolidinediones, antihistamines, antidepressants) and synthetic hormones (e.g., ethynilestradiol). Their lipophilic nature, allied with extreme resistance to chemical and biological degradation, are responsible for their persistence in environment and consequent harmful effects (Li, et al., 2006). As lipophilic compounds, POPs are bioaccumulated in fatty tissues (Maia, et al., 2020), and their levels increase along the trophic chain (biomagnification).

Our initial works were focused on EDCs as responsible for increased metabolic dysfunction in obese adult individuals and their effect on decreased

metabolic improvement after bariatric surgery (Faria, et al., 2014; Pestana, et al., 2014b; Teixeira, et al., 2015a). More recently, research from our group demonstrated the involvement of EDCs in aggravation of inflammation and cardiovascular risk in a context of obesity (Pestana, et al., 2014b; Pestana, et al., 2017; Teixeira, et al., 2015a). Indeed, we were one of the firsts to propose the redefinition of obesogens to that of metabolic disruptors, to encompass chemicals that play a role in altered susceptibility to obesity, diabetes and related metabolic disorders (Sá, et al., 2018; Teixeira, et al., 2015a).

Some of our works have studied the distribution of EDCs among different fat depots. In fact, possibly due to its particular localization and unique metabolic characteristics (Palaniappan, et al., 2004), visceral obesity is considered determinant in the causal pathway of dysmetabolic obesity and metabolic syndrome (Bluher, 2010; Wernstedt, et al., 2014). In turn, data from obese individuals report that higher visceral adipose tissue (AT) EDCs levels, the main reservoir, are associated with metabolic abnormalities, inflammation and higher cardiovascular risk (Pestana, et al., 2014a; Teixeira, et al., 2015b). Using an animal model, it was possible to show that *p,p'*-DDE exposure exacerbates the metabolic impact of a high-fat (HF) diet as a consequence of an impairment of the mesenteric visceral AT normal function and its growth ability (Pestana, et al., 2017). Altogether, these mechanisms could ultimately lead to AT dysfunction, disturbing its toxicological functions, namely of protection (La Merrill, et al., 2013), and having local and systemic consequences (Regnier & Sargis, 2014; Sargis, 2014; Ukropec, et al., 2008), affecting the interplay with central (brain) and peripheral organs (gut, liver, muscle) (Bluher, 2010; Wernstedt, et al., 2014) and contributing to the dysfunction of other metabolically active organs (Pestana, et al., 2017; Rodriguez-Alcala, et al., 2015). Despite seeming paradoxical, AT plays a central yin-yang role in EDC toxicology, acting as a protector/modulator and as a target of toxicity (La Merrill, et al., 2013) (Figure 2).

With respect to vulnerable groups, children have been identified as one of the higher exposed sub-groups and specifically vulnerable for certain groups of chemicals, including some with food as major exposure source, like phthalates and bisfenol A. We have been focused not only in evaluating EDCs effects in children, but also how to intervene for exposure reduction (Castro-Correia, et al., 2018; Correia-Sa, et al., 2018; Correia-Sa, et al., 2017; Correia-Sa, et al., 2017; Lessmann et al., 2017). Strategies that modulate the effect of toxicants on pathophysiologic processes involved in disease etiology and progression will be of public health importance.

Recent findings implicate the importance of an individual's nutritional status and the use of protective bioactive food components to decrease the overall toxicity of environmental pollutants (Petriello, Newsome, & Hennig, 2014).

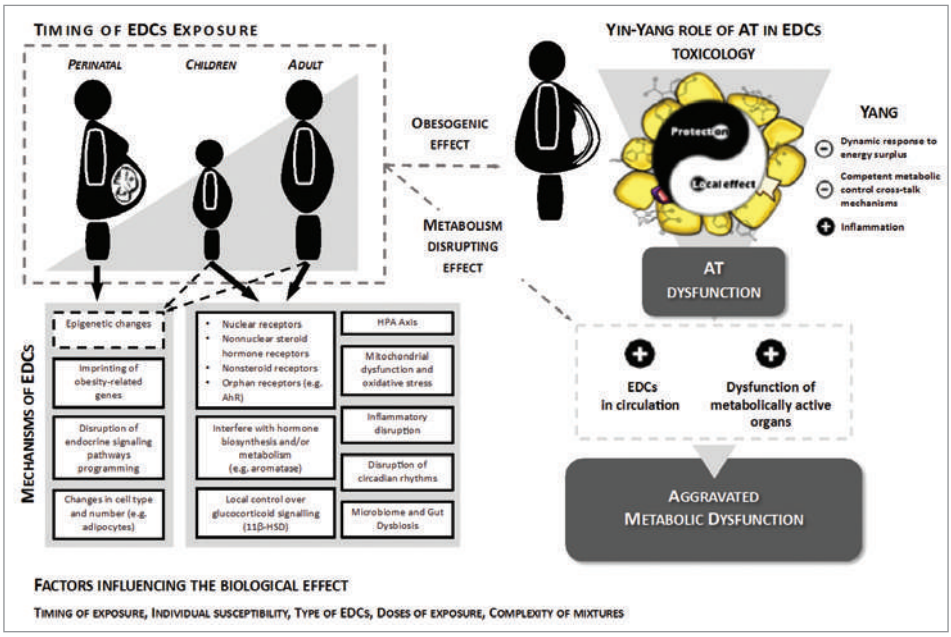

Figure 2 – Schematic representation of the proposed mechanisms for the obesogenic and metabolism disruption effects of endocrine disrupting chemicals (EDCs). 11β-HSD, 11β-hydroxysteroid dehydrogenase; AT, adipose tissue; AhR, aryl hydrocarbon receptor; HPA, hypothalamic-pituitary-adrenal axis.

Overall, there is a need to biomonitor and evaluate all the exposures across the lifespan and how they interact with our own unique characteristics. The 'exposome' encompasses exposures to environmental factors throughout life, starting from conception and pregnancy, however, the knowledge integrating the metabolomic, gut microbiome and epigenetic signatures related with disease progression and interaction with EDCs exposures is still scarce. We are committed to this goal to create new avenues of intervention and prevention, such as nutrition. This knowledge will allow to create an innovative approach that will empower different stakeholders with guidelines of action that take into consideration and integrate EDCs exposures and biomarkers in the clinical practice (Teixeira D, 2015; Teixeira D, Pestana P, Calhau C, & Graça P, 2015).

SUMMARY

In conclusion, our research group has contributed to significant advances in the area of nutrition, lifestyles and health. It has provided more detailed our knowledge about how lifestyle can affect health and its link with the diseases that are most prevalent in the world today. Even in the COVID context, the group's recent advances showed that patients infected with the new coronavirus had a worse prognosis where there was dysbiosis and therefore less gut microbiota diversity; this confirms the reports published at the start of the pandemic that patients infected with associated metabolic disease presented the most serious clinical condition. In fact, the microbiota together with the organic pollutants in the environment appear to be central to most diseases. The link with lifestyle and dietary choices is decisive and polyphenols in particular are of great interest.

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MOLECULAR NUTRITION AND HEALTH: DIETARY POLYPHENOLS AND NEURODEGENERATIVE DISEASES

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INTRODUCTION
NUTRITION AND HEALTH

According to WHO nutrition is a critical part of health and development for humans. Better nutrition is related to safer pregnancy and childbirth, improved infant, child and maternal health, stronger immune systems, longevity and lower risk of chronic diseases (such as diabetes and cardiovascular diseases).

Chronic diseases result from a combination of genetic, physiological, environmental and behaviour factors and are driven by forces that include unhealthy lifestyles and population aging. Some of these factors are not modifiable, such as the genetic ones, while lifestyle associated factors like nutrition, physical and social activity are potentially modifiable. In this sense, nutrition and other environmental factors have a huge impact on health and wellbeing. Therefore nutrition emerges as a critical modifiable risk factor to be exploited in policy strategies to prevent or delay the onset of chronic diseases.

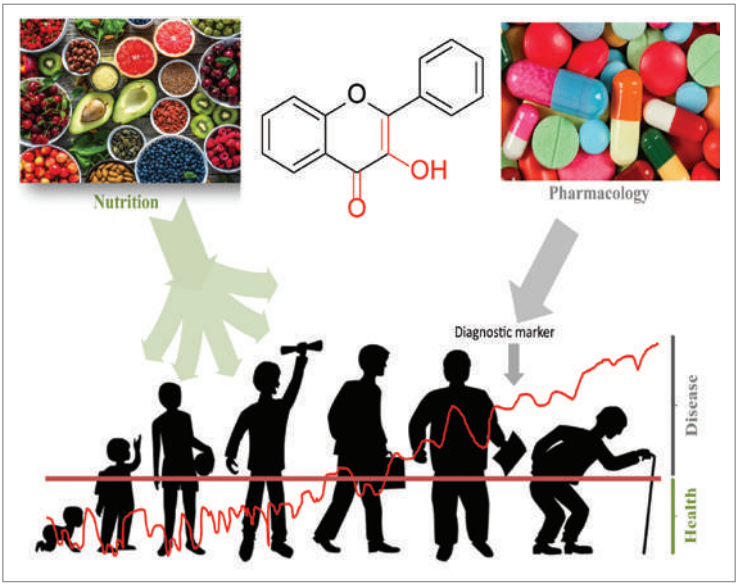


Figure 1 – (Poly)phenols in a nutritional or pharmacological approach: disease progression and transition from a healthy to a disease state. Through nutrition (poly)phenols can act in the disease prevention, or in the restoration of a healthy state in earlier disease stages, even before detection of diagnostic markers or drug administration. (Poly)phenols can also be the basis for developing new therapeutic compounds and therefore contribute to pharmacological interventions.

Several epidemiological studies from the last decades illustrate that a continuous and prolonged intake of fruits and vegetables, rich sources of compounds named (poly)phenols, can have beneficial effects in humans health (Li et al., 2014; Medina-Remón et al., 2015; Rodriguez-Mateos, Heiss, et al., 2014), preventing degenerative pathologies such as diabetes, cardiovascular diseases, neurodegenerative diseases and cancer, and to prevent effects associated to aging and menopause (Del Rio et al., 2013; Vauzour et al., 2010). If we consider the normal process of evolution of a healthy state to a disease state, we may resume that the evidences indicate an active role of dietary (poly)phenols to homeostasis maintenance (Figure 1). Based on this we may expect an active role of dietary (poly)phenols in delaying or even reversing the transition from a healthy to a pathological state (Figure 1). Then, nutrition and in particular bioactive (poly)phenols identified in the diet, are strong contributors to the maintenance of a healthy condition. Moreover, (poly)phenols can also constitute a lead compounds as a basis for developing new drugs and new pharmacological approaches (Figure 1). Thus, pharmacological and nutritional approaches can be considered for the study of (poly)phenols.

STATE OF THE ART REVIEW (POLY)PHENOLS AND NEURODEGENERATIVE DISEASES

Phenolic compounds, commonly referred to as polyphenols, constitute one of the most extensive and ubiquitous groups of secondary metabolites in the plant kingdom. These compounds are characterized structurally by the presence of, at least, one hydroxyl functional group (-HO) linked to an aromatic ring (Tsao, 2010). Some compounds that do not present structural characteristics of polyphenols are commonly integrated into the group of polyphenols as "honorary", such as phenolic acids or stilbenes. For this reason, recently the term "polyphenols" has been rewritten as "(poly)phenols" (Diederich, 2012; Rodriguez-Mateos, Heiss, et al., 2014; Zanotti et al., 2015). (Poly)phenols represent an extremely differentiated group, both in terms of a chemical structure and biological activity, and present themselves conjugated with sugars, carboxylic and organic acids, amines, lipids and other phenols. Different groups are classified in terms of the number of phenol rings that they encompass and according to the structural elements binding these rings (Pandey & Rizvi, 2009; R.C. Pimpão, 2014). The main classes are represented in Figure 2 and include phenolic acids, stilbenes, flavonoids, coumarins and tanins.

Phenolic acids comprise two different groups that are represented by hydroxybenzoic and hydroxycinnamic acids. The first ones can be found in plants, both free and esterified. Examples are gallic acid, present in fruits, herbs, tea and wine, or more complex compounds, the hydrolyzable tannins such as ellagic acid, gallotannins and ellagitannins (Manach et al., 2004). Hydroxycinnamic acids are generally represented by p-coumaric, caffeic, ferulic and sinapic acids, usually found glycosylated or conjugated with quinic, shikimic and tartaric acids (Manach et al., 2004; Pandey & Rizvi, 2009). For example, chlorogenic acid is an ester of caffeic and quinic acids that can be found in several fruits and vegetables and is highly abundant in coffee. Ferulic acid is present in large amounts in cereal grains (Bisson et al., 2008; Manach et al., 2004). Most stilbenes are present in roots, barks, rhizomes and leaves, and are not routinely consumed. By opposition we have a highly valued stilbene, resveratrol, present in grapes and in red wine (Cassidy et al., 2000).

The largest group of phenolic compounds in plants are flavonoids, with more than 10,000 different compounds being identified (Cheynier et al., 2013). The main classes of flavonoids are flavonols, flavones, isoflavones, flavanones, anthocyanins and flavanols. Monomers of flavanols (catechins), such as (+)-catechin and (-)-epicatechin, are relatively abundant in fruits, wine, chocolate and green tea (Hollman & Arts, 2000; Manach et al., 2004). Proanthocyanidins, also known as condensed tannins, are constituted by dimers, oligomers and polymers of catechins. Proanthocyanidins can be found in fruits such as apples and grapes, in wine, cider, tea and beer, and also in cocoa (Santos-Buelga & Scalbert, 2000). Anthocyanins are glycosylated pigments, responsible for the colours of some flowers and fruits.

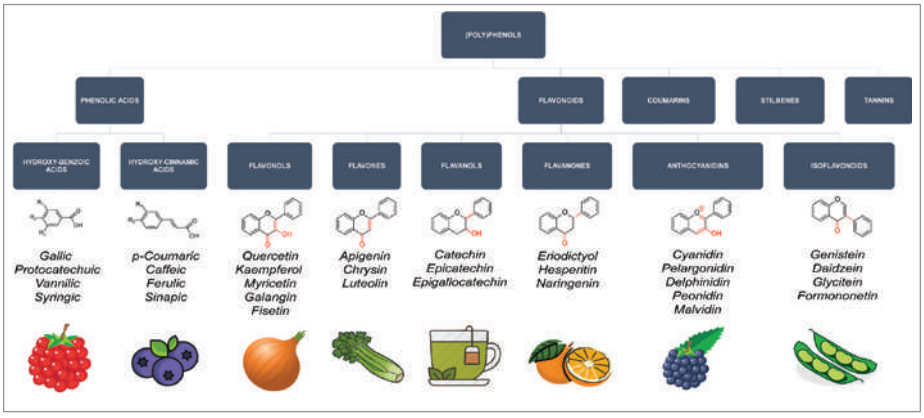


Figure 2 – Main (poly)phenol classes with structure, name of representative compounds (in italic) and examples of food sources.

Although (poly)phenols are not essential nutrients for humans, they have a positive impact on human nutrition, as already mentioned, and in fact the amounts normally included in our diet are considerable. (Poly)phenols are widely spread in food, and the total (poly)phenols dietary intake can be as high as 100-150 mg per day, which is much higher than that of all other classes of phytochemicals (Manach et al., 2004). Just for perspective, this is one order of magnitude higher than the intake of vitamin C and two orders of magnitude higher than the intake of vitamin E and carotenoids (Manach et al., 2005; Scalbert et al., 2005).

Neurodegenerative disorders (NDs) are collectively refered to debilitating, life-threatening conditions that affect neural cells. Nowadays we estimate that there are nearly 46.6 million people worldwide living with dementia, constituting the greatest burden of NDs (Prince et al., 2013). Chronic and progressive neurological diseases are caused by nervous system dysfunction leading to neural cell failure (Brettschneider et al., 2015), resulting in impaired cognitive function (dementia) or movement complications/disorders. These diseases can arise from hereditary or sporadic conditions, having complex pathogenesis that triggers dysfunction of central or peripheral structures of the nervous system (Soto, 2003). These pathologies, with a significant projected rise in incidence, are among the most burdensome age-related chronic diseases, with both social and economic consequences. Currently, there are no disease-modifying therapies to delay or reverse disease progression, hence we rely only on a paucity of pharmacotherapy strategies focused on symptomatic relief. Thus, preventive strategies are urgently needed to tackle the growing burden of neurodegenerative diseases.

(Poly)phenols are emerging as effective agents in slowing cognitive decline, preventing or postponing the onset of neurodegenerative diseases and delaying or slowing their progression. Besides the epidemiological evidences nutritional studies show also significant cognitive benefits of phenolics consumption in humans (Kennedy, 2014; Macready et al., 2009; Nehlig, 2013; Rendeiro et al., 2015). It has been described that dietary phenolics may modulate different aspects of synaptic plasticity, e.g. memory and/or learning improvement in both animals and humans (Rodriguez-Mateos, Vauzour, et al., 2014; Spencer, 2008; Williams & Spencer, 2012), even if their mechanism of action on central nervous system remains poorly understood (Figueira et al., 2017). Long-term supplementation with (poly)phenols in animal models, suggests that they can activate neuronal receptors, interact with signaling pathways (e.g. MAP kinases) and control the expression of specific genes (Rendeiro et al., 2015). Their ability to directly modulate brain plasticity is

dependent on their accessibility to the brain, which in turn is defined by the structural characteristics of the circulating phenolic metabolites (Youdim et al., 2003, 2004). As such, whether the effects induced by dietary phenolics on brain functions are mediated directly in the brain or involve other mechanisms triggered from cells in the periphery remains unclear.

METABOLISM AND BIOAVAILABILITY OF (POLY)PHENOLS

Numerous research emerged in the past years enable us to better understand (poly)phenols' health benefits disclosing a glimpse of the huge potential they may present (Arts & Hollman, 2005; Parr & Bolwell, 2000; Peterson et al., 2012). However, for a more profound understanding of the effect of (poly)phenols on human health, further studies concerning their absorption, distribution, metabolism and excretion need to be conducted. The most common (poly)phenols present in the human diet are not necessarily the most active inside the body, either due to low inherent activity or due to their poor absorption, extensive metabolization and rapid excretion (Manach et al., 2004). Throughout digestion, (poly)phenols suffer several chemical modifications and metabolism resulting from the digestive and hepatic activity, and the bioavailable metabolites found in blood and tissues diverge from the parent compounds in terms of biological role. Absorption events are accompanied by multiple metabolic reactions, which occur in the small and large intestine, in the liver and in the cells throughout the body, resulting in a wide variety of derivatives, e.g. sulfated, methylated and glucuronidated. In the colon, bacterial enzymes from local microbiota promote the aromatic ring fission of the compounds into low-molecular-weight metabolites. The correlation between parent compounds and derived gut metabolites is puzzling: the large majority of the known gut (poly)phenol metabolites derives from a group of structurally diverse parent compounds (e.g. chlorogenic acids, flavanols, proanthocyanidins, theaflavins and thearubigins) that undergo modifications converging to the formation of aromatic/phenolic acids with hydroxyls substituents whereas fewer are associated with a unique gut metabolite (e.g. urolithins from ellagitannins, S-equol from isoflavones) (Williamson & Clifford, 2017). For some parent compounds data about their metabolism is still lacking (e.g. pyranoanthocyanins, coumarins and other minor dietary components) (Williamson & Clifford, 2017).

Bioavailability of (poly)phenols is thus a multi-stage process comprising de-conjugation and possible catabolism, absorption, conjugation and excretion

(Figure 3). Moreover, the possible sequestration of some (poly)phenol metabolites inside tissues has been recurrently undetermined and may contribute to underestimation of the their bioavailability. The accessibility of (poly)phenol metabolites to the central nervous system is an example where the presence of an additional barrier, the blood-brain barrier (BBB), reduces even more (poly)phenols bioavailability, in addition to the described metabolism.

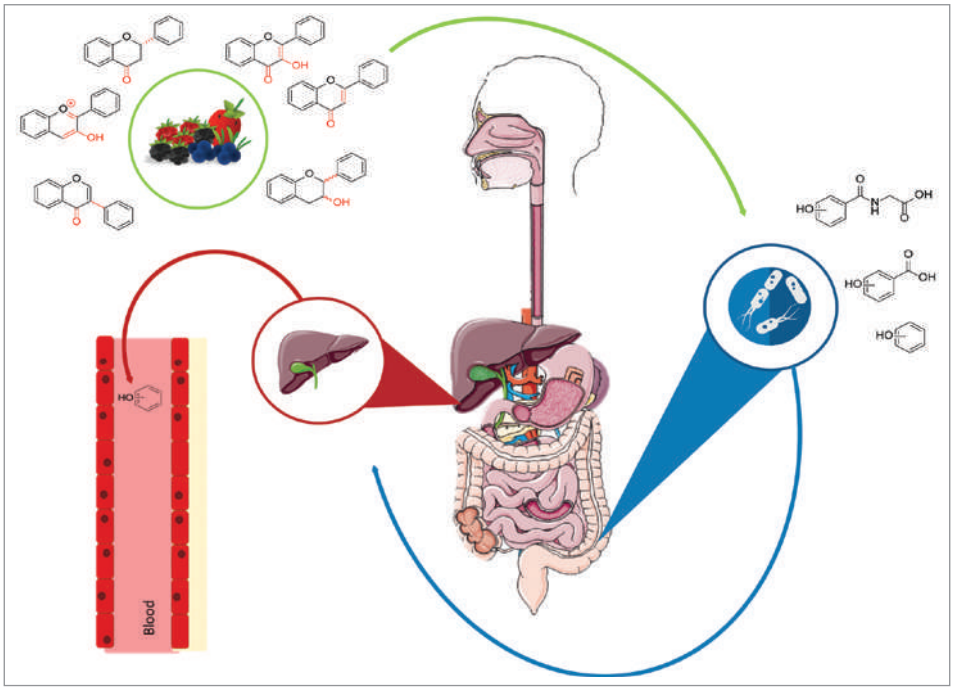


Figura 3 – General diagram of absorption and biotransformation of (poly)phenols in the human body. The gastrointestinal tract functions as physical barrier, determining bioavailability of xenobiotics, like (poly)phenols. Absorption occurs at the small intestine but compounds not absorbed travel to the large intestine and at the colon can be extensively metabolized by the resident microbiota. (Poly)phenols can undergo phase I (oxidative and reductive reactions) and phase II reactions (conjugation to glucuronic acid, sulphate, methyl, acyl, glycine or reduced glutathione) in liver when enter circulation.

The BBB is a dynamic interface that limits and regulates molecular exchanges between the blood and the neural tissue, hence playing a key role in the control of the accessibility of nutrients and other compounds to the brain (Abbott, 2002; Cardoso et al., 2010). Evidence of BBB capacity to uptake some (poly)phenol metabolites is

growing in literature; consistent with (poly)phenol metabolites' potential activity in the brain (Chen et al., 2015; Faria et al., 2011, 2014; Gasperotti et al., 2015; Ho et al., 2013; Ishisaka et al., 2011; Milbury & Kalt, 2010; Wu et al., 2012). The majority of these studies that are focussed on oral administration of dietary phenolics, directly or in their derived (poly)phenols metabolites, have reported their capacity to reach the brain, e.g. (-)epicatechin (Abd El Mohsen et al., 2002; Lin et al., 2007; van Praag et al., 2007), hesperetin and naringenin (El Mohsen et al., 2004; Peng et al., 1998), quercetin (Bieger et al., 2008; Ho et al., 2013; Huebbe et al., 2010; Ishisaka et al., 2011) and anthocyanins (Andres-Lacueva et al., 2005; El Mohsen et al., 2006; Kalt et al., 2008; Milbury & Kalt, 2010; Talavéra et al., 2005; Wang et al., 2014). To date, despite the fact that gut (poly)phenol metabolites are in general accepted to comprise a considerable percentage of the total diversity of (poly)phenol metabolites (e.g. (Czank et al., 2013; Pereira-Caro et al., 2014; Rui C Pimpão et al., 2015)), only a very limited number of studies have specifically focussed on gut (poly)phenol metabolites accessibility to the brain (e.g. valerolactones and phenolic acids) (Del Bò et al., 2010). In a very recent pharmacokinetic study in rats, upon intravenous administration, some gut (poly)phenol metabolites were observed to reach the brain (Gasperotti et al., 2015).

RELEVANT CONTRIBUTIONS FROM NOVA

At the Molecular Nutrition and Health laboratory from CEDOC of NOVA Medical School and in collaboration with iBET-Instituto de Biologia Experimental e Tecnológica, we have contributed to resolving the kinetics of the absorption of dietary phenolics by measuring plasma concentrations and/or urinary excretion in adults upon ingestion of pure phenolic compounds, plant extracts or whole beverage (single dose) (Del Rio et al., 2013; Feliciano et al., 2016; Rui C Pimpão et al., 2014, 2015). This strategy has allowed us to identify specific (poly)phenol metabolites as well as their physiological "circulating" levels (Del Rio et al., 2013; Feliciano et al., 2016; Rui C Pimpão et al., 2014, 2015).

We have positively identified metabolites and conjugates, some of them novel, in the urine of healthy volunteers after intake of multiple phenolics from a mixed puree of berry fruits, with each being excreted at specific and signature times (Rui C Pimpão et al., 2014). Although conjugated phenolic metabolites derived from colonic metabolism have been identified in the urine, the quantification and

appearance of these compounds in plasma is less well studied. To address this gap, we performed a cross-over intervention study where a mixed fruit puree (blueberry, blackberry, raspberry, strawberry tree fruit and Portuguese crowberry) or a standard polyphenol-free meal was given to thirteen volunteers (ten females and three males), who received each test meal once, and plasma metabolites were identified by HPLC–MS/MS. Sulfated compounds were chemically synthesized and used as standards to facilitate quantification. Gallic and caffeic acid conjugates were absorbed rapidly, reaching a maximum concentration between 1 and 2 h. The concentrations of sulfated metabolites resulting from the colonic degradation of more complex (poly)phenols increased in plasma from 4 h, and pyrogallol sulfate and catechol sulfate reached concentrations ranging from 5 to 20 mM at 6 h. This study allows to conclude that phenolic sulfates reached high concentrations in plasma, as opposed to their undetected parent compounds (Rui C Pimpão et al., 2015). At that time this identification, plasma quantification and the ability to synthesize these colonic metabolites open us the avenue for the assessment of the biological activities on the brain-related mechanisms. But before assessing their neuroprotective ability we used an *in vitro* model of the human blood-brain barrier to evaluate the permeability of some of the novel gut (poly)phenol metabolites at physiological levels. Transport through the BBB varies between 5-10% for the tested gut (poly)phenol metabolites (Figueira et al., 2017). Inspired by these early findings, we also analyzed if they could undergo further metabolism in the brain cells. Surprisingly, we have built evidence that two gut (poly)phenol metabolites, namely catechol-sulfate and pyrogallol-sulfate, originate new end-route metabolites in human brain endothelial cells (Figueira et al., 2017).

We also showed that amongst the gut (poly)phenol metabolites tested so far, 9 of them display cytoprotective activities in diferrent cell systems (brain endothelial cells, neurons, astrocytes and microglia cells) (Figueira et al., 2017). Concerning our dataset on pyrogallol-sulfate and catechol-sulfate ability to attenuate neuroinflammation, we observed that only the first compound was able to inhibit the release of the pro-inflammatory TNF-α from lipopolysaccharide (LPS) induced microglial cells (Figueira et al., 2017). TNF-α is known to be transcriptionally regulated by NF-kB and we have observed that NF-κB translocation to the nucleus is blocked by pyrogallol-sulfate (Figueira et al., 2017). Moreover, some of the tested gut (poly) phenol metabolites are able to protect brain endothelial cells from an oxidative insult even before being transported through the BBB (Figueira et al., 2017). Additionally, we detected strong cytoprotection by pyrogallol-sulfate and catechol-sulfate in 3D

neurospheroids cultures of differentiated human NT2 cells (mixed culture of neurons and astrocytes differentiated from the human pluripotent NT2 cell line) towards an oxidative insult. Physiologically relevant concentrations of both compounds promoted significant neuroprotection that was reflected in a higher survival rate of cells when exposed to an oxidative insult (Figueira et al., 2017).

IMPACTS TO SCIENCE AND SOCIETY

The pleiotropic action of dietary phenolics make them attractive candidates to approach for multifactorial diseases such as neurodegeneration, therefore the exploitation of the gut (poly)phenol metabolites as novel neuroactive compounds is emerging. The legacy of our research will be essential for the knowledge-based development of (poly)phenol metabolites for nutritional and/or clinical applications for a healthy brain. This research will help to bridge the gap between nutrition and cell biology and clearly goes beyond the state-of-art on the effects of (poly) phenol metabolites in the brain. Applications are also expected in the food sciences for the study of new bioactive compounds that could constitute either new functional ingredients or nutraceutical formulations. The concepts that we develop could contribute to designing new dietary recommendations, and the established knowledge on (poly)phenol metabolites bioactivity can leverage future development of cost-effective nutraceutical/pharmaceutical therapies. Both endings will contribute to increasing healthy life expectancy by delaying the onset of neurodegenerative diseases. Therefore, increasing quality of life and having a significant social-economic impact.

Our societal priority is to contribute to the delay of the onset and/or progression of neurodegenerative diseases showing increasing incidence in developing countries. Discovery of (poly)phenol metabolites capable to strongly attenuate neuroinflammation, can be patented as they can be the basis for the development of new pharmaceutical/nutraceutical therapies.

Additionally, insights from our outcomes will enhance our ability to delivering strategic solutions for healthy foods and diets. By linking dietary phenolics with neurodegenerative diseases we contribute for dietary solutions and innovations leading to improvements in health and well-being as well in disease states of people affected by NDs. These outcomes are central for policy-making of governmental and non-governmental organizations to promote healthy eating and improve food security.

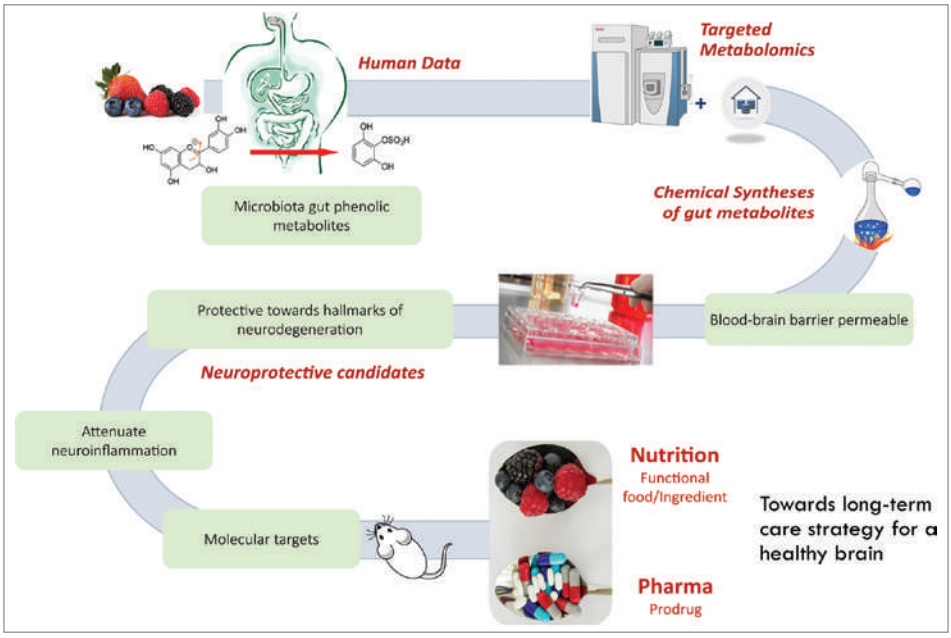
Since we deliver new insights on the health potential of phenolics from diet, this will increase the visibility of the diet effects on health. This has considerable educational value, making possible to communicate: the impact of nutritional practices in a healthy lifestyle; the causes of the benefits on brain health and the merits of more rational recommendations. This is also a great opportunity to deliver science impact into networks other than academic institutions, such as businesses, policymakers, stakeholders, schools or the wider public. Dissemination activities within the public are very important and helpful to contribute for informed consumer choices. Moreover, it will also present an impact by reducing the gap in knowledge transfer through enhancing the communication between consumers, food industry and the research community.

ONGOING RESEARCH

The mechanism of action of the most abundant (poly)phenol metabolites originated from our diet in our gut and detected in circulation in humans – the low molecular weight (LMW) (poly)phenol metabolites – is still unknown. Absorption and blood concentrations of some of these metabolites are very high (10-30 microM) and we and others have shown their ability to cross the BBB (Angelino et al., 2019; Feliciano et al., 2016; Figueira et al., 2017; Rui C Pimpão et al., 2014, 2015). Yet, our understanding of their molecular effects once inside the brain is still unknown. In the Molecular Nutrition and Health laboratory from CEDOC of NOVA Medical School we aim to determine the ability of several LMW (poly)phenol metabolites, to modulate neuroinflammation provoked and propagated by microglial cells (the brain immune cells), as well as to understand their mechanisms of action. By now, our team has listed more than 100 LMW (poly)phenol metabolites present in either blood or urine, thus with physiological relevance (Carregosa et al., 2020). We gathered all described metabolites, either from commercial sources or made its synthesis, thanks to methods developed by the team, to constitute our in-house library (Almeida et al., 2017). Currently, we are evaluating all metabolites for their ability to modulate the microglial inflammatory response, at several physiologically relevant concentrations and timepoints to reveal the most potent and effective ones. One important aspect that we are also foreseeing is to consider that the appearance in circulation is simultaneous for more than one LMW (poly)phenol metabolite and accordingly a time course of increasing concentrations. This is an aspect that has

not been considered in current approaches that only test isolated compounds at static concentrations in cells.

The transport across the BBB will also be investigated using *in silico*, *in vitro*, and *in vivo* methods. Meanwhile, the molecular mechanisms associated with the anti-inflammatory properties of these compounds, which are still unknown, will concentrate our efforts. The potential of revealing such protective mechanisms will facilitate the understanding of how LMW (poly)phenol metabolites could be neuroprotective and how we could benefit from them either from diet or as an effective therapy in the future.



Overall the Molecular Nutrition and Health team aims to decipher the role of LMW (poly)phenol metabolites at physiologically relevant conditions, tracking the compounds with the greater anti-inflammatory effects, and to explore the mechanism(s) behind this effect in the model systems. We will also acquire critical knowledge about the *in vivo* behavior of these compounds in animal models. Finally, by studying the impact of dietary (poly)phenols in inflammation-associated neurodegeneration, we will ensure the translation of obtained results to humans. Only by unlocking the mechanism of action of specific (poly)phenol metabolites the established knowledge can be translated into specific dietary

recommendations, particularly valued to delay/postpone the development of neurodegenerative diseases.

The Molecular Nutrition and Health laboratory from CEDOC of NOVA Medical School hopes to understand how we can impact the prevention and progression of NDs through nutrition. Moreover, by correlating brain beneficial effects with physiological relevant (poly)phenolic compounds we will establish the grounds for the development of novel food products enriched in specific (poly)phenols or nutraceutical/pharmacologic applications.

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BIODIVERSITY AND HEALTH: INVESTING IN BIODIVERSITY PROTECTION TOWARDS HEALTH GAINS

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ABSTRACT

Biodiversity is declining faster than at any time in human history and the direct drivers of change in nature with the largest global impact are related to human activities: land and sea use changes; direct exploitation of organisms; climate change; pollution; and invasion of alien species. The One Health approach, and other holistic approaches, integrates human, animal, and plant health, as well as the health of their shared environment, informing and supporting a multidisciplinary and holistic approach that integrates monitoring, planning, and evaluation to optimize co-benefits and outcomes for public health. This chapter intends to provide a systematic overview on how conserving nature and biodiversity can contribute to improve the implementation of the One Health and other holistic approaches, to prevent new pandemics and to promote well-being. A detailed analysis regarding how the targets in the updated zero draft of the Post-2020 Global Biodiversity Framework can contribute to improve the implementation of the One Health or other holistic approaches was performed, aiming to support the ambition and commitment needed. Additionally, a list of indicators is proposed to guarantee a suitable monitoring framework and to adequately incorporate the value of biodiversity for health, well-being, and more specifically contributing to the reduction of the risk of new pandemics. This work highlights the importance of preventing biodiversity loss for human health and well-being. The linkages between biodiversity and human health reinforce the need of holistic approaches such as One Health to understand the intricate linkages between the health of plants, animals, humans, and our shared environment.

Keywords: One Health approach; Biodiversity; Research and Innovation; Public health; Global biodiversity framework

1. BACKGROUND

Nature is essential for human existence and good quality of life. Most of nature's contributions to people are not fully replaceable, and some are irreplaceable. Nature plays a critical role in providing food and feed, energy, medicines and genetic resources, clean air, fresh water, climate regulation, pest and disease regulation, disaster risk reduction, as well as spiritual and cultural values, all of

them fundamental for people's physical and mental well-being and for maintaining culture [1].

Biodiversity can be defined as the variability among all living organisms including, *inter alia*, terrestrial, marine, and other aquatic ecosystems and the ecological complexes of which they are part; this includes diversity within species, between species and of ecosystems [2]. Biodiversity is declining faster than at any time in human history [1]. The direct drivers of change in nature with the largest global impact are related to human activities: land and sea use changes; direct exploitation of organisms; climate change; pollution; and invasion of alien species. The impact of human activities is driving a global increase in species extinction risk [3] and an overall decline in species population abundance [1]. Up to 1 million species (approximately 25% of the known species) are already threatened with extinction across terrestrial, freshwater, and marine vertebrate, invertebrate and plant groups that have been studied in sufficient detail. Additionally, the mammal biomass on Earth is composed mainly of humans and livestock (96%), 36% corresponding to *Homo sapiens*. Wild mammals, both terrestrial and aquatic, represent only 4% of the total amount of mammal biomass [4].

The diversity of functional groups and traits of species and populations is essential for ecosystem integrity and for the generation of ecosystem services [5,6,7]. Biodiversity also underpins ecosystem functioning providing food, medicines, goods, and services that are critical to human health and well-being. Biodiversity, besides other important roles, is highly relevant for buffering shocks and extreme events, which will become more and more frequent in the face of the current climate change scenarios [8] and increasing environmental pollution. Thus, biodiversity is a key environmental determinant of human health and well-being. Its conservation and sustainable use are crucial to maintain ecosystem services [2].

Biodiversity loss can destabilize ecosystems, promote outbreaks of infectious disease, increase the incidence, mortality and prevalence of non-communicable diseases, and undermine nutrition security and protection from natural disasters. Protecting public health from these risks lies outside the traditional roles of the health sector and depends on working with partners engaged in conservation, and the sustainable use and management of natural resources [2]. However, these benefits are not consistently considered in health and development decision-making processes. With the increasing frequency and impact of disease emergencies linked to environmental and animal sources, expertise from environmental science and management is critically needed to improve prevention and detection of disease

threats. These issues have been pointed out as key areas of research in the context of the COVID-19 pandemic [9].

The One Health approach integrates human, animal, and plant health, as well as the health of their shared environment, informing and supporting a multidisciplinary and holistic approach that integrates monitoring, planning, and evaluation to optimize co-benefits and outcomes for public health [10]. Furthermore, the One Health approach supports global health by improving coordination, collaboration and communication between multiple disciplines and sectors at the human-animal-plant-microbiome- environment interface to tackle shared health threats such as antimicrobial resistance, food safety, zoonotic diseases, and many others [11].

In this scope and with the same aims, other holistic approaches besides One Health can be considered. In 2015, the Rockefeller Foundation-Lancet Commission introduced a novel approach called Planetary Health concerning the health of human civilization and the state of the natural systems on which it depends, and proposed a concept, a strategy, and a course of action. The Planetary Health strategy includes the need to act urgently, a need that has been highlighted by the 2018 report of the Intergovernmental Panel on Climate Change (IPCC) [12] and by the 2019 report of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services [1]. The urgency to act is also embraced in the framework of the United Nations 2030 Agenda for Sustainable Development.

The Sustainable Development Goals (SDG) are an urgent call for action, recognizing that ending poverty and other deprivations must go hand-in-hand with strategies that improve health and education, reduce inequality, and boost economic growth, while tackling climate change and working to preserve our oceans and forests [2]. Although these goals have attracted wide interest and commitment from many organizations and stakeholders, current trends in terms of action to achieve them are not encouraging. The first Global Sustainable Development Report [13] presents alarming data, such as rising inequalities, climate change, biodiversity loss, and increasing amounts of waste. However, a systemic approach such as Planetary Health could be taken to redirect the key development activities in our societies towards a more sustainable path [14].

Recently, a conceptual framework was proposed to improve this linkage between health and biodiversity, proving that this is an area of research demanding for attention [15]. This framework considers four domains in the interaction between biodiversity and human health: (i) reducing harm (e.g. provision of medicines, decreasing exposure to environmental pollution); (ii) restoring capacities (e.g.

attention restoration, mental health); (iii) building capacities (e.g. facilitating physical and mental activity, spiritual experiences); and (iv) causing harm (e.g. exposure to dangerous wildlife, chronic and infectious diseases or allergens) [16].

The increasing importance of this linkage means it is urgent for it to be considered in the worldwide institutional agendas. All governments and decision makers need to address health impacts of major environmental threats on a regular basis, to prompt timely and corrective actions. A shift from fragmented to systematic actions will promote Human and Planetary Health, conserving nature and biodiversity, and slowing down climate change. Improving cooperation between various sectors including health, environment, energy, agriculture, trade, and transport, as well as chemical and other industries, is crucial. To achieve this, we need a multidisciplinary approach in the European Union (EU) with full commitment of the relevant sectors. There is substantial evidence that initiating a European Strategy for Planetary Health in support of the Green Deal [17] could help to achieve the United Nations' SDG. Now is the moment to unite, from individual, to planetary scale, in shared actions [14]. Now is the moment to do more and better towards health gains!

1.1. Biodiversity, health, and human well-being

There is a close interrelationship between global environmental issues, such as the crisis associated with biodiversity loss and climate change, and health issues [2, 10, 15, 18]. The health sector depends on biodiversity and ecosystem functions and services and, on the other hand, the health sector has potential impacts on biodiversity that may threaten the provision of ecosystem services. Additionally, health has an important role in the 2030 Agenda, with SDG 3 calling on all stakeholders to "ensure healthy lives and promote wellbeing for all at all ages". In addition to socio-economic determinants of human health, the impact of environmental, climate, ecosystem change and degradation on health is increasingly recognized. Human health and livelihood ultimately depends upon ecosystem products and services [15]. The World Health Organization (WHO) periodically produces data on the global burden of disease attributable to the environment, with the most recent available data, published in 2018, indicating 630 000 deaths in the EU in 2012 [19]. Thus, tackling the upstream drivers of environmental degradation and biodiversity loss is of utmost importance.

The incidence of infectious diseases is influenced by biodiversity at the level of pathogens, vectors, and reservoir hosts, including livestock; by the diversity of

habitats; and by human movements, wildlife-livestock-human contact interface and behaviour. Ecosystem disturbances can affect the risk of acquiring infectious diseases by promoting the direct contact between human and microbes, or they can do so indirectly through their impact on infectious agents, reservoirs, and vectors [2,10,18]. Biodiversity conservation reduces the risk of zoonotic diseases by providing or maintaining habitats for wild species, thus minimising the potential contact between wildlife, livestock, and humans. The interlinkages between biodiversity and human health and well-being have been extensively reported [2, 20]. Some of them can be summarised as follows:

- Biodiversity, be it in the form of pollinators (Figure 1), soil biota or natural pest controllers, for example, plays a critical role in supporting food production. A broad diversity of species, varieties and breeds underpins good nutrition and varied diets.

Figure 1 – Butterflies play an important role in pollination. A Silver-studded Blue (*Plebejus argus*) in Alvão Natural Park.
Source: Photo provided by João Pargana

- Terrestrial and freshwater ecosystems underpin the water cycle and the provision of clean water supplies, regulating nutrient cycling, soil erosion and water purification.

- Marine biodiversity is essential for a healthy planet. It plays a vital role in maintaining the functionality and productivity of ecosystems. Oceans represent a significant source of water, food, biomass, oxygen, and other important aspects to human health, as well as having an important role in carbon sequestration.
- Many medicines, such as some antibiotics, are derived from naturally occurring substances. A large proportion of antibacterial drugs can be traced back to chemicals of natural origin. Plant, microbial and marine species hold vast potential for new medicinal products.
- The human body depends on its microbiota to support the function of the gastrointestinal tract, the regulation of the immune system and the prevention of infections. Reduced contact with healthy ecosystems can reduce diversity in this human microbiota and lead to immune dysfunction and diseases, such as allergies and intestinal disorders.

Figure 2 – Landscape in Peneda-Gerês National Park and one of the natural environments available for health benefits.
Source: Photo provided by João Pargana

- Spending time in natural environments is also associated with improved mental health and increased levels of physical activity with consequent

health benefits The benefits of access to biodiverse green or blue spaces are particularly significant for urban residents of low socio-economic status. Interaction with nature can contribute to treatment for depression, anxiety, and behaviour problems, including in children (Figure 2).

- Biodiversity contributes to ecosystem resilience and is essential for enabling the adaptation of our agricultural production systems to climate change. For example, new species may be drawn into agricultural production as the climate shifts. Vegetation reduces erosion and plays a role in flood mitigation, reducing the impact of natural disasters on health and well-being.
- Living organisms act as bio-indicators of human health stressors. For example, lichen act as indicators of air pollution, while crustaceans are an indicator group of species for water quality.

1.2. Biodiversity and pandemics

Pandemics have their origin in diverse microbes carried by animal hosts, but their emergence is entirely driven by human activities. The fundamental causes of pandemics are the same global environmental changes that cause biodiversity loss and climate change [10]. These include deforestation, land- and sea-use change, agricultural expansion and intensification, and wildlife trade and consumption. These activities bring wildlife, livestock, and people into closer contact, allowing animal microbes to spillover into people and causing infections, sometimes outbreaks, and more rarely epidemics and pandemics [10]. The recent increase in global consumption and trade, caused by demand in developed countries and emerging economies, as well as by demographic pressure, has led to a series of emerging diseases that originate mainly in developing countries [10].

New diseases in humans can emerge either as a result of a modification in the nature or behaviour of commensal microorganisms that lead to a disease, or through infection by novel organisms, usually through contact with animals and the environment [18, 21]. About 60% of human infections are estimated to have an animal origin, and 75% of emerging human infectious diseases "jump species" from (non-human) animals to people [21, 22]. In fact, the majority (70%) of emerging infectious diseases such as Ebola, Zika, Nipah encephalitis originate from microbes found in nature. Almost all known pandemics, namely influenza, HIV/AIDS and COVID-19 are zoonoses since their natural hosts are animals. Around 80% of pathogens infecting animals are "multi-host," since they survive among different animal hosts [21, 23], rarely including humans (Figure 3). Domestic animals and peri-domestic wildlife also

have a role in creating bridges for the emergence of human diseases, since this can happen in an evolutionary sense, or the animal could serve as a physical transmitter. Additionally, an estimated 1.7 million still unknown viruses are thought to exist in mammal and avian hosts and up to half of these could have the ability to infect humans. The most important reservoirs of pathogens with pandemic potential are mammals (in particular bats, rodents and primates) and some birds (in particular water birds), as well as livestock (e.g. pigs, camels, mink, poultry) [10,21].

Figure 3 – Horseshoe bats are believed to be the primary hosts of *SARS-CoV-2*. A colony of the horseshoe bat *Rhinolophus ferrumequinum* found in a cave roost in Vila Pouca de Aguiar.
Source: Photo provided by João Pargana

Unsustainable exploitation of the environment due to land- and sea-use change, intensive animal breeding, deforestation, agricultural expansion and intensification, wildlife trade and consumption, and other activities, disrupts natural interactions among wildlife and their microbes, increasing the contact among people, livestock, wildlife, and their pathogens [10, 18]. This proximity with humans (unnatural hosts) increases the spillover risk and has led to almost all known pandemics [10, 18, 24, 25].

In 2020, the COVID-19 pandemic raised global attention to the consequences of the emergence of zoonotic diseases. The response to the current acute pandemic

has mainly focused on containment measures that have shown a dramatic social and economic impact [10]. Healthy and resilient societies depend on giving nature the space it needs. This ongoing pandemic makes the need to protect and restore nature even more urgent. The pandemic is raising awareness to the links between our own health and the health of ecosystems, and it is demonstrating the need for sustainable supply chains and consumption patterns that do not exceed the planetary limits [10]. This reflects the fact that the risk of emergence and spread of infectious diseases increases as nature is destroyed [10, 18, 24, 25]. Protecting and restoring biodiversity and well-functioning ecosystems is therefore key to boost our resilience and prevent the emergence and spread of future diseases [1, 2, 10, 21]. Unfortunately, according to several published reports, at the global level, none of the 20 Aichi targets included in the Strategic Plan for Biodiversity 2011-2020 (adopted by decision X/2 at the tenth meeting of the Conference of the Parties of the Convention on Biological Diversity) have been fully achieved, though six targets have been partially achieved [26].

Strengthening health systems and increasing epidemiological surveillance of wild and domesticated animals focused on "highly probable pathogen reservoirs", as well as of humans, using modern science-based technologies, may help to predict and detect at early stages incidents and potential spillovers. However, we must also be aware that the identification of these potential intermediary hosts or wild reservoirs may take a long time or not happen at all, so the best option is to act in a preventive way, eliminating or minimizing the risks [2, 18, 21]. The One Health approach, which recognizes the interconnection between health in people, animals, plants, and their shared environment, is, together with other integrated approaches, a fundamental pillar of prevention and early detection. With this approach in mind, ten science-based policy recommendations were proposed [21]:

- Raise awareness and increase zoonotic literacy and emerging disease risks and prevention at all society levels to build extensive support for risk-reduction strategies;
- Increase investments in holistic approaches, including the One Health perspective, strengthening the integration of environmental considerations in the World Health Organization (WHO) and in other health organizations;
- Boost scientific enquiry into all the dimensions of emerging diseases (health, social and economic), including zoonoses, to assess risks and develop interventions at the interface of the environment, animal health and human health;
- Improve cost-benefit analyses of emerging diseases prevention interventions to include full cost of all the emerging diseases dimensions

and guarantee ongoing and well-resourced preparedness and response mechanisms;
- Develop effective means of monitoring and regulating practices associated with zoonotic disease, including food systems from farm to fork and improving sanitary measures;
- Include health considerations in incentives for (sustainable) food systems, including wildlife source foods;
- Identify key drivers of emerging diseases in intensive animal production and smallholder production;
- Support integrated management of landscapes and seascapes that enhance sustainable co-existence of agriculture and wildlife, including investing in sustainable methods of food production that diminish pollution while reducing risk of zoonotic disease transmission;
- Reinforce the existing and build new capacities among health stakeholders to improve outcomes and to increase literacy in the human, animal and environment health dimensions of zoonotic and other diseases;
- Mainstream and implement the One Health approach, or other holistic approaches, in land-use and sustainable development planning, implementation and monitoring.
- In spite of these already identified science-based policy recommendations, regardless the enormous real and potential socio-economic impacts of emerging zoonotic diseases, and although the consensus that prevention is better than cure, investments and political will to control them at their source have been unsatisfactory until now [21].). Therefore, the current pandemic should be considered the "cost of inaction" in biodiversity protection and a higher ambition and stronger commitments should be the focus when discussing the Post-2020 Global Biodiversity Framework respective targets [27].

1.3. Post 2020 Global Biodiversity Framework

To date, the most remarkable global commitment to safeguard species has been Aichi Target 12 of the Strategic Plan for Biodiversity 2011–2020 (the "Strategic Plan") under the Convention on Biological Diversity (CBD) [28]. This global commitment states the following: "By 2020, the extinction of known threatened species has been prevented and their conservation status, particularly of those most in decline, has been improved and sustained." Unfortunately, the ongoing situation (decline in species populations, and many species facing extinction) shows a clear failure of this global commitment [26, 29].

The Post-2020 Global Biodiversity Framework (Post-2020 GBF) is a stepping-stone towards achieving the CBD's 2050 Vision of 'Living in harmony with nature', where "by 2050, biodiversity is valued, conserved, restored and wisely used, maintaining ecosystem services, sustaining a healthy planet and delivering benefits essential for all people". This framework will replace the Strategic Plan for Biodiversity 2011-2020 and will set the basis for shaping policies and guiding efforts and resources to halt and reverse biodiversity loss worldwide.

Like the Paris Agreement, with its clear statement on limiting carbon emissions, the Post-2020 GBF will establish a set of goals and targets intending to preserve and restore the diversity of life on Earth, with strong commitments of all governments, and is due to be adopted by the Conference of the Parties of the CBD, at its fifteenth meeting.

The updated Zero Draft of this framework proposed four outcome goals—three of those relating to different levels of ecological organization (ecosystems, species, and genetic diversity), and the other the means of implementation are available to achieve all goals and targets in the framework. To accomplish these four goals, the framework proposes 20 action targets and related indicators —an appropriate, potentially powerful and ambitious framework that needs a strong commitment at political level [30].

This chapter intends to provide a systematic overview on how conserving nature and biodiversity can contribute to improve the implementation of the One Health and other holistic approaches, to prevent new pandemics and to promote well-being.

2. THE ROLE OF THE POST-2020 GLOBAL BIODIVERSITY FRAMEWORK IN PROMOTING HUMAN HEALTH AND WELL-BEING

A detailed analysis regarding how the targets in the updated zero draft of the Post-2020 Global Biodiversity Framework can contribute to improve the implementation of the One Health or other holistic approaches, to prevent new pandemics and to promote well-being, was performed, aiming to support the ambition and commitment needed. Table 1 presents the results of this analysis and intends to describe with some detail how each target can influence human's health and well-being and which actions should be taken to promote health in the scope of each target.

Additionally, a list of specific indicators is proposed to guarantee that the proposed Post-2020 Global Biodiversity Framework monitoring framework adequately incorporate the value of biodiversity for health, well-being, and more specifically contributing to the reduction of the risk of new pandemics.

Targets	Influence on health	Actions to take	Possible indicators
(a) Reducing threats to biodiversity			
Target 1. By 2030, [50%] of **land and sea areas** globally are under **spatial planning** addressing land/sea use change, **retaining most of the existing intact and wilderness areas**, and allow to **restore** [%] of degraded freshwater, marine and terrestrial natural ecosystems, and connectivity among them.	- Degradation of ecosystems can lead to increased emerging disease risk [10]. - Pathogens of aquatic ecosystems can directly threaten biodiversity [10]. - Conservation is the key to sustainability and Protected Areas (PA) remains the core of global conservation strategies [31].	- Ensure ecosystem connectivity, which is critical for maintaining important ecological and evolutionary processes [32]. - Implement adequate land and sea spatial management planning, considering "One health" approach and the information provided by health surveillance reports for zoonosis [32]. - Promote the active restoration and remediation of degraded ecosystems [32]. - Conserve and protect the mainly intact ecosystems [33].	– Number of zoonoses outbreaks per year provided by health surveillance reports - Area (ha) of restored and remediated ecosystems - Degree of coverage and effectiveness of spatial planning measures to protect biodiversity (%)
Target 2. By 2030, protect and conserve through **well connected** and effective system of **protected areas** and **other effective area-based conservation measures** at least **30** per cent of the planet with the focus on **areas particularly important** for biodiversity.	- PA represent a fundamental tool in nature conservation policies. Some species rely on efficient PA management for their survival [10]. - PA are important to increment the global area free of harmful anthropogenic activities, reducing the wildlife-livestock-human contact interface and hence the risk of spillovers [10]. - Ensuring connectivity is essential for managing healthy ecosystems, conserving biodiversity, and adapting to climate change across all biomes and spatial scales. - Well-connected ecosystems support a diversity of ecological functions such as migration, hydrology, nutrient cycling, pollination, seed dispersal, food security, climate resilience and disease resistance [34].	- Ensure a network of, well planned and well managed, ecologically representative PA. - Assure by the Governments area-based conservation [32]. - Conserve protected areas to reduce the wildlife-livestock-human contact interface [10].	- Extent of protected areas and OECMs (ha) and their connectivity (%) - Results on management and effectiveness of PA - *Degree (%) of particularly important areas for biodiversity covered by PA.*

Targets	Influence on health	Actions to take	Possible indicators
Target 3. By 2030, ensure **active management** actions to enable **wild species of fauna and flora** recovery and conservation, and **reduce human-wildlife conflict** by [X%].	- Environmental changes causing biodiversity loss on a global scale are increasing the risk of spillover [10]. - Rapidly growing human populations are often on the frontline of disease emergence [10]. - Underlying drivers of almost all recent Emergent Infectious Diseases (EIDs) are anthropogenic environmental changes [10].	- Avoid wildlife contact/conflict [10].	- Number of zoonoses outbreaks per year provided by health surveillance reports - *Number of endangered species with effective management plans and recovery progress.* - Number of PA management plans effectively addressing and reporting human-wildlife conflict.
Target 4. By 2030, ensure that the **harvesting, trade and use** of **wild species of fauna and flora** is legal, at sustainable levels and safe.	- Farming, trade and unsustainable consumption of wildlife and wildlife derived products led to biodiversity loss and emerging diseases including COVID-19 [10]. - Trade in mammals and birds poses a high risk for disease emergence [10]. - Many plant species are threatened by unsustainable harvest for horticulture, food, or medicine.	- Implement rigorous enforcement of existing laws, regulations, and international treaties. - Limit or prohibit the trade of endangered species and highly probable pathogen host species. - Strengthen the sanitary and phytosanitary measures of the World Trade Organization [35]. - Develop One Health preparedness and effective response strategies. - Ensure that harvesting, trade and use of wild species of fauna and flora is legal, at sustainable levels, safeguarding indigenous peoples and local communities. - Modify previous calls for taxes, or levies on meat consumption or livestock production [10].	- Number of zoonoses outbreaks per year provided by health surveillance reports - *Proportion of traded wildlife that is legal and safe (not poached, illicitly trafficked or unsustainable)* - Proportion of harvested wildlife (both terrestrial and marine) which populations are within biologically sustainable levels
Target 5. By 2030, manage, and where possible control, pathways for the introduction of **invasive alien species,** achieving [50%] reduction in the rate of **new introductions**, ...	- Invasive alien species (IAS) have been recognized as one of the main causes for biodiversity loss globally [36].	- Increase awareness of people working in the fields of animal and public health [36].	- Rate of new introductions of alien species - *Rate of invasive alien species spread*

Targets	Influence on health	Actions to take	Possible indicators
... and control or **eradicate invasive** alien species to **eliminate** or reduce their impacts, including in at least [50%] of priority sites.	- IAS may promote pathogen pollution, leading to the emergence of diseases and potentially affecting the economy.	- Improve the understanding of the key epidemiological events and factors driving the emergence of infectious diseases [36].	- Risk level of introduction of invasive alien species.
	- Animal IAS should be the focus of intense study by epidemiologists due the risk for zoonotic pathogen emergence [36].	- Apply preventive and precautionary principles in addressing issues related to IAS [37]. - Implement flexible tools able to prioritize IAS [36]. - Promote public awareness and education on IAS. - Involve citizens in IAS control and eradication programs [36]. - Establish a monitoring framework capable of early detection of invasion and rapid intervention.	- Number of actions to control and/or eradicate IAS
Target 6. By 2030, reduce pollution from all sources, including reducing excess **nutrients** [by x%], **biocides** [by x%], **plastic waste** [by x%] to levels that are not harmful to **biodiversity** and **ecosystem functions** and **human health**.	- Pollution is one of the drivers of biodiversity loss and disease emergence [10]. - Pollution prevention leads to Environmental Sustainability [38]. - More than 90% of biodiversity loss come from resource extraction and processing of materials, fuels, and food. Exposure to harmful chemicals is a threat to human health and to biodiversity. Examples include negative effects on pollinators, insects, aquatic ecosystems, and bird populations [39].	- Promote education to avoid pollution (EcoSchools) [40]. - Promote companies' certification on ecosystems (Blue Flag) [41] and industries (ISO14001.2015) [42]. - Implement measures of prevention at source [35]. - Implement measures to comply with Circular Economy Action Plan. - Enhance circular economy to achieve a positive impact on the ecological systems, avoiding their depletion or overload. - Minimize the environmental footprint of chemicals on climate change, resource use, ecosystems and biodiversity from a lifecycle perspective [39].	- Evolution reports concerning waste and wastewater management - Evolution reports concerning water (covering biocides) and air quality - Evolution reports concerning marine litter, including plastic debris and micro plastics - Evolution reports on pesticides load - Number of establishments certified by EcoSchools, Blue Flag and ISO14001.2015

Targets	Influence on health	Actions to take	Possible indicators
Target 7. By 2030, increase contributions to **climate change mitigation, adaption and disaster risk reduction** from **nature-based solutions** and **ecosystem-based approaches**, ensuring resilience, and minimizing any negative impacts on biodiversity.	- Land-use change creates synergistic effects with climate change and biodiversity loss, leading to important emerging diseases [10]. - Biodiversity provides numerous ecosystem services necessary to human well-being. - Climate change can lead to the dominance of some parasitic species in crops that will threat food safety, as well as the ecosystems sustainability. - Climate change can promote invasion by alien species or changes in wild species dominance, as well as in their pathogens [39].	- Promote Climate action in the scope of European Green Deal [43]. - Promote synergies in the protection and restoration of ecosystems that contribute to reversing biodiversity loss and simultaneously mitigate climate change, either through biomass and soil carbon storage or in blue carbon.	-Actions on implemented NBS and EBA that contribute to adaptation and mitigation of CC. - Actions on implementation on NBS and EBA towards disaster risk reduction outcomes with co-benefits for biodiversity and human health and well-being. - % of public transport increase - % of decrease in fossil fuels use - % of increase in the level of buildings energetic certification - % of increase of sustainable farming and livestock activities – ISO 14001 – 2015 certification)
(b) Meeting people's needs through sustainable use and benefit-sharing			
Target 8. By 2030, ensure benefits, including **nutrition, food security**, **livelihoods**, **health,** and **well-being for people**, especially for the **most vulnerable** through sustainable management of **wild species of fauna and flora**.	- Human activities have substantially impacted ecosystems [44]. - Unsustainable trade and consumption of wildlife, agricultural intensification, globalized trade, and travel are driving the increasing spillover and spread of novel infectious diseases. - Biodiversity is necessary for food security, dietary health and to sustainable livelihood. - A proper diet is necessary to maintain micro-nutrient balance in the body [45]. - The unsustainable exploitation of marine ecosystems for food and energy resources could lead to disease emergence [10].	- Implement healthy diets by 2050 through substantial dietary shifts [45]. - Improve information and food marketing, investing in public health information and education, aiming the shift to a healthier and more sustainable diet [45]. - Increase the overlap between protected areas, endangered ecosystems and endangered species, aiming towards an effective protection of natural populations and habitats. - Reinforce local governance and land rights, focusing on helping the most vulnerable, including indigenous peoples and marginalized populations.	- Percentage of the population in sustainable traditional occupations, using wild resources for energy, food, or culture (including firewood collection, hunting, and fishing, gathering, medicinal use, craft making, etc) - Number of schools that implemented sustainable diet in the scope of the Eco-schools - Number of companies that implemented new food-based dietary guidelines

Targets	Influence on health	Actions to take	Possible indicators
Target 9. By 2030, support the productivity, sustainability, and resilience of biodiversity in **agricultural and other managed ecosystems** through **conservation and sustainable use** of such ecosystems, reducing **productivity gaps** by at least [50%].	- Landscape mosaics and ecological corridors are essential features for the promotion of biodiversity. - The requirement by many species for multiple habitats suggests that their conservation will be most effective in a mosaic environment [46]. - Management regimes that result in homogenization of habitats should be avoided [46].	- Reinforce efforts to drive sustainable agricultural and fisheries practices. - Improve the provisioning of ecosystem services. - Develop efforts to protect the soil and its biodiversity. - Create an agricultural revolution based and driven by sustainability and system innovation [45].	- *Proportion of agricultural area under productive and sustainable agriculture* - % of increase of more sustainable farming and livestock activities – ISO 14001 – 2015 certification - Reports on conservation and sustainable use of marine productivity - Number of sustainably managed fisheries
Target 10. By 2030, ensure that **nature-based solutions** and **ecosystem approach** contribute to regulation of **air quality**, **hazards and extreme events** and **quality and quantity of water** for at least [XXX million] people.	- Besides causing biodiversity loss, environmental changes are also driving the increasing spillover of viral diseases or other public health threat arising from zoonotic diseases [10]. - The same human activities that are destabilizing the Earth's climate also contribute directly to poor health [47]. - Nature based solutions (NbS) are essential to protect, sustainably manage, and restore natural and modified ecosystems (landscapes, seascapes, and cities) providing environmental, social, and economic benefits and help build resilience. - NbS support the delivery of a range of ecosystem services providing human health and well-being.	- Effectively implement the European Directives on the protection of groundwater and on Drinking Water. - Effectively implement the Thematic Strategy on Air Pollution, to improve air quality in Europe. - Effectively implement the Clean Air Programme for Europe. - Adopt nature-based solutions for energy supply, transport, and food systems, as far as possible. - Switch to low-carbon energy sources.	- Analysis of costs and effectiveness of NbS implemented and/or in place - Population affected by respiratory and cardiovascular diseases (burden of disease) - Data evolution of air pollution - Data evolution on waste and wastewater production and management - Data evolution on water supply, quality and underground water availability - Reports on clean energy sources (wind, waves, ...) - Number of companies certified by ISO 14001:2015
Target 11. By 2030, increase benefits from biodiversity and **green/blue spaces** for human health and well-being, including the proportion of **people with access** to such spaces by at least [100%], especially for **urban dwellers**.	- Green/blue spaces benefit nature and provide services regarding people health: mitigate urban heat island effect, absorb CO_2 and NO_2, increase air quality, absorb noise, help prevent the effects of drought, secure soil, increase water retention, reduce flood risk, improve water quality, and may be used to food production [48].	- Implement more green/blue spaces in urban areas to avoid contact with wildlife in non-urban environment [10]. - Promote Green and Blue areas through Blue Flag certification [41], Green Flag for parks and green spaces [51] and ISO 14001:2015 for companies [42].	- Proportion of total urban area corresponding to green/blue spaces for public use or benefit. - Area (m^2) of green spaces per citizen (for cities with more than X thousand people) - Maximum distance between green spaces

Targets	Influence on health	Actions to take	Possible indicators
Target 11. (cont.)	- Proximity to green and blue spaces is good for our physical and mental health as it reduces our stress levels and provides space for outdoor activity [49]. - Exposure to lakes, rivers and the sea was found to have a positive impact on mental health and to promote physical activity [50].	- Promote and support investments in green and blue infrastructure and cooperation across borders through the European Territorial Cooperation.	- % of increase in green/ blue spaces (referenced to a baseline) - Companies/spaces certified by Blue Flag, Green Flag, ISO 14001: 2015 - Evolution reports on Europe Green capital Award
Target 12. By 2030, increase by [X] benefits shared for the **conservation and sustainable use of biodiversity** through ensuring access to and the **fair and equitable sharing of benefits** arising from utilization of **genetic resources** and associated **traditional knowledge.**	- Most of the world's genetic resources are located in low and middle-income countries. - Indigenous peoples and other communities in low and middle-income countries have an advanced knowledge on how to use local genetic resources for agricultural or medicinal purposes. - Access to genetic resources should only be allowed after the consent of their providers, who should also obtain a share of the benefits. - Traditional knowledge systems highlight the importance of equitable "access and benefit sharing" (ABS) [10].	- Allow the access to traditional knowledge associated with genetic resources. - Share benefits from the use of those genetic resources and knowledge. - Implement measures for providers of genetic resources and knowledge: PIC (Prior Informed Consent) and MAT (Mutually Agreed Terms). - Strengthen the work between the European networks for genetic resources and biodiversity networks [20].	- *Number of users that have shared benefits from the utilization of genetic resources and/or traditional knowledge associated with genetic resources with the providers of the resources and/or knowledge.*
(c) Tools and solutions for implementation and mainstreaming			
Target 13. By 2030, integrate biodiversity values into **policies, regulations, planning, development processes**, **poverty reduction strategies** and **accounts** at all levels, ensuring that **biodiversity values are mainstreamed across all sectors** and **integrated** into **assessments of environmental impacts**.	- Human activities are increasingly disturbing both the structure and functions of ecosystems and altering native biodiversity. - Ecosystem disturbances can affect the risk of acquiring infectious diseases directly or they can do so indirectly through their impact on the biodiversity of infectious agents, hosts, reservoirs, and vectors. - Exposure to harmful chemicals is a threat to human health and to biodiversity.	- Implement cross-sectoral knowledge development and knowledge co-production. - Increase awareness of biodiversity values for people working in the fields of animal and public health [36]. - Promote the implementation of One Health Approach - Implement strategies complying with the concept of "Biodiversity in all politics", ensuring gains in all sectors.	- Number of references to biodiversity in the national health plans - Financial impact of environment-related diseases - Evolution reports on waste and wastewater production and management . - % of harmful chemicals banned from consumer products (ECHA and SAICM reports available and regularly published)

Targets	Influence on health	Actions to take	Possible indicators
	- About 40% of cancer occurrences in the EU are preventable. Prevention is also the most cost-efficient long-term cancer control strategy. E**xposure to pollution, carcinogenic substances,** and radiation, as well as infectious agents, are risk factors for cancer [52]. - Some of the environmental triggers (pollution) for cancer are also drivers for biodiversity loss. The reduction or elimination of these triggers will simultaneously contribute for the prevention of cancer and to reduce biodiversity loss.	- Promote education and awareness-raising about the conservation of biodiversity. - Monitor and evaluate the biodiversity mainstreaming in nutrition and health sectors [53]. - Drive change through knowledge and research to better understand **cancer risk factors,** as well as improving diagnoses, therapies, treatments, and **prevention policies** [52]. - Create new eco-friendly jobs, support educational opportunities, and train people in new skills. - Identifying synergies and trade-offs of biodiversity with other environmental issues such as climate mitigation and adaptation policies, air pollution, freshwater, and marine environment. - Assess the benefits to health and well-being that can be delivered by healthy ecosystems, and conversely identifying emerging risks and health impacts linked to ecosystems degradation (cost of inaction).	- Report the positive impact on health and well-being by maintaining healthy ecosystems. - Report the negative impact on health due to ecosystems degradation
Target 14. By 2030, achieve **reduction** of at least [50%] in negative impacts on **biodiversity by ensuring production practices** and **supply chains are sustainable**.	- The food supply industry is tightly linked to raw materials consumption derived from living systems. - Considerable quantities of these resources marketed every day, are linked with Habitat fragmentation; Land use change; Water scarcity; Climate change; Overexploitation of fishery resources; Deforestation; Pollution and waste production [54]. - These factors impact negatively on Biodiversity and should be top priority issues for Biodiversity protection.	- Support businesses solutions to biodiversity challenges related to their activities [54]. - Integrate the Biodiversity issue into sustainable business strategies. - Increase sustainable business strategies, through Companies' certification on ISO14001:2015 [42] with increased Biodiversity protection goals.	- Agriculture sector reports regarding sustainable production practices - % of increase of more sustainable farming and livestock activities – ISO 14001 – 2015 certification - Trends in food losses and waste reduction-reports on diet at schools in the scope of the Eco-schools

Targets	Influence on health	Actions to take	Possible indicators
Target 15. By 2030, **eliminate unsustainable consumption patterns**, ensuring that **people everywhere understand** and **appreciate** the **value of biodiversity**, and thus make **responsible choices** commensurate with 2050 biodiversity vision, considering individual and national cultural and socioeconomic conditions.	- Europe consumes more resources than most other regions; More consumption implies more water scarcity. - Current consumption leads to unsustainable waste levels. - Intensive animal production (e.g. mink, poultry, pigs, camels) can also trigger the closer contact with humans and potentiate spillover [55]. - Antimicrobial resistance (AMR) threatens the effective prevention and treatment of an ever-increasing range of infections caused by bacteria, parasites, viruses, and fungi [56].	- Implement programmes to support national and regional initiatives to accelerate the shift towards sustainable consumption and production [57]. - Increase public awareness of zoonotic diseases and of pandemic prevention and control strategies [55]. - Implement better monitoring and better knowledge on which viruses are circulating [33]. - Accelerate our transition to a sustainable food system [58]. - Review of the EU school scheme legal framework with a view to refocus the scheme on healthy and sustainable food.	- Health surveillance reports regarding zoonoses and microbial resistance profile; - Eco-schools reports on diet and food loss -Reports on shifting to healthier and more sustainable diets through National Food Consumption surveys. - Reports on ISO14001:2015 implementation regarding the goals in the scope of sustainability and biodiversity protection
Target 16. By 2030, establish and implement measures to prevent, manage or control **potential adverse impacts** of **biotechnology** on **biodiversity** and **human health reducing these impacts** by [X].	- Application of biotechnology to improve crop plants, medicinal plants, livestock, and microbes and to get new products from various biological systems is a fast-growing sector [59]. - There are adverse impacts of Biotechnology on Biodiversity, such as the introduction of genetically modified organisms (GMO) into natural ecosystems [60]. Adverse impacts on biodiversity through the introduction of GMOS may also result from disturbance of the dynamic population equilibrium of ecosystems [59]. - Another direct impact of biotechnology could be episodic genetic erosion, which could threaten the genetic diversity on which this technology depends [59].	- Implement sustainable strategy to provide food security for a growing population promoting biodiversity conservation and avoid further habitat loss in natural ecosystems. - Apply agricultural biotechnology tools, implementing bioprospecting activities by establishing partnerships with public and private sector institutions in industrial and developing countries [60]. - Ensure the implementation of regulation that promotes the adequate management of risks associated with Living Modified Organisms (LMOs) resulting from biotechnology which are likely to have impacts on biodiversity and human health [61].	- Extent to which necessary legal, administrative, technical, and other biosafety measures are in place to prevent, manage and control potential adverse impacts of biotechnology on biodiversity

Targets	Influence on health	Actions to take	Possible indicators
	- Awareness of the rapid expansion of modern biotechnology and the growing public concern over its potential adverse effects on biological diversity, taking also into account risks to human health. - Recognizing that modern biotechnology has great potential for human well-being if developed and used with adequate safety measures for the environment and human health. - Other potentially significant risks were identified, such as [62]: • transfer of genetic material to wild populations, leading to a loss of biodiversity; • diffusion of toxic effects on other organisms; • invasive effects on native species; • introduction of new diseases by replacing the population of the original disease vector with another [2]; • negative economic effects on small-scale farmers, challenged by the production of synthetic alternatives to their natural products; • privatisation of nature and restricted access for public benefit; • changes in people's perception of nature and biodiversity (e.g. focus on commercial value and profit potential); • policymakers, scientists, and industry being distracted from addressing the deeper underlying causes of biodiversity loss, including potentially less commitment to protecting endangered species (if extinct species can be restored synthetically).		

Targets	Influence on health	Actions to take	Possible indicators
Target 17. By 2030, redirect, repurpose, reform, or eliminate **incentives harmful for biodiversity,** including [X] reduction in the most harmful subsidies, ensuring that incentives, including public and private economic and regulatory incentives, are either **positive or neutral** for biodiversity.	- Price control and subsidies in agriculture, urban development, water provision, transport, energy and forestry can distort the costs of the use of biological resources [63]. - Another source of biodiversity loss can be unawareness of the ecosystems function and structure, and lack of hard data to demonstrate their importance [63].	– Implement positive incentives: monetary or non-monetary inducements which encourage or motivate governments, organisations, and individuals to safeguard biological diversity. – Implement disincentives: mechanisms that internalise the costs of use of and/ or damage to biological resources to discourage activities that deplete it. - Implement indirect incentives: trading mechanisms and other institutional arrangements that create or improve upon markets and price signals for biological resources, encouraging the conservation and sustainable use of biological diversity. - Eliminate perverse incentives: incentives which induce behaviour that reduce biodiversity.	– Biodiversity-relevant taxes, charges, and fees on payments for ecosystem services and on biodiversity relevant tradable implemented - Reports on ISO14001:2015 implementation regarding the goals in the scope of sustainability and biodiversity protection
Target 18. By 2030, **increase** by [X%] **financial resources** from all **international** and **domestic sources**, through new, additional, and effective financial resources commensurate with the ambition of the goals and targets of the framework and implement the **strategy for capacity-building** and **technology transfer** and **scientific cooperation** to meet the needs for implementing the post-2020 global biodiversity framework.	- Environmental changes that drive biodiversity loss also drive disease emergence [19]. - Changes in the incidence of infectious diseases are known to accompany changes in biodiversity and ecosystem disturbances, such as deforestation, dam and irrigation systems, and agricultural development.	- Implement incentive measures that can be directed at three main target groups [63]: 1) people whose behaviour enhances biodiversity related goods and services; 2) people who benefit from biodiversity-related goods and services and who attach value to biodiversity; 3) those whose behaviour diminishes or harms biodiversity- related goods and services affecting both groups 1) and 2).	- Health surveillance reports regarding zoonoses - Reports on ISO14001:2015 implementation regarding the goals in the scope of sustainability and biodiversity protection
Target 19: By 2030, ensure that **quality information**, including **traditional knowledge**, is **available to decision makers** and **public** for the effective management …	- Poor information on the importance of biodiversity often leads to bad choices in biodiversity protection and, consequently in the rising of pandemic risk.	- Establish education and awareness campaigns	- Reports on ISO14001:2015 implementation regarding the goals in the scope of sustainability and biodiversity protection

Targets	Influence on health	Actions to take	Possible indicators
... of biodiversity through **promoting awareness**, **education,** and **research**.		- Inform about the economic benefits of maintaining biodiversity and the costs of biodiversity loss. - Establish international monitoring programs on ecosystem disturbance and the resultant effects on human health.	- Eco-Schools and Blue Flag certification
Target 20. By 2030, ensure **equitable participation in decision-making related to biodiversity and ensure rights over relevant resources of indigenous peoples and local communities, women, and girls as well as youth**, in accordance with national circumstances.	- Traditional knowledge associated with genetic resources that comes from indigenous peoples and local communities (IPLCs) provides valuable information regarding the particular properties and value of these resources and their potential use for the development of new medicines [64]. - Benefits derived from the use of genetic resources may include the sharing of the results of research and development carried out on genetic resources, the transfer of technologies which make use of those resources, and participation in biotechnological research activities [64]. -Benefits may also be monetary when products based on genetic resources are commercialized [64].	- Promote research exchanges: a researcher from a provider country collaborates with research staff from the user country [64]. - Promote provision of equipment, improvement of infrastructure and sharing of technologies: the user of genetic resources sets up laboratories or a drug manufacturing facility in the provider country [64]. - Implement payment of royalties: royalties generated from the commercialization of a product based on genetic resources are shared between the provider and the user of genetic resources and associated traditional knowledge [64]. - Establish preferential access for the provider country to any medicine derived from genetic resources and associated traditional knowledge: preferential rates to purchase medicine [64].	- The percentage of surface lands legally controlled by the inhabitants through formal and native title.

Table 1 – Targets to support the ambition on Biodiversity and Health. Elements between square brackets are still under discussion, and indicators that are part of GBF monitoring framework are in italics.

3. CONCLUSIONS AND WAY FORWARD

This work highlights the importance of preventing biodiversity loss for human health and well-being. The linkages between biodiversity and human health reinforce the need of holistic approaches such as One Health to understand the intricate linkages between the health of plants, animals, humans, and our shared environment. The One Health approach and others, such as Planetary Health, can address the common drivers of biodiversity loss, disease risk and negative health outcomes by reducing the loss and degradation of biodiversity, enhancing human health and well-being, and preventing future pandemics. The identification and implementation of policies that will bring benefits to both health and sustainability should be a driver for the next years. We need to act now and promote the mainstream of biodiversity and health linkages into national policies, strategies, programmes, and accounts. This will imply ambition, commitment, and dedication.

ACKNOWLEDGMENTS

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CONFLICT OF INTEREST

None.

I have full control of all primary data and permission is given to the journal to review the data if requested.

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CHAPTER IV

HUMAN DISEASES

A

AGE-RELATED DISEASES: COMMON MECHANISMS AND THERAPEUTIC APPROACHES

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Paulo Pereira
Otília V. Vieira
Cláudia Guimas Almeida
Hugo Vicente Miranda
Helena L. A. Vieira
Miguel C. Seabra

INTRODUCTION

According to the World Health Organization of the United Nations (WHO), health is inseparable from wellbeing, both of which are essential elements for the existence of more prosperous and more just societies, therefore transversal to several of the UN Sustainable Development Goals for 2030. Disease presentation is changing, and traditional organ-centered, symptom-based approaches to diseases are no longer helpful. Health interventions will become increasingly personal and age-specific, and treatment should start in the earliest phase of life as possible. Simultaneously, new discoveries clarified that disease mechanisms are more complex than anticipated by traditional reductionist approaches. This complexity calls for integrated comprehensive and transdisciplinary approaches to guide our future endeavors in biomedical research. In particular, there is an urgent necessity of moving from one discovery in the laboratory to a clinical application (or otherwise a health solution) and back to the lab, to inform a new cycle of fundamental discoveries.

Another important emerging concept is the realization that shared mechanisms of disease are likely to underpin a plethora of apparently different age-related diseases. Although these are multifactorial diseases, an important drive of current biomedical research in the field is the identification of common mechanisms in these diseases, which can be described as cellular ageing. Cellular ageing is defined as the progressive decline in the resistance and adaptation to stress and other cellular damages, leading to a gradual loss of cellular functions, eventually resulting in cell death (Figure 1). Age represents the most important and common risk factor of chronic degenerative diseases of the developed world, including age-related macular degeneration (AMD), Alzheimer's disease (AD), Parkinson's disease (PD), atherosclerosis, and stroke. Importantly, atherosclerosis and hypertension associated with stroke represent the 1st single most frequent cause of mortality in Portugal (and 2nd worldwide) in people older than 60 years-old. With the increase in lifespan during the last decades and consequently the increased percentage of elderly population, especially in the developed countries, age-related diseases now represent perhaps the biggest challenge for public health systems worldwide.

At CEDOC-Nova Medical School, several research groups are working towards understanding critical biological processes that underpin the most common age-related diseases attempting to identify common biological mechanisms and therefore attempt to discover novel therapeutic approaches for various chronic age-related diseases such as AMD, AD, PD, atherosclerosis, and stroke. These common

mechanisms are cellular stress, age-related dysregulation of proteostasis, and lysosomal dysfunction, and are highlighted here. Our biomedical research is fully aligned with UN Sustainable Development Goal #3 Good Health and Well-being and the Cluster Health of the Horizon Europe Pillar 2, Global Challenges and Industrial Competitiveness. It is also aligned with the national strategy for research and innovation, specifically with the Agenda for Health, Clinical Research, and Translation.

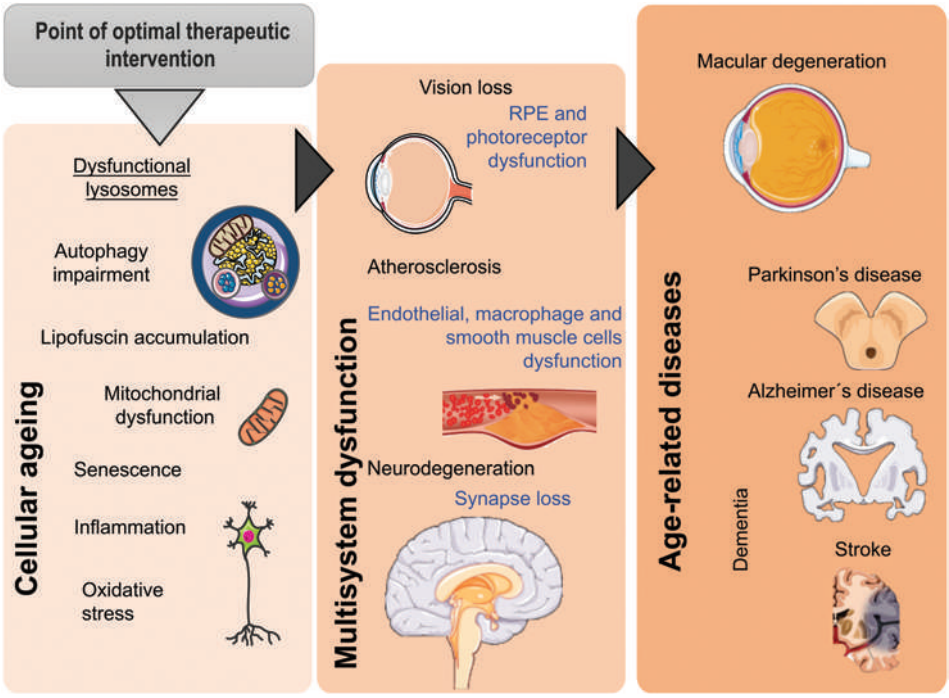

Figure 1 – Ageing events starting at cellular level and evolving to multimorbidities at the organ level. Cellular dysfunction causes a series of cascade events which result in tissue function loss or impairment, which accumulate with age resulting in organ failure and disease. To treat these types of diseases, the optimal point of therapeutic intervention is at the cellular level.

STATE OF THE ART REVIEW

Cellular ageing encompasses mechanisms shared by age-related diseases with higher prevalence worldwide involving neurodegeneration, such as AD and PD, vision loss as is the case of AMD, atherosclerosis, and stroke. In 2013, a seminal

review paper summarized the common mechanisms associated with cellular ageing [1] in nine distinct processes, namely: genomic instability, telomere attrition, epigenetic alterations, loss of proteostasis, deregulated nutrient sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion, and altered intercellular communication.

Among the hallmarks of cellular ageing is loss of proteostasis. Proteostasis can be broadly defined as the cellular ability to maintain protein homeostasis or a functional proteome. Research on proteostasis has traditionally focused on the age, stress, and genetic impact on protein synthesis, posttranslational modifications, folding, transport, and degradation. Several reports show that the proteostasis network declines with age in most, if not all, animals, leading to the accumulation of damaged and misfolded proteins, loss of molecular chaperones, decreased proteasome activity, and lysosomal dysfunction. Multicellular organisms, however, consist of different cell types that are structurally and functionally diverse. Cell specialization impacts the ability of tissues and organs to cope differently with a decline in proteostasis networks. For example, post-mitotic cells such as neurons, photoreceptors, and the retinal pigment epithelium cannot dilute proteotoxic material by asymmetric cell division, thus suggesting that alternative mechanisms may have evolved to alleviate proteotoxic stress [2-4]. Consistently, post-mitotic cells are often more resilient to stress than anticipated by the simple estimation of their individual proteostasis network strength.

During Ageing, lysosomes, responsible for the degradation of endocytic cargo, build up lipofuscin, the hallmark of cellular ageing composed of undegradable cellular waste [5]. In the ageing brain, neurons are particularly susceptible to lysosomal defects since they endure a lifespan in a post-mitotic differentiated state, facing a high cellular demand to maintain a life-long cognitive capacity [5]. Although present in every cell type, genetic mutations in lysosomal enzymes and regulators of lysosomal function cause neurodegenerative diseases. Significantly, one-third of the genes associated with late-onset AD are involved the endolysosomal trafficking [6, 7]. Strong genetic evidence connects lysosomal and proteasome impairment to PD, with more than half of the genes associated with the familial forms involved in autophagy-lysosomal and proteasome pathways [8]. Both AD and PD pathogenesis are associated with misfolding and aggregation of several proteins in brain-specific regions due to loss of proteostasis. A common feature underlying these diseases is lysosomal dysfunction, typically characterized by increased lysosomal pH and accumulation of unprocessed cargo within these

organelles. Finally, dysfunction of the lysosomal compartment has a central role in the etiology and pathogenesis of Atherosclerosis. The disease is initiated by uncontrolled uptake of modified lipoproteins by arterial phagocytic cells, namely macrophages and vascular smooth muscle cells to form lipid-laden, so-called "foam cells", whose accumulation and death culminates in the formation of atheromata. Lysosomal dysfunction, a characteristic of "foam cells", and consequent inefficient degradation of oxidized low-density lipoproteins (oxLDL) and apoptotic cells in atherosclerotic lesions have numerous deleterious consequences for cellular homeostasis and disease progression [9, 10].

RELEVANT CONTRIBUTIONS FROM NOVA

Retinal Degeneration. In the visual system, lysosomal dysfunction and loss of proteostasis have been proposed by Seabra Lab and Pereira Lab as key mechanisms underlying AMD and Choroideremia (CHM) [11-14]. For more than 20 years, the Seabra Lab has been studying mechanisms of retinal degeneration such as those occurring in the rare X-linked retinal degenerative disease CHM, leading to an entire body of fundamental and pre-clinical work, including pathogenesis studies, creation of complex conditional KO mice, viral gene therapy development and proof-of-concept studies for the disease. This work culminated in a phase I/II pioneering trial that showed gene therapy's effectiveness in the retina in humans [15, 16]. Moreover, it resulted in the spin-off of a UK-based company, Nightstar Therapeutics (recently purchased by Biogen after a successful listing on NASDAQ). CHM studies also impacted the AMD knowledge field as several features are shared between both diseases, with the emerging idea that in CHM, there is accelerated ageing resembling what is observed in AMD over the years. This motivated Seabra Lab to shift its focus towards AMD, covering from basic to clinical aspects.

The Pereira Lab is working on proteostasis and proteolytic signaling and how these processes are affected by ageing and disease. For example, the team have identified a new pathway for the degradation of the transcription factor HIF-1alpha [17] and how this might impact cell ability to respond to hypoxia conditions, which is a main feature of many ageing microenvironments. More recently, they focused on the role of extracellular vesicles in maintaining intercellular communication and how these vesicles can be engineered to deliver drugs or other active biomolecules with increased efficiency and decreased tissue toxicity [18]. They have also

shown that cell stress impairs intracellular proteostasis and induces secretion of exosomes loaded with proteotoxic material [19], presumably as means to alleviate intracellular proteostasis networks (Figure 2). Their work on extracellular vesicles biogenesis and selective sorting of proteins into exosomes also led to a patent (PCT/IB2020/051341) describing a method to selectively load proteins into exosomes in living cells by developing improved exosomal targeting signal-peptides (ExoSignals). For example, these can be used in innovative therapeutic approaches by providing an alternative means to deliver currently undruggable proteins.

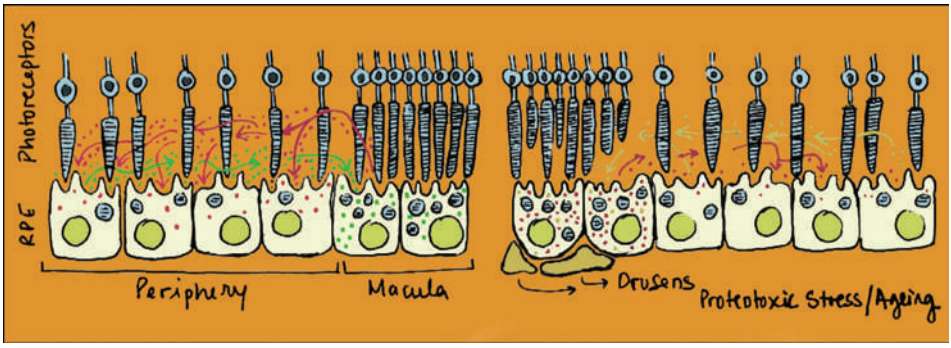

Figure 2 – Exosomes sustain transcellular regulation of proteostasis in the retina. Proposed model for transcellular regulation of proteostasis in the retina. RPE use exosomes to share and redistribute both proteotoxic material and chaperone machinery across the RPE monolayer so that cells with intact PN assist overburden RPE in the macula to cope with proteotoxic material either by uptaking exoxomes containing proteotoxic material (red arrows) or by releasing exosomes containing machinery that supports proteostasis (green arrows).

Neurodegenerative Diseases. The loss of synaptic function and the increased vulnerability of neurons to proteome stressors have been proposed by Cláudia G. Almeida and Hugo Vicente Miranda labs as early mechanisms underlying AD and PD pathology, respectively [20-29] (Figure 3). The Almeida lab investigates endolysosomal mechanisms of ageing and AD underlying synaptic decline in neurons with a focus on two late-onset AD GWAS genes, Bin1 and CD2AP, that are regulators of endosomal trafficking but with an unknown role in synapses. First, they discovered that Bin1 and CD2AP loss of function increases endogenous Aβ production. Bin1 through the endosomal sorting of BACE1, potentiating Aβ generation in axons and CD2AP via the endosomal sorting of the amyloid precursor protein for lysosomes in dendrites [24]. Multiple mechanisms intrinsic to ageing neurons likely drive synaptic

decline. The Almeida Lab found that APP endocytosis increases in aged neurons in vivo and in vitro, thus contributing to the Aβ accumulation by aged neurons since APP endocytosis is required for Aβ production [30]. Furthermore the Lab discovered that the age-associated Aβ is synaptotoxic and that if endocytosis alone is increased, it is sufficient to decrease synapses [30].

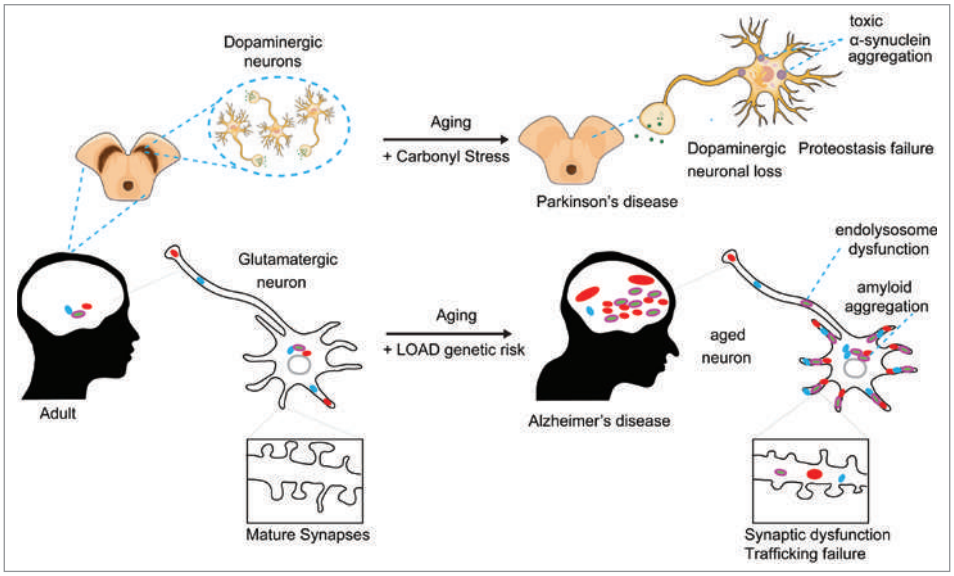

Figure 3 – Early mechanisms of neurodegenerative diseases. Working hypothesis for dopaminergic neuronal loss driven by alpha-synuclein aggregation triggered by ageing and carbonyl stress as a mechanisms of neurodegeneration under research in Vicente Miranda Lab; and for synapse dysfunction of glutamatergic neurons driven by endolysosome dysfunction and amyloid aggregation triggered by ageing and late-onset Alzheimer's disease (LOAD) genetic risk factors as a mechanism of neurodegeneration in Alzheimer's disease under research in the Almeida lab.

The Vicente Miranda Lab has been investigating the molecular mechanisms underlying PD. Provided the low incidence of familial PD, his lab has recently focused on the impact of given risk factors on PD development. In particular, they explored the association between type-II diabetes mellitus (T2D) and PD, which increases the risk of disease development up to 380%. Since hyperglycaemia is a major consequence of T2D, they explored the impact that carbonyl stress, via glycation (reaction between reducing sugars and biomolecules such as proteins), on alpha-synuclein and huntingtin. These proteins are the major components of the classical proteinaceous aggregates found in the brain of PD (Lewy bodies) and

Huntington's disease patients, respectively. The team showed that glycation triggers the accumulation of alpha-synuclein and huntingtin and their pathogenesis, inducing aggregation and cytotoxicity [26, 27, 29]. They also found that glycation promotes the failure of cellular protein clearance mechanisms such as the proteasome and the autophagy-lysosome system [26, 27] and the chaperone heat shock protein 27 (Hsp27) [29]. This carbonyl stress induces classical hallmarks of PD in animal models, such as the loss of dopaminergic neurons (mice) and the impairment of motor performance (flies) [26]. In contrast, the suppression of carbonyl stress [26] or the compensation of Hsp27 loss [29] prevent the proteostasis network's dysregulation and have therapeutic potential.

Atherosclerosis and Stroke. The Otilia Vieira (OV) Lab main focus is 1) Identification of new biomarkers in cardiovascular diseases (or improving the functional logic of the existing biomarkers), 2) Improving the understanding of the molecular and cellular pathogenesis of atherosclerosis, and eventually, 3) Identifying new drug targets. Within the framework of the first goal and, based on the data obtained by OV Lab, the team propose that the plasma lipidome profile is a powerful tool for atherosclerosis diagnosis [31] and the concentration of free cholesterol, cholesteryl esters, phosphatidylcholine, and lyso-phosphatidylcoline in blood plasma are a better measure for identification of the risk of cardiovascular disease than measurement of HDL-cholesterol [32]. Other results also indicate that the quantitative measurement of cholesteryl hemiesters, identified for the first time in plasma lipidomic analysis by OV Lab, could be an excellent indicator of atheromatal load and risk of cardiovascular disease [33]. Their recent studies also suggest that chlolesteryl hemiesters, may be responsible for lysosome dysfunction [33, 34].

The Helena Vieira (HV) Lab main interest is the study of the cellular mechanisms underlying stroke, including apoptosis, autophagy, metabolic shifts, and neuronal differentiation. The objective is to integrate all these cellular processes, focusing on mitochondria and redox signaling. Two different main approaches to protect the brain against stroke are followed based on (i) the cytoprotective function of carbon monoxide and (ii) remote ischemic conditioning.

(i) Novel carbon monoxide-based strategies against ischemic stroke: Carbon monoxide (CO) is an endogenous gasotransmitter produced by the activity of heme-oxygenase (HO), a stress response enzyme. CO is associated with the maintenance of homeostasis and cytoprotection in several tissues, including the brain. HV Lab and others have demonstrated that CO has anti-apoptotic properties in neurons

[35, 36] and in astrocytes [37-39], promotes neurogenesis [40-42], and reduces neuroinflammation in microglia [43]. Recently, HV Lab was a pioneer in establishing a new biological property of CO: modulation of cell metabolism, which is involved in preventing cell death [38] and control of neurogenesis [41, 42].

(ii) Remote ischemic conditioning in ischemic stroke – disclosing the underlying signalling mechanisms: (Pre)-conditioning is promoted by a stimulus that is usually hazard (as ischemia). Still, when applied below the damage threshold, it promotes cytoprotection by activation of endogenous mechanisms of defence. Remote ischemic conditioning (RIC) is the ischemic conditioning of non-vital organs (as arms) that protect other organs, like the brain. In experimental models, there is clear evidence of RIC protection in stroke. Nevertheless, in clinical settings, less clear evidence exists, and more clinical trials are needed. Furthermore, there is very little data about how inter-organ communication occurs. In fact, how short cycles of ischemia in the arm can protect the brain in humans is still unclear. Three mains hypotheses exist: circulating biochemical factors, immune cells, and activation of the autonomous nervous system. Thus, human interventional studies with healthy volunteers were undertaken to understand the autonomous nervous system's role [44].

IMPACTS TO SCIENCE AND SOCIETY

The traditional paradigm on approaching age-related diseases has been largely driven by observation, medical practice, and research, fragmented in many specialities and sub-specialities. However, in recent years, several mechanistic studies have challenged this paradigm, suggesting that ageing and age-related diseases share molecular players and signalling pathways. The impact of such observations is likely to lead to innovative therapies that may target mechanisms that are not disease-specific but instead shared between many age-related diseases. On the other hand, it is conceivable that age-related diseases result from unique combinations and particular flavours of the same basic players and pathways that regulate ageing. If this is the case, it is critical to understand which and when those combinations occur, lead to the disease, and identify biomarkers that distinguish between chronological and biological ageing. Furthermore, these common mechanisms may represent effective druggable targets, which, by preserving cell homeostasis, may lead to effective treatments aimed to delay or prevent the onset

of the debilitating chronic age-related diseases. The current efforts with societal impact are summarized below as we described our ongoing research.

ONGOING RESEARCH

The Seabra Lab current research focuses on fundamental, translational and clinical aspects of the AMD pathobiology. At the fundamental level, the focus is to get insights into lysosomal dysfunction in the retinal pigmented epithelium (RPE) cells, the primarily affected cells in AMD, on cellular ageing and lipofuscin accumulation, an essential feature of the disease. At translational/applied level, drug repurposing strategies are being explored based on solid and promising preliminary data. Finally, at a clinical level, studies are being conducted in collaboration with CHULC and CHLO to identify early biomarkers and prognostic markers for AMD, including developing new imageing analysis tools and using artificial intelligence, aiming at overcoming limitations faced by ophthalmologists in clinical practice.

The Pereira Lab uses the retina as a privileged model to address the hypothesis that dysregulation of proteostasis is a prominent feature of many age-related diseases, including AMD. In this model, cells in tissues and organs exchange both proteotoxic material and components of the proteostasis networks, with the ultimate goal of maintaining tissue homeostasis. This strengthening of proteostasis networks via extracellular vesicles may provide unique opportunities to tackle the age-related diseases that affect pos-mitotic tissues associated with an age-dependent disruption of proteostasis such as age-related macular degeneration. The Pereira Lab is addressing the hypothesis that extracellular vesicles (such as exosomes) are critical players transcellular regulation of proteostasis. By using animal models of tissue regeneration, we are also addressing the hypothesis that extracellular vesicles can carry HIF-1alpha and modulate hypoxia signaling in various conditions, including wound healing, which is also perturbed upon ageing and is the main feature of many ageing microenvironments

The Almeida lab is collaborating with the Brito lab at ITQB/iBET to setup human neurospheroids edited with LOAD patients mutations in Bin1 to recapitulate AD pathology, hoping to translate in 3D the genotype into the AD phenotype and establish a preclinical model for drug screening and validation. In another project, the Almeida lab is collaborating with the Seabra lab to establish common mechanisms of lysosomal dysfunction with neuronal and RPE ageing, relevant for

synapse and photoreceptor function in AD and AMD. Finally, the Almeida lab is also developing sensitive methods of microscopy-based assays of AD cytopathology that can eventually be translated into novel diagnostic tools[10, 11].

Provided that mice under increased glycation present classical symptoms of PD, the Vicente Miranda Lab is investigating the functional impact of glycation in the brain. In collaboration with Patrícia Gomes-Alves (ITQB, NOVA University), they are performing a proteomics study that allows to identify the dysregulated cellular functions at brain region level, and to identify druggable protein targets. The lab also takes advantage of their drug screening platform to test the therapeutic efficacy of compounds in improving cell proteostasis networks and in preventing alpha-synuclein toxicity and aggregation. The potential of anti-diabetic compounds in suppressing carbonyl stress and in alleviating alpha-synuclein pathogenicity was already tested, and their therapeutic potential in suppressing neuronal loss and in alleviating or preventing behavioural changes will be analysed in their developed animal mouse model. Altogether, these studies may unveil novel therapeutic approaches for PD that also hold potential for the treatment of Huntington's and AD.

The OV Lab, together with medical doctors from Hospital Santa Maria, is trying to assess and understand how cholesteryl hemiesters could be used as indicators of atherosclerosis and risk of cardiovascular diseases. Furthermore, OV Lab is currently addressing the role of cholesteryl hemiesters in the etiology and in the pathogenesis of atherosclerosis. Specifically, the Lab is devoted to study the molecular mechanisms behind lysosome dysfunction and how this event impacts inflammasome activation/inflammaging and cell senescence. With this fundamental research the Lab aims to pinpoint new druggable targets to prevent, delay or treat atherosclerosis.

The HV Lab is now very interested in disclosing the signaling factors generated by ischemic remote conditioning in the arm that will promote neuroprotection. Based on human volunteers and sample collection, we have ongoing work that aims (i) at exploring changes in circulating immune cells and (ii) the identification of biochemical factors in the plasma by proteomic, gasometry, and metabolomics, along with functional validation of conditioned human plasma in experimental models. Additionally, HV Lab works to understand how glial cells support and protect neuronal function in response to stress. Moreover, how CO improves glia-to-neurons communication is another critical scientific question addressed in ongoing projects concerning CO modulation (i) of neuroinflammation and microglial phagocytosis, and (ii) microglial and astrocytic metabolism, with a particular focus on mitochondria function.

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B

FROM A CLASSICAL TO A NEW^{NOVA} PERSPECTIVE ON CARDIOMETABOLIC DISEASES

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INTRODUCTION

Cardiovascular diseases represent a great part of the human burden of disease due to their high prevalence, high mortality, associated incapacity, poor mental health and well-being and economic costs to health services and society as a whole. It is now known that these conditions are frequently associated to a set of metabolic conditions as is the case of dysglycemic states (pre-diabetes and diabetes) and dyslipidemia, obesity and hypertension and all these concur in a vicious and perpetual cycle that is still difficult to address in its full dimension.

The scientific knowledge coming from epidemiological, fundamental, clinical and translational research has provided different physiopathological explanations that have evolved from a classical perspective of parallel, almost independent mechanisms to a merged, unified set of different factors. The different balance and weight of these factors will produce different phenotypic expressions that will also lead to different patterns of diseases including the severity level, evolution to complications and response to pharmacological and non-pharmacological interventions. The pandemic of cardiometabolic diseases is growing and there is a need to know which alternative and new explanations may exist (1).

The improvement in health outcomes is associated to the need of a collaborative effort of clinicians, researchers, health systems, governments, nongovernmental organizations, community-based organizations and the public (2).

In this chapter we intend to demonstrate on how this philosophy was translated into different research lines and teams centered at CEDOC – the Center for the Study of Chronic Diseases at

Nova Medical School, in a strong collaboration with a large network of research centers, hospitals, patient associations and community.

THE SITUATIONAL DIAGNOSIS

The economic burden of the cardiometabolic diseases has to be measured considering direct medical costs and the associated reduced productivity, being also important to calculate the cost of the undiagnosed cases – the cost of delaying a diagnosis.

The economic burden of pre-diabetes and diabetes in the USA in 2007 reached $218 billions, almost $700 annually for each American with or without diabetes (3). The global burden of diabetes has been continuously increasing. In

2017, the global incidence, prevalence, death and disability-adjusted life years (DALYs) were 22.9 million, 476 million, 1.37 million and 67.9 million respectively. These numbers will be expected to grow to 26.6 million, 570.9 million, 1.59 million and 79.3 million in 2025. The behavioral associated factors and BMI were the main contributors to attributable death and DALYs (4).

To have a better forecast of next years' situation it is also important to analyze the pediatric situation. In 1975, the prevalence of obesity in children was below 2% in 8 European countries for girls and in 5 for boys and in 2015 the estimated proportion of obese girls was 8,9% and 10% for boys in high income countries (5). This problem is particularly aggravated in developing countries where the overnutrition and associated obesity and diabetes is still joined by undernutrition and its consequences – the "double burden of malnutrition" (6).

The calculus for assessing the economic burden of obesity are more difficult since they have to include also other associated diseases apart from diabetes, cardiovascular diseases and hypertension, as is the case of certain cancers, respiratory diseases, skeletal-muscle disorders, mental disorders and digestive diseases among many others (7) giving disparate results. OECD has calculated that 8,4% of the health budget of OECD countries will be spent to treat the consequences of overweight and obesity in the next 30 years and that all these chronic non-transmissible diseases will reduce the life-expectancy in these countries in 2.7 years (8).

But still cardiovascular diseases remain the leading cause of disease burden in the world with an expected number of 523 million people living with them in 2019 also causing 18.6 million deaths and 182 million DALYs for ischemic heart disease with a known need to intervene in modifiable risk factors: high blood pressure, high fasting plasma glucose, high low-density lipoprotein cholesterol, impaired kidney function, ambient and household air pollution, tobacco, dietary risks and low physical activity (9).

A specific cluster of several metabolic factors (elevated waist circumference, elevated triglycerides, reduced HDL-cholesterol, elevated blood pressure and elevated fasting plasma glucose) have been named metabolic syndrome and this is associated to increase risk to develop cardiovascular disease (relative risk 1.65-1.93) and diabetes (relative risk 3-6). While the reasons behind the different proportions that each factor contributes to the individual risk are not known, this concept was important to demonstrate the importance of multiple targets for preventive interventions realizing that the outcomes are also dependent of biological risk factors, adverse social conditions and unhealthy lifestyle (10). This definition has also risen the relevance for the heterogeneity concept of risk which has also to take into

account the different regions of the globe where clearly different patterns of the association of risk factors (age, smoking status, systolic blood pressure, history of diabetes, total cholesterol) led to different survival estimates of cardiovascular death – the same set of factors may be associated to a 11% cardiovascular risk in Latin America and 30% in central Asia (11). A profile analysis of different risk factors of the population of NHANES 2001-2002 has demonstrated different progression rates to diabetes (11% and 31% at 3 and 7 years) and cardiovascular events (4% at 10 years) with higher risks associated to the metabolic syndrome cohort demonstrating the amplifying effect of the association of these risk factors and the relevance of identifying subpopulations at increased risk and associated costs (12).

Another new research area in cardiometabolic diseases is on how to define and measure the patient perspective and aligning it with the concept of health value and the impact of interventions. Recently a set of patient related outcomes measures were introduced for diabetes (13) with the inclusion of measures of quality of life, depression and distress. Behavioral interventions were found as key factors for success on results with a stronghold on the communication setting (14,15) for cardiovascular patients and for diabetes (16) and also on a different perspective of the metabolic syndrome (17).

In our days a more advance and precise vision should move to take into account a deeper understanding of each of these variables, profiling different needs for each patient and incorporating them in a full therapeutic strategy.

RELEVANT CONTRIBUTIONS FROM NOVA AND ONGOING RESEARCH

The relevance of the cardiometabolic field at Campus Santana Medical School started at least 100 years ago. In the year of the insulin centennial discovery, it is of upmost importance to remember Dr Ernesto Roma who was a medical doctor that took his degree at Lisbon Medical School at the “new building” at Campo Santana campus. Roma went to Boston in 1922 for a medical residency at Peter Brigham Hospital and at Massachusetts General Hospital, visiting Joslin Clinic where he was introduced to a new treatment for diabetes – insulin. Back in Portugal in 1926 he founded APDP – the Portuguese Diabetes Association (the oldest diabetes patient association in the world). Roma recognized that insulin treatment changed the paradigm of the doctor-patient relationship with a new role for the healthcare providers – the educator. He created the first integrated care outpatient clinic for

people with diabetes with a stronghold component on Education, integrating also the associative role with the training center for healthcare providers and a research center that maintained a continuous growth up to now and internationally recognized as still an innovative model of organization (18).

In the late '90s of the last century, Maria Paula Macedo implemented the new area of metabolism at the NOVA medical school. Professors Antonio Rendas and Pedro Costa facilitated the instalment of the Macedo Laboratory. With experience in the cardiovascular field from her PhD, she became interested in understanding the mechanisms that could explain the high postmeal glucose excursions in individuals with prediabetes. At the time, the majority of what was known in the field was essentially related to the fasting glucose alterations when glucose levels are at a steady state. Importantly, not only the mechanisms to explain these high post-meal glucose excursions were barely known but also the diagnosis was and still is problematic in our days due to methodological limitations, resulting in a high predominance of undetected cases (19).This unchecked progressive rise in glucose excursions after a meal has been tightly associated with postprandial insulin resistance (20). The inability to adequately dispose of ingested nutrients is expected to lead to glucose intolerance and favors the development of disease if postprandial glucose clearance is not effective. The Macedo laboratory also latter denominated as the Metabolic Disorders Research Group (MEDIR) unveiled the dogmatic role of the liver in postprandial insulin sensitivity and its association with the vagus nerve. Consequently, they uncovered the behaviors of metabolic pathways determined by hepatic parasympathetic function status, in physiology and pathophysiology. On this basis, they revealed that hepatic parasympathetic nerve tonus regulates insulin sensitivity at the periphery, mainly in skeletal muscle, through the action of a hepatic factor called hepatic insulin sensitizing substance (HISS)(21). Relevantly, hepatic parasympathetic nerve ablation induces insulin resistance at soleus and extensor digitorum longus skeletal muscles, heart, and kidney, but not at liver or adipose tissue (22) (Fig 1 A). This data support the hypothesis that skeletal-muscle postprandial glucose clearance depends on the integrity of the hepatic parasympathetic nerve (HPN) changing between 38 and 69% of whole-body glucose clearance (22).

The mechanisms through which the HPN promotes postprandial glucose clearance and disposal involve the release of acetylcholine and subsequent activation of muscarinic receptors [39] leading to the liver production of NO, shown to be critical for secretion of the hepatic insulin sensitizing substance (HISS) from the liver (23).

The other main factor to support the HPN-mediated increase in peripheral insulin sensitivity is Glutathione (GSH). The Macedo Lab further revealed that depletion of GSH decreases insulin sensitivity and causes impairment in insulin signaling. They disclosed that hepatic parasympathetic nerves/NO axis is also dependent on increased hepatic GSH levels (24,25). It was possible to restore postprandial insulin sensitivity by boosting NO and GSH levels. Interestingly, they observed that glucagon controls postprandial GSH levels, consequently the hyperglucagonemia observed in T2D can affect postprandial glucose clearance through this mechanism. Based on the fact that GSH regulates insulin sensitivity, they described that a meal rich in cysteine leads to an increase in postprandial insulin sensitivity (fig 1 A). Of note, HPN dysfunction is now associated with the insulin resistance observed in the cardiometabolic setting – obesity, hypertension and in ageing process.

The Macedo lab has now been working on the hypothesis that the above-described mechanism is fundamental for the breakdown of insulin into A and B insulin chains, which suffer S-nitrosylation to stabilize the insulin chains. Crucially, it was observed that B-nitrosylated insulin chain is as potent as insulin with the highest activity for glucose uptake at the skeletal muscle; in contrast, A chain has low activity in the skeletal muscle but very high at the adipose tissue. This mechanism is then capable of directing glucose towards the skeletal muscle or to the adipose tissue depending on the needs as insulin is pleiotropic and its effects cannot selectively distinguish one action from the other (Fig 1 A) (26).

Nitric oxide gained a leading role in the liver having pleiotropic effects not only on glucose homeostasis but also in the regulation of insulin bioavailability in the systemic circulation. Indeed, the MEDIR group unveiled that nitric oxide is a primary regulator of insulin clearance (IC) in the liver in humans (27). Of major relevance, the lab pinpointed that this regulatory effect is essential in physiological prandial regulation allowing that the first pass effect that insulin suffers in the liver just after being secreted by removing between 50-70% of the secreted insulin is mediated by nitric oxide. This is a result of the capacity of nitric oxide to regulate the insulin-degrading enzyme (IDE) activity, a major enzyme responsible for insulin degradation. Hypothalamic nitric oxide specifically at the ventromedial nucleus also regulates hepatic insulin clearance. On the other hand, in an inflammatory milieu, inflammatory nitric oxide synthase (iNOS) releases abnormal levels of nitric oxide and therefore persistently suppresses insulin clearance. This mechanism originates primary hyperinsulinemia, a feature of prediabetes, which we now know to drive insulin resistance, which is associated with cardiovascular disorders,

neurodegenerative progression and obesity. For understanding the IDE role in postprandial insulin clearance, Macedo lab uncovered IC dynamic processes, where key modulators of IC have quite distinct impacts in fasting or in the postprandial state. Thereafter they evaluated the role of Insulin-Degrading Enzyme on postprandial IC and of hepatic IDE dysfunction on successive mal-adaptive responses of prediabetes, such as hepatic steatosis. The genetic control of postprandial IC in a human cohort revealed in a robust genetic approach that IDE controls IC and regulates postprandial glucose excursion in humans as well as in mice. Strikingly, IC genetic control by IDE is prominent in men but less in women and is reduced under prediabetes and other metabolic disturbances such as hepatic steatosis (Fig 1 A) (28).

Other molecules are known to control insulin levels, as dipeptidyl peptidase 4 (DPP4). The MEDIR group disclosed that independently of insulin levels DPP4 controls glucose excursions by controlling GLP-1 glucose output in the liver, being this mechanism abrogated in the prediabetic state. DPP4 and triggering receptor expressed on myeloid cells 2 (TREM-2) were revealed by the Macedo lab to also be involved in liver fibrosis regression (29,30). The liver has a tremendous impact not only on glucose but also on lipid homeostasis. Lately, an atlas of organ interplay (liver, skeletal muscle, white and brown adipose tissue) was established by using metabolomics and lipidomics after exposure to dissimilar types of diets. Moreover, by evaluating paraoxonase-1 (PON1), known to play a role in lipid metabolism and homeostasis, it was perceived that reductions of PON1 activity led to a more profound dysglycemic state, both in mice and human.

Dyslipidemia is highly prevalent in individuals with prediabetes or type 2 diabetes. Knowing which lipids' patterns are characteristics of either the diabetes profile progression per se or related to specific diabetes-associated pathologies are central issues that need to be disclosed if we aim at a precision medicine approach. The entero-hepatic axis has acquired more relevance in the diabetes scenario. Recently the MEDIR lab has established the role of this axis by addressing the role of gut permeability in the glucose and lipid metabolic interplay. The other recent branch of research on this axis has been to understand if and how gut derived exosomes (GDE) are protagonists of liver dysmetabolism. Indeed, these GDE appear to significantly impact liver lipid metabolism (Fig 1 A). Taken all these research lines the lab has been challenging the current concept of type 2 diabetes based on glycemia recognizing that its heterogeneity can only be captured through metabolic milieu, mechanisms and etiology profiling, a novel perspective that we denominated as metabolic footprint (Fig 1 B) (31,32).

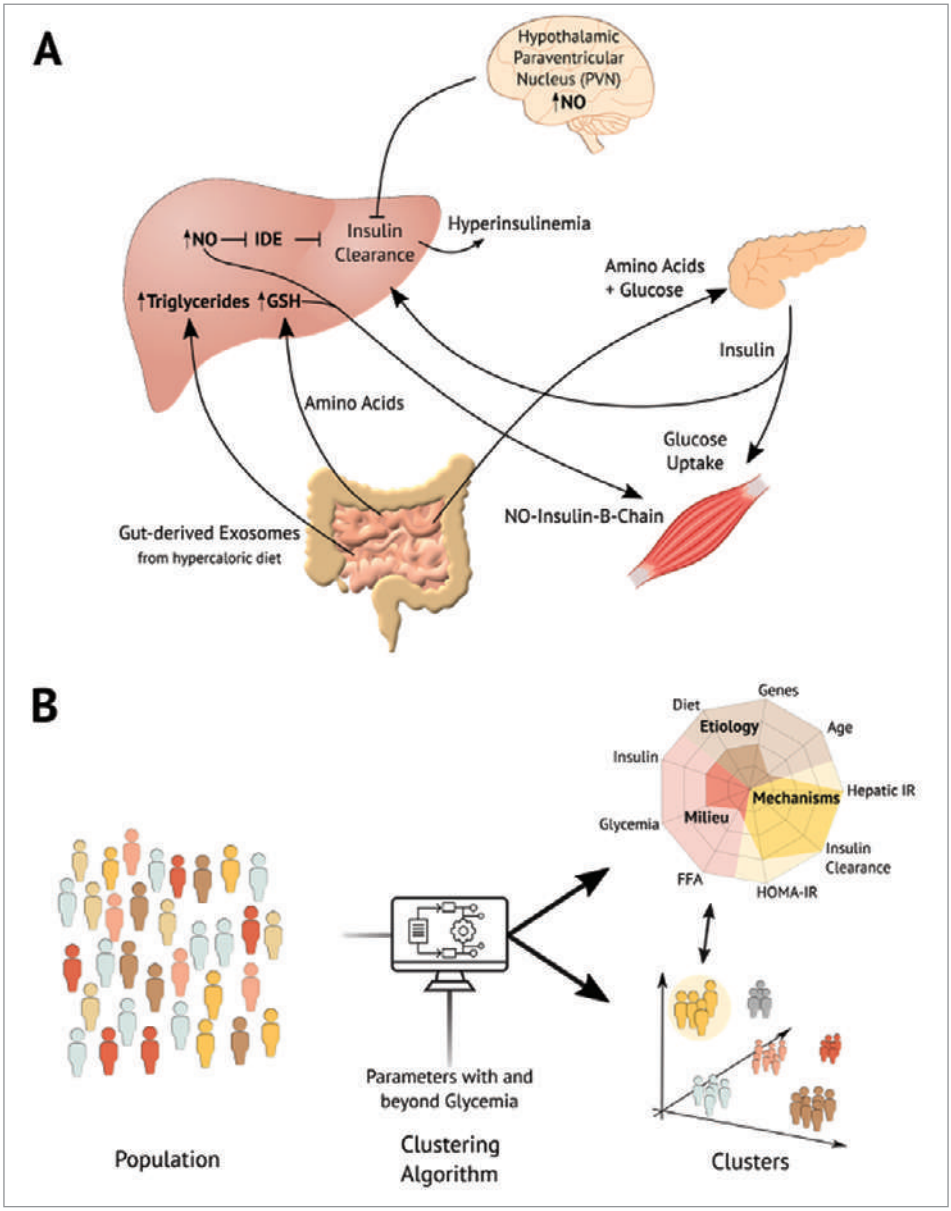

Figure 1 – The postprandial livercentric hypothesis interplays the relationship of organs and how the liver acts as a maestro for the regulation of plasma glucose levels and of insulin sensitivity. The Macedo laboratory unveil several of the mechanisms that are reflected in this fig A. The gut responds to a meal by realizing hormones and exosomes and by activating the vagus to the hypothalamus. Thereafter, efferent vagus nerve signaling can be triggered by sensing metabolic alterations in the brainstem and the hypothalamus

regulating hepatic glucose production glycogen synthesis, insulin clearance as well as pancreatic endocrine function (insulin). Vagal activation to the liver in the postprandial state allows hormonal communications from the liver to skeletal muscle, heart, and kidney to increment glucose clearance by these organs. This mechanism is proposed to be dependent on the hepatic produced NO-insulin-B-Chain as a consequence of part of the insulin that is uptake by the liver. The liver also plays a major role in insulin clearance by regulating the first pass effect and thereafter the levels of the hormone that reaches the periphery. This mechanism is regulated genetically through the insulin degrading enzyme and by nitric oxide in physiology and pathophysiology. B) Depicts the basis of Type 2 Diabetes heterogeneity dependent on metabolic milieu, mechanisms and aetiologia profiling, a novel perspective that we denominated as metabolic footprint.

All this work was only possible with the work of a full team that contributed to different moments of the lab which names are patent in the manuscripts referenced and in several PhD thesis as well as on the contribution of young and senior researchers. Other scientific advancements are not described herein as they are collaborative works with groups in different parts of the world.

From a historical perspective, all the clinical studies achieved in this lab were performed at the APDP-Diabetes Portugal. The strong scientific relationship of the NOVA Medical School and APDP was allowed by both past and present presidents of APDP, Luis Gardete Correia and José Manuel Boavida, as well as the clinical director João Filipe Raposo presently and for a decade professor at the NOVA Medical School. Raposo also attains other relevant positions where he has been playing a role with impact in the medical school and society, as clinical director of APDP and as external consultant for the WHO besides presently being the president of the Portuguese Diabetes Society. His interests have been related since a long time to real world evidence on NCD management and design of personalised interventions. Unmet needs represented by lack of adequate control not only of glycaemic target values, but also lipids, frame a considerably high risk of cardiovascular disease (CVD) in the diabetes population. Although recent guidelines cover therapeutic goals, effective lipid management of patients with diabetes to reduce CVD risk is still largely unattained, both in type 1 and type 2 diabetes (33). João Raposo's team explore these aspects through large electronic health records (EHR), both for epidemiological and big data studies. These have simultaneously highlighted issues of data quality, processing and interpretation, especially considering the level of register of prior CVD episodes (34). The knowledge derived from these approaches is also translated into awareness actions, involving the awareness and education of patients and healthcare

professionals regarding diabetes co-morbidities which increase CVD risk, like fatty liver (35). Further, the group manages a living lab associated with APDP outpatient clinic, where it is able to integrate clinical data, provided by EHR, with new digital technologies to co-develop with diabetes patients and healthcare professionals a strategy to follow diabetes management and patient behaviour through digital biomarkers on an European Union's Horizon 2020 research and innovation programme (grant agreement 727409). This is currently being expanded through a grant from DGS, the Portuguese Directorate-General of Health.

Another research focus has being taken by José Silva Nunes who joined the NOVA Medical School recently. His group has been working in endocrine diseases in general with a particular emphasis in obesity and its related conditions. The team has been studying the genetic basis for the development of excessive fat mass accumulation (36,37) and the influence of several hormones on excessive adiposity itself (38) on carbohydrate dysmetabolism (39), on non-alcoholic fatty liver disease (40–42) and on blood pressure profile (43). With the expertise in obesity and type 2 diabetes management the group has been collaborating in several national (44–51) and international guidelines (52,53). Recently, the team is working in a project that studies determinants of type 2 diabetes remission in patients subjected to metabolic surgery.

Deepening the concept behind neurological metabolic control, Silvia Conde started her research work with Professor Emilia Monteiro. After her PhD in Spain, Silvia became interested in metabolism research and is now dedicated to exploring the pathophysiological mechanisms behind the development of metabolic diseases. The main interest is to investigate how alterations in the nervous system contribute to metabolic deregulation aiming the development of prevention strategies and treatment interventions that would help stem this epidemic. The group primary focus has been the carotid body (CB), a peripheral chemoreceptor, classically defined as a hypoxia sensor. They have discovered that the CB also senses insulinemia and leptin, key signaling hormones known to be involved in autonomic overactivation and dysmetabolism (54,55) and whose effect on the CB probably contributes to metabolic diseases. Additionally, they found that CB dysfunction contributes to metabolic diseases (Fig 2A), since CB activity is increased in prediabetes and type 2 diabetes (T2D) animal models (54,56–58) and prediabetes patients (59) (Fig 2A) and by the demonstration that the resection of the carotid sinus nerve (CSN), the CB sensitive nerve, prevents and reverses the pathological characteristics in prediabetes and T2D animal models (54,56,60) (Fig 2B).

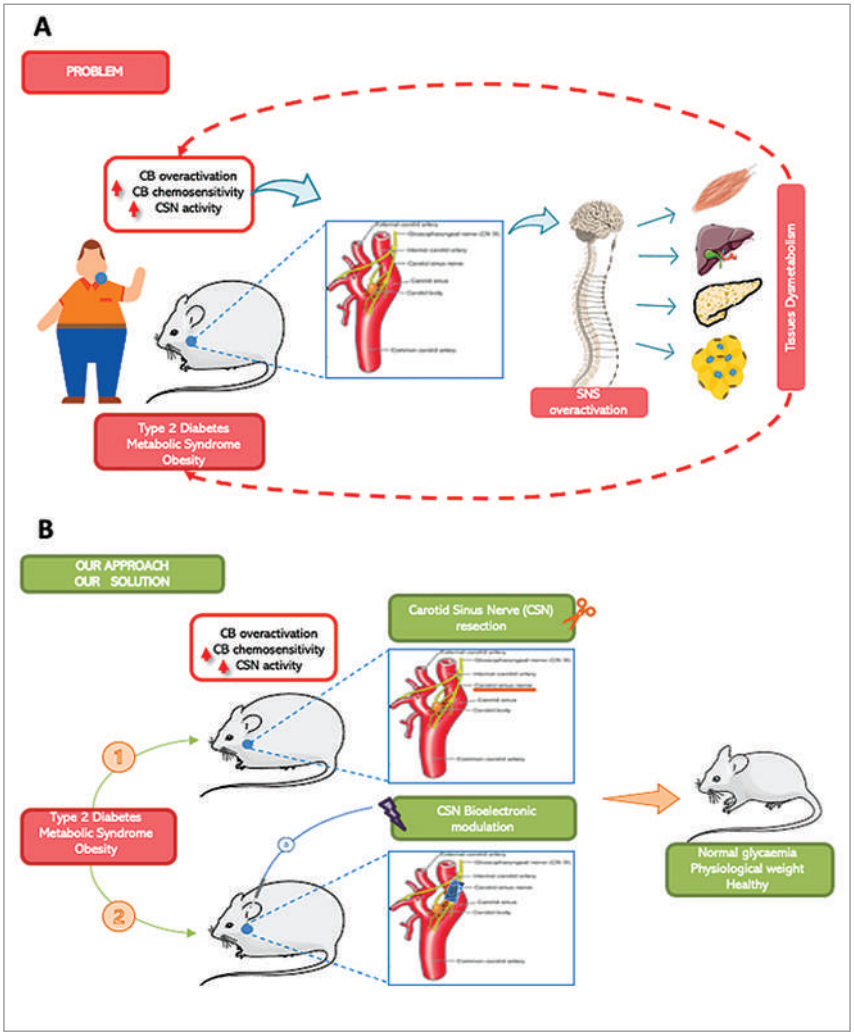

Figure 2 – Findings supporting the role of carotid body (CB) in the development of metabolic diseases (A) and the experimental methodologies used to prove the work hypothesis and as therapeutic approaches. A) Metabolic diseases both in animals and humans are characterized by increased CB activity. CB signals to the sympathetic nervous system (SNS) promoting a sustained SNS activation that leads to and aggravates metabolic dysfunction in insulin-sensitive tissues, as the liver, skeletal muscle, intestine, adipose tissue and the pancreas. This generates a vicious cycle CB-SNS-metabolic dysfunction leading to metabolic diseases as obesity, T2D and metabolic syndrome. B) Methodological approaches to improve dysmetabolic states: carotid sinus nerve (CSN) bilateral resection or CSN bioelectronic modulation reverted clinical T2D pathological features in rats.

In agreement with the pre-clinical data, the group have also showed that hyperbaric oxygen therapy (HOT), an intervention that dramatically reduces CB activity frequently used to promote wound healing in diabetic foot, improves glucose homeostasis in T2D patients (61). Moreover, and pointing towards the use of the modulation of CB activity for the treatment of metabolic diseases, Conde lab showed that the continuous blocking of the CSN activity, using kilohertz frequency alternating current, delivered through an electrode implanted on the CSN, reversed pathological clinical features of T2D in rats (Fig 1B) (56). Aiming to facilitate the emergence of this therapeutic to address the unmet medical needs for T2D, they are now mapping carotid body neural pathways, characterizing the CSN nerve fibers (62,63) and disclosing the molecular mechanisms involved in the etiology of insulin resistance and obesity.

Additionally, Conde's lab has a second line of research to investigate if the modulation of adenosine signaling or metabolism in insulin-sensitive tissues ameliorates glucose metabolism and tissue function in metabolic diseases (64,65). The mapping of neural pathways and the disclosure of the molecular mechanisms involved in T2D and related co-morbidities should reveal new targets for therapeutic intervention.

In the line of this translational research, the Translational Pharmacology Lab is one of the oldest laboratories of the NOVA Medical School. The lab is headed by Emilia Carreira Monteiro and Sofia Pereira. The group has been teaming up for tailoring pharmacological therapies to particularly subgroup of patients (phenotypes) – precision pharmacology – visualized in Figure 3, instead of a one-drug-fits-all model.

The pharmacology lab focus is the individual's metabolic phenotype that arises from the interaction between environmental factors, microbiota, lifestyle factors and particular (epi)genetic/physiological backgrounds. These phenotypes favor clusters of patients with different susceptibility to develop cardiometabolic diseases and variable response to drug therapy. These clusters reflect inter-person differences in deleterious vs. protective metabolites and, therefore, in pathways/pathophysiologic mechanisms, justifying variable symptoms and prognosis. The enzymatic activities responsible for metabolic phenotype (eg. NAT8, SULT, CYP1A1, PON-1) also contribute for drug metabolism. Both factors amplify the interindividual variability in drug response that is a major issue in clinical practice and drug development. These premises are highly present in arterial hypertension (HTN), a burden where genetic background is inconclusive and is highly dependent on environmental influences.

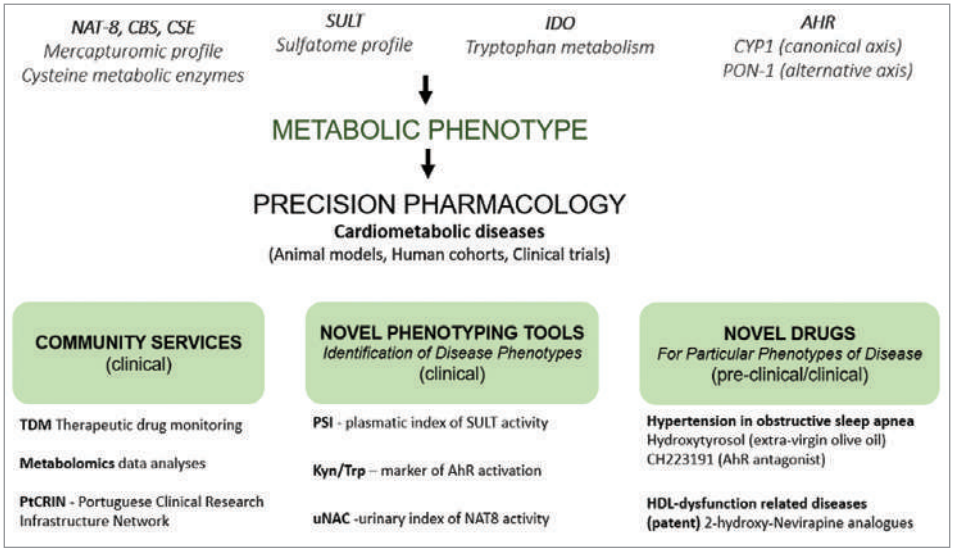

Figure 3

In their vision, the umbrella of "essential" HTN covers different mechanisms and vulnerabilities for HTN (phenotypes). In fact, secondary causes of HTN, as obstructive sleep apnea (OSA, a major cause of drug resistant HTN) while out of the tag "essential" HTN, faces similar challenges and no differential therapeutic management. From a translational perspective, perceived innovation in HTN aspires, above all, for phenotyping tools to dissect the "essential" concept and guide therapeutic decisions and preventive measures while discovering novel mechanisms of drug action for particular phenotypes of HTN.

To clustering patients according to enzymatic metabolic activity, the group has been researching for the development of phenotyping tools in a minimal invasive way through quantification of ratios of metabolites that allow patient stratification. An example is NAT8, a N-acetyltransferase that acetylate drugs and endogenous metabolites, that is genetically associated to HTN and regulation of renal function. High and low NAT8-metabolizers might be differentiated by an in-house identified urinary indicator – the uNAC, (66–68), developed in a collaborative study with the Nephrology Department of Hospital Prof. Doutor Fernando Fonseca. This indicator reflects kidney tubular function, whose contribution for cardiometabolic diseases is being increasingly recognized (67,69). We have also developed translational models for identification of metabolic phenotypes in drug-induced renal tubular injury (70,71). Other enzymes related to cysteine metabolism that are associated with HTN

and drug detoxification (CBS, CSE) are also being addressed in collaboration with Structural genomics lab of Instituto de Tecnologia Química e Biológica – ITQB, a work from Dalila Fernandes PhD project.

SULT1A1 is a sulfotransferase genetically associated to body weight control that promotes the sulfonation of drugs and endogenous metabolites. SULT1A1 display wide interindividual variability, explained only partially by genetic variation, suggesting that other non-genetic, epigenetic, and environmental influences could be major determinants of its variability. Studies of sulfonation in humans are lacking, although they are of key importance in assessing the functional consequences of individual variation (72). We developed using paracetamol as probe substrate, the paracetamol sulfonation index – PSI – a valuable new tool to investigate the clinical significance of variation of SULT1A1 activity in a collaborative study with Hospital da Luz (73). The first non-randomized clinical trial to phenotype SULT1A1 activity in healthy volunteers was concluded (NCT03182595) and another clinical trial (EudraCT 2019-002266-12) is ongoing to study interindividual variation in a larger sample, including subjects with HTN and on medication, part of Natália Marto PhD project. The team work in drug metabolism also allowed them to identify a metabolite that recently was patented for diseases related to high density lipoproteins dysfunction (patent Nº WO/2019/229565), a hallmark of metabolic endothelial dysfunction and arterial diseases (75), as demonstrated by Aline Marinho PhD thesis.

The team has also a focus on HTN related to obstructive sleep apnea (OSA) as this is a relevant cause for resistant HTN (76–79). OSA is a highly prevalent disease associated to head and neck anatomical variables, that limits an etiological treatment, where HTN is concomitant with other cardiometabolic comorbidities (obesity, kidney and liver disease, dyslipidaemia, insulin resistance and cancer) that might also contribute for resistant HTN. Inspired by the association of pollution, microbiota, lifestyle factors and individual susceptibility to develop HTN the group was pioneer in linking the aryl-hydrocarbon receptor (AhR, ligand activated transcription factor) to OSA-HTN by proposing the existence of an AhR-related phenotype of HTN that may contribute to explain why some persons are more sensible to environmental factors than others and/or why the response to anti-hypertensive drugs is so variable among HTN patients (80). Using a rat model of OSA-HTN resistant to ß-blockers (77,81), two AhR-related compounds with putative anti-hypertensive efficacy for OSA-HTN were identified: CH223191 (82,83), in a collaborative project with Université Paris-Descartes) and hydroxytyrosol, a work project of Maria João Correia PhD thesis in a collaborative project with Food Functionality and Bioactives Laboratory, ITQB,

that may deal, not only with the complexity of OSA-HTN, but also with other OSA comorbidities. Currently the group has an ongoing clinical study (NCT04646902) with participation of five Hospitals in Lisbon area (CHLO, CHULC, Hospital da Luz, HFF & HBA) to measure IDO/TDO activity, which metabolizes tryptophan and generates AhR agonists, for an approximation to the AhR-related phenotype of HTN.

Additionally, the team has been running services to the community: they coordinate the Portuguese National Roadmap infrastructure for clinical research PtCRIN – Portuguese Clinical Research Infrastructure Network, host the PtCRIN European Portuguese correspondent, Joana Batuca (postdoc) and run a Therapeutic Drug Monitoring unit for dose individualization and optimization of drug response. Currently a new unit dedicated to metabolomic clinical data analyses, part of Judit Morello's postdoc, was set.

With the implementation of CEDOC, new researchers arrived and expanded the previous groups of research. In the Cardiometabolic field, Gabriela Silva and José Belo enriched the group.

Gabriela A. Silva is now addressing the role of gene therapy for diabetic retinopathy.

Diabetic Retinopathy (DR) is one of the main complications associated with the progression of Diabetes Mellitus (DM), and its prevalence has been increasing in recent years(84). DR is the main cause of vision loss in working-age individuals worldwide, being directly related to the duration of diabetes. In the first years after the diagnosis of type 1 DM the prevalence of RD is very low; however, 20 years after the disease, most patients with diabetes end up developing some form of DR (85,86). Individuals with type 2 DM may be affected with DR at the same time that they are diagnosed with DM, due to the number of years they have lived with undiagnosed DM. 60% of patients with type 2 DM for over 20 years develop some form of DR (85,86). Thus, this disease is associated with a significant socio-economic impact.

DR is a disease with a progressive evolution that, in earlier stages, is characterized by the appearance of acellular capillaries and microaneurysms, as well as the breakdown of the hemato-retinal barrier (87,88). All of these processes are known to be associated with inflammatory responses, resulting from chronic hyperglycemia in the retina caused by DM (89). In a more advanced stage of the disease, macular edema can occur, that is, thickening of the retina in the macular area, which can cause visual loss, and in more advanced stages, proliferative DR may involve neovascularization, hemorrhagic new blood vessels, detachment of the retina and neovascular glaucoma(90).

It is known that Vascular Endothelial Growth Factor (VEGF), the main regulator of angiogenesis, is deregulated in DR (91) and hence a primary therapeutic target. To date, anti-VEGF therapy is common, but with the need of repeated intraocular administrations. In addition, about 30% of patients do not respond to therapy (92), and thus there is an urgent need to find other therapeutic targets (93). In recent years, other molecules have emerged as associated with the development and progression of DR, namely growth factors, cytokines and pro-inflammatory chemokines (91). One of the molecules is Placental Growth Factor (PlGF) which acts synergistically with VEGF (94) to activate the angiogenesis signaling pathway. PlGF is increased in the vitreous of patients with non-proliferative (RDNP) and proliferative (RDP) diabetic retinopathy, but its expression is not detected in healthy control individuals (95). An endogenous factor – the Pigment Epithelium Derived Factor (PEDF) – with multiple functions in retinal homeostasis, has been found in low levels in the vitreous of patients with DR (96,97).

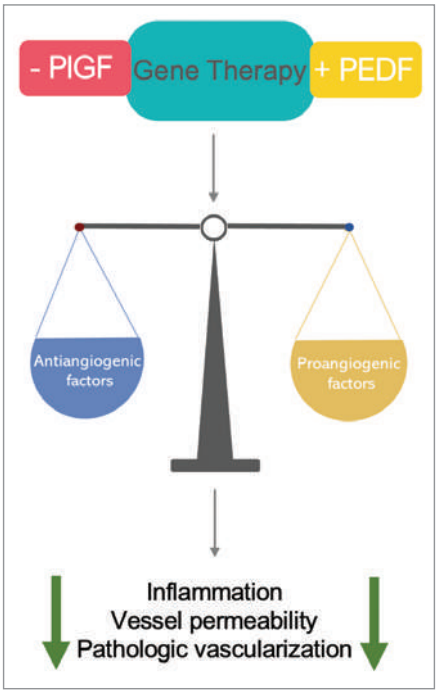

Figure 4 – Gene therapy strategy for the treatment of diabetic retinopathy. This strategy is based on the restoration of the balance of trophic factors, which are highly dysregulated in diabetic retinopathy. With this dual gene therapy approach a marked decrease in inflammation, vascular leakage and pathological neovascularization.

Hence, the group strategy was to restore the imbalance between pro- and anti-angiogenic factors, by using gene therapy to reduce the expression of pro-angiogenic PlGF and increase the expression of anti-angiogenic PEDF (98) (Fig.4). They have tested this approach in a mouse model of DR that has previously shown to reflect this imbalance between pro- and anti-angiogenic factors in the retina(99). By restoring the balance between PlGF and PEDF using a dual gene therapy approach, it was possible to significantly reduce retinal inflammation, neovascularization and hemorrhages in an animal model of diabetic retinopathy (100).

This innovative approach has the potential to be a long-lasting therapeutic alternative to frequently administered anti-VEGF therapies, and also a much-needed therapy for non-responders.

In the cardiovascular field José António Belo was the first senior principal investigator to join the recent formed CEDOC and he is devoted to investigating heart development and diseases. Coronary heart disease (CHD) may lead to heart failure, as a consequence of cardiomyocyte (CM) death or dysfunction due to Myocardial Infarct (MI) that constitutes the leading cause of morbidity and mortality in developed countries(101). Unfortunately, the adult human heart does not self-regenerate, and current therapies for heart failure are limited (e.g., heart transplantation) and non-curative (e.g., damage control (102,103). An enticing more permanent solution is the design of therapies for heart (coronary vessels and CM) regeneration or reconstitution. From all proposed strategies and approaches, cell replacement is the most intuitive and probably the most effective for the remuscularization and revascularization of fibrotic tissue associated with an injury. For this end, the detailed knowledge of the developmental signaling pathways governing heart development/cardiogenesis is paramount. The Lab of José António Belo (Belo Lab) at CEDOC/NMS is actively pursuing this strategy.

Heart development is a complex process in which the primitive heart tube is formed from cardiogenic mesoderm of the cardiac crescents, i.e., first heart field (FHF), while anterior and venous poles are derived from a subsequent subset of cardiogenic cells located medial to the cardiac crescents, dubbed second heart field (SHF) (104). Then, the developing heart will receive cellular contribution from extra-cardiac sources. On the one hand, cardiac neural crest will colonize the most anterior parts of the heart playing a pivotal role on aortic-pulmonary septation (105). On the other hand, cells originating from the proepicardium (PE) will cover the developing heart forming the epicardium, which will infiltrate into the myocardial space leading to distinct cellular subpopulations, such as endothelial (ECs) and smooth muscle

cells (SMC) forming the coronary vasculature, endocardial cushion mesenchyme, cardiac fibroblasts, and of course the adult epicardial lining (106). Several studies have shown that besides the contribution from the proepicardium-derived endothelial cells (ECs), the remaining coronary vessels are derived from the sinus venosus (SV) and the ventricular endocardium. Regardless of all those different origins, VEGF/VEGFC signaling has been indicated as the main player in this angiogenic process.

In the course of a differential screen for cardiogenic genes (107), the Belo Lab isolated and initially characterized the novel gene CCBE1. In mouse and in chick, expression of Ccbe1 in heart forming regions co-localizes with the first heart field (FHF) and second heart field (SHF) progenitors and also in the proepicardium (108,109). In addition, defective heart tube formation is observed in knockdown of Ccbe1 in chicken (109), thus supporting a role of this gene in cardiogenesis. Moreover, we demonstrated that CCBE1 is required for cardiac mesoderm (Mesp1+) and multipotent cardiovascular progenitors (Isl1+) production during differentiation of mouse and human ESCs (110).

CCBE1 has been shown to be required for the maturation of VEGFC leading to activation of VEGFR3. Novel expression domains for Ccbe1 in the SV, epicardium, and proepicardium have been uncovered by the Belo Lab (111) at the stage when VEGFC-dependent coronary vessels start to form (E11.5) (112). On the other hand, and in a similar fashion as it has been described in VEGFC mutants, we have observed that the full CCBE1 KO leads to underdeveloped coronary vessels with a significant accumulation of immature of VEGFC pro-peptide (Fig.5) (111). These data strongly indicate that CCBE1 is required for the proper sprouting of the developing coronary vasculature through enhancing of VEGFC signaling.

During embryogenesis, the correct development of the anatomic asymmetries on the visceral organs, and in particular in cardiac development, relies on the intensity and duration of the asymmetric left-sided expression of Nodal-Lefty-Pitx2c and further genetic programs must be restricted in time and space (reviewed in (113)).

The group has isolated and for the first time described the role a novel gene that was denominated Cerberus-like 2 (Cerl2; now also designated DAND5). We defined Cerl2/DAND5 as an inhibitor of Nodal and demonstrated its role in the generation of asymmetries in the early vertebrate embryo (reviewed in (114)). In mice, Cerl2 controls Nodal signaling at the node and the transmission of the LR asymmetry information to the left-lateral plate mesoderm (LPM) in a precise time window (115) and this process is crucial for the correct positioning and anatomical development of the heart.

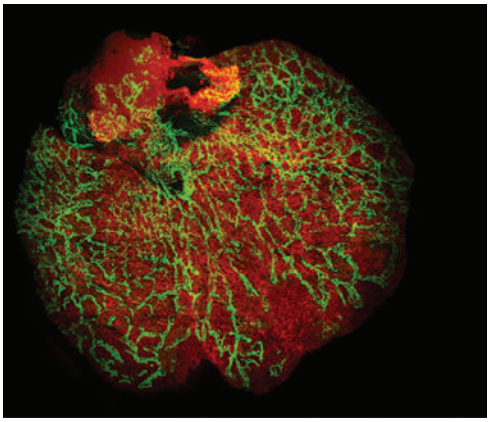

Figure 5 – The correct formation of the complex coronary vasculature network requires the activity of CCBE1. Research from the Belo Lab (Stem Cells and Development Lab, CEDOC/NMS) firstly identified the gene CCBE1 and demonstrated that its inactivation in mouse hearts or in Embryonic Stem cells, dramatically impairs the formation of coronary vessels. Confocal image of the coronary vessel network on an embryonic day 14.0 mouse heart immunostained for VE-cadherin (green, vessels) and Cardiac Troponin (Red, cardiomyocyte).

The absence of Cerl2/DAND5 results on a wide spectrum of malformations commonly known as heterotaxia, which comprises defects in either global organ position (e.g. situs inversus totalis), reversed orientation of at least one organ (e.g. situs ambiguus), and mirror images of usually asymmetric paired organs (e.g. left or right isomerisms of the lungs). Moreover, these laterality defects are frequently associated with Congenital Heart Diseases (e,g. transposition of the great arteries, or atrioventricular septal defects).

Cerl2/DAND5 is not involved in the looping process per se, only indirectly by influencing Nodal LPM pathway program; Cerl2, unlike Pitx2c, is not expressed in anterior Left-Lateral Plate Mesoderm (L-LPM) or in the primitive heart tube at these stages. However, from E10.5 to E13 its expression can be detected in the developing heart (116).

This later expression uncovered a role for Cerl2 during cardiac development that is independent of its function in the early events of LR asymmetry establishment (117). The cardiac malformations observed in Cerl2 null mice result from a combination of both disturbances of laterality and of disruption of the contribution from intrinsic cardiac lineages independent from laterality leading to increased ventricle wall thickness. 40% of Cerl2 KO mice die at birth due to a massive increase in heart

ventricular walls (117). This is caused by CM hyperplasia due to increased pSMAD2 (TGF-ß/Nodal) and nuclear β-catenin signaling. Also, differentiated Cerl2/DAND5 KO mESC presented a dramatic increase in the number of generated beating foci and up-regulation of early cardiac genes (Mesp-1, Nkx2.5, and Isl1) and mature cardiac marker α-MHC (118).

The Belo Lab has recently driven a translational project involving academia and hospital organizations for screening a cohort of human patients with congenital heart disease. A DAND5 variant (c.455G>A) was identified on these patients with LR defects and ventricular hypertrophy that leads to an increased ectopic Nodal signaling (119). From those patients, using the iPS cell technology we generated iPSCs lines (120). Furthermore, using CRISPR/Cas9 technology, we CRISPR-corrected isogenic and DAND5-KO cell lines (121,122). These iPS cell lines were further successfully differentiated to Cardiac Precursor Cells (CPC) and to Cardiomyocytes (CMs). We verified that the variant-derived and KO-derived CMs have a higher proliferation index at days 10 and 20 of CM differentiation protocol and up-regulation of the cardiac gene networks, as observed in mCerl2 KO ES cells (118). Our data indicates that reduced levels of DAND5 lead to increased cardiovascular progenitors' numbers and, in addition, extend their progenitor state for a longer period (Fig.6).

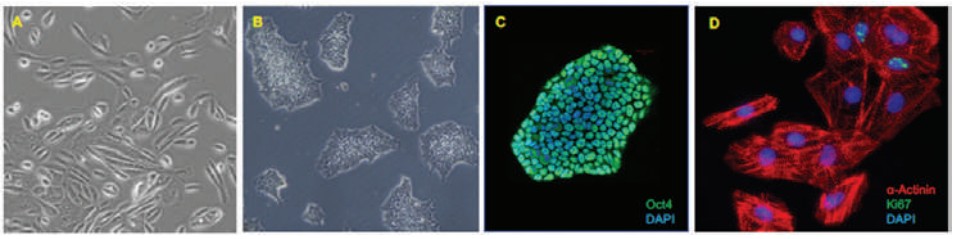

Figure 6 – Cardiovascular disease modeling using patient-specific DAND5 iPSC lines. A. Exfoliated renal epithelia (ERE) cells isolated from patient's urine (10X); B. Bright-field image of the DAND5 iPSC line colony morphology (5X), showing a typical human embryonic stem cell (hESC) colony-like morphology with a high nucleus/cytoplasm ratio (generated using the reprogramming factors SOX2, OCT3/4, c-MYC and KLF4 on the ERE cells); C.Immunodetection of the pluripotency marker OCT4 (green) on a DAND5 iPSC colony (10); D. DAND5 iPSCs-derived Cardiomyocytes. Immunofluorescence (63X) for α-Actinin (red, sarcomeric α-actin), Ki67 (green, mitosis). Nuclei were stained with DAPI. Research from the Belo Lab (Stem Cells and Development Lab, CEDOC/NMS).

Among the multiple signaling pathways that have been identified in cardiac development, particular attention has been given to TGF-β/Nodal and Wnt/β-catenin

signaling pathways, especially in the in vitro differentiation of hiPSC-CMs. TGF-β/Nodal cascade is involved in the molecular specification and formation of myocardial precursors, cell movement, proliferation, and in activities such as stress or heart injury response. On the other hand, the manipulation of Wnt/β-catenin signaling has been explored given its biphasic role during cardiogenesis: an early activation induces myocyte proliferation, but further CM differentiation and maturation requires the inhibition of Wnt/β-catenin.

The Belo Lab demonstrated that DAND5 is the only member of the Cerberus/DAN family of secreted antagonists of Nodal and Wnt signaling cascades with expression in the heart (114). Bearing in mind the role that was established for DAND5, both in development and in cardiogenesis using Pluripotent Stem Cells (PSCs), the group wants now to take advantage of these functional capabilities.

Using a combination of our unique-generated genetic tools, DAND5 KO mESCs; DAND5-KO/KD hiPSCs lines; DAND5 KO mice, the Belo Lab is performing studies of patient specific disease modeling to better understand cardiomyocyte differentiation, proliferation and maturation. Firstly, to decipher the different cellular and molecular mechanisms that promote CM maturation during heart development; and then unveil the molecular mechanisms responsible for the fading of proliferative capacity in the adult heart. The Belo Lab aims to understand the etiology and treatment of Cardio-Vascular Disease, from a Developmental Biology, Genetics and physiology perspective, with Regenerative Medicine purposes.

IMPACTS TO SCIENCE AND SOCIETY

The examples shown in this chapter clearly demonstrate the relevance of a translational and integrated approach to cardiometabolic diseases. We are all in a transition research phase evolving from a classical perspective of simple, linear disease mechanisms as exemplified in Fig.7A for diabetes and cardiovascular diseases, to a unified model with more complex interactions between different sets of individual factors and its relationship with environment resulting in different phenotypic expressions (Fig.7C).

This complexity is clearly demonstrated in the research lines here described with the proposed roles of the gut and liver for energy metabolism and the importance of the neuronal control in this field as well as in the unveiling mechanisms of heart formation and retinopathy development. These research lines are also

focused on therapeutic advances as is also the case of the precision pharmacology described and the translational studies on diabetes and obesity.

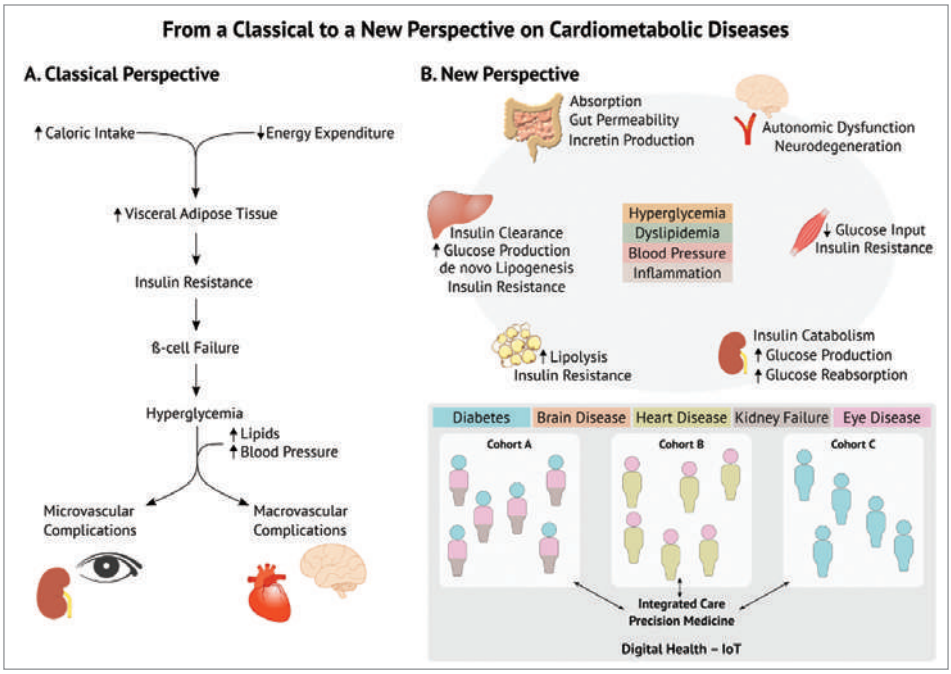

Figure 7 – Different perspectives on disease mechanisms' definition. Panel A – The classical approach in cardiometabolic diseases – a linear, sequential explanation of different factors leading to disease where global, disease-centered therapeutic recommendations are the cornerstone. Panel B – The new perspective highlighting precision medicine approach – A complex interaction of different factors contribute to different disease phenotypes and subsequent therapeutic responses. Complex models of data analysis will be implemented to support algorithms facilitating therapeutic decisions and follow-up control (digital health) – the person-centered approach.

The new[NOVA] model of healthcare advocates the personalized approach with the creation of an individualized plan of care that will include not only the results of this characterization but also the role of the social and environmental determinants in individual contexts.

All these processes will run simultaneously with strategies to increase the health literacy level of the individual and the population, and to promote people's engagement in health prevention and disease management. A significant decrease in cardiometabolic diseases and its consequences will happen when we'll master

socio-environmental interventions in parallel with actions at individual level. The chronicity of these diseases has to be leveraged with better results in health promotion and disease prevention so we will have more resources to adequately treat patients with these conditions.

The success of this model will be only achievable with an efficient and effective process of integrated care within the patient perspective with new intervenients apart from the classical roles of health care providers, working in new models of health organization, with the research targeted to the needs risen from patients and society, as demonstrated here. All these should be based in adequate information systems with the capacity to analyze data, and assist and support providers and patients to have their best decisions to achieve the best outcomes.

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C

MUSCULOSKELETAL DISEASES

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INTRODUCTION

According to World Health Organization (WHO), musculoskeletal conditions (MSK) comprise over 150 diseases and syndromes, which are usually progressive and associated with pain. They can broadly be categorized as joint diseases, physical disability, spinal disorders, and conditions resulting from trauma. Musculoskeletal conditions are leading causes of morbidity and disability, giving rise to enormous healthcare expenditures and loss of work (WHO, 2021).

The conditions with the greatest impact on society include Rheumatoid Arthritis (prevalence varies between 0.3% and 1%), osteoarthritis (9.6% of men and 18.0% of women aged over 60 years have symptoms), spinal disorders (low back pain is the most common and affects over 80% of persons at some point in their life) and severe limb trauma (requiring hospitalization in 50% of the time from falls, 15–20% from road traffic accidents, and about 20% from machinery and tool usage (WHO, 2021). The disease-specific prevalence rates of MSK in the adult portuguese population were: 26.4% low back pain, 15.8% periarticular disease, 12.4% knee osteoarthritis (OA), 10.2% osteoporosis (OP), 8.7% hand OA, 2.9% hip OA, 1.7% fibromyalgia (FM), 1.6% spondyloarthritis (SpA), 1.3% gout, 0.7% rheumatoid arthritis (RA), 0.1% systemic lupus erythematosus (SLE), and 0.1% rheumatic polymyalgia (Branco et al., 2016).

The musculoskeletal group of NOVA Medical School is based at Chronic Diseases Study Center (CEDOC), an established centre which aims the excellence in Biomedical, Translational and Clinical Research on chronic diseases. The teams are distributed through two research units- The Comprehensive Health Research Centre (CHRC), a multidisciplinary, multi-institutional and comprehensive new research centre aimed at supporting, developing and fostering clinical, public health and health services research and iNOVA4HEALTH, a translational medicine programme organizing the efforts of biomedical researchers involved in i) biological understanding of disease, lead compounds and biopharmaceuticals pre-discovery, ii) preclinical development, and iii) early clinical and first-in-man clinical trials (http://cedoc.unl.pt/clinical-translational/).

STATE OF THE ART REVIEW

In recent years, classification criteria and therapeutic guidelines have been reviewed for several MSK, namely for OA (Kolasinski et al., 2020; National Institute

for Health and Care Excellence, 2014); OP (Camacho et al., 2020; Kanis et al., 2019; Rodrigues et al., 2018a), FM (Arnold et al., 2019; Wolfe et al., 2016), Juvenile idiopathic arthritis (JIA) (Ringold et al., 2019), SpA (Branco et al., 2016; Van Der Heijde et al., 2017; Rudwaleit et al., 2009, 2011) and Sjögren syndrome (Shiboski et al., 2017).

In parallel, innovative methods such as -omic approaches (genomics, transcriptomics, proteomics, metabolomics, others) have been generated a huge amount of data. Their integration with clinical and epidemiological information from large databases (big data) and the development of information technology (artificial intelligence) allowed an increase of the physiopathology knowledge and the identification of new therapeutic targets. New therapies with different mechanisms of action are now available in the therapeutic armamentarium (Van Vollenhoven et al., 2018). This progress, translated to clinical practice, improves the diagnosis and the selection of the most effective therapeutic intervention. The main interest is the identification of preventive / therapeutic approaches that should be effective in preventing or inducing early and sustainable clinical remissions (Lever et al., 2020).

"The Global Burden of Diseases, Injuries, and Risk Factors Study 2016 (GBD 2016)" provides a comprehensive assessment of prevalence, incidence, and years lived with disability (YLDs) for 328 causes in 195 countries and territories from 1990 to 2016. Low back pain contributed with 57·6 million of total YLDs, occupying the first place before migraine, age-related and other hearing loss, iron-deficiency anaemia, and major depressive disorder (Vos et al., 2017).

Musculoskeletal diseases, as a whole, were the main cause of disability in Europe, accounting for about 30% of YLDs (EULAR, 2017; Vos et al., 2017). Studies carried out in Canada, USA and Western Europe, estimated the prevalence of physical problems caused by a musculoskeletal disease, in 4-5% of the adult population, increasing among women and in particular, in the elderly (Reynolds et al., 1992). This justified the urgent unmet need increase research in this field.

RELEVANT CONTRIBUTIONS FROM NOVA

Musculoskeletal Databases

Our team has been developing research work in different areas of rheumatology focused in the study of the epidemiology of rheumatic and musculoskeletal diseases (RMD). In an initiative promoted by Portuguese Society of Rheumatology (SPR), we developed Reuma.pt, the Portuguese Register of Rheumatic Diseases

(Canhão et al., 2011). This project was started in 2006, made accessible to rheumatology centers in 2008 and released online in 2012. With this register, rheumatologists across the country, collect over time, individual information in a systematic and comprehensive way about several rheumatic diseases, using dedicated and specific protocols. Patients have also access to a dedicate area to self-report patient outcomes. Reuma.pt is well recognized as a robust and very detailed registry, with more than 25 000 patients and more than 218 000 appointments included, contributing to leverage the quality of rheumatologic research with several international and national collaborations and peer- reviewed publications (Mourão et al., 2017; Romão et al., 2015).

EpiDoC team has also led the first national epidemiologic study of rheumatic diseases (EpiReumaPt) (Dias et al., 2018; Rodrigues et al., 2015). This study collected information of 10.661 dwelling adults living in Mainland, Azores and Madeira Islands, in a national representative sample, characterizing the prevalence of the major RMD, but also evaluating socio-economic and other clinical variables. A subsample of 3886 participants was observed in a medical appointment, with blood collection and imaging data. This study performed in 2011-2013 was the first (EpiDoC 1), of a series of national studies (EpiDoC 2 in 2013-2015 and EpiDoC 3 in 2015-2016), evaluating the same individuals, constituting the EpiDoC cohort. The EpiReumaPt was awarded by Grande Premio Bial de Medicina in 2016 (Branco et al., 2016).

After 10 years, we are developing EpiDoC 4, the fourth and last wave of EpiDoC 4. We plan to finish the interviews up to June 2021. This study will allow to have a ten-year perspective of clinical, social and economic data of a representative sample of the Portuguese population. During the COVID era we have 3 ongoing funded studies Fast track COVID and OSCAR by ANI-COMPETE and Serologies follow-up by Fundação Alvaro Carvalho, after characterizing SARS COV2 serology profile of NOVA community.

Funded projects using new ICTs and AI are FrailCareAI and PrevOccupAI by FCT, Healthy bone by Amgen International, Leaves and Cotidiana, two European projects funded by AAL, Patient2Entrepreuneurship funded by FCT, Digiadherence funded by DGS, Patient Innovation Bootcamp funded by EIT Health. Other projects are more focused in chronic diseases like diabetes, heart failure, back pain and OA.

We are also very committed in developing and boosting clinical, translational and public health research by establishing the FCT research unit scored excellent, Comprehensive Health Research Center (CHRC) and the new Associated Laboratory in Translation and Innovation Towards Global Health (REAL).

Osteoarthritis

In the field of OA we have a wide and varied production, which is based on a long and consolidated international collaboration within ESCEO – European Society for Clinical and Economic Aspects of Osteoporosis and Osteoarthritis.

In the 18 OA-related articles published during the last five years, the pathophysiology of OA was studied, in particular the association with type 2 diabetes mellitus and the importance of bone microarchitecture for its development and identification (Ljuhar et al., 2017; Veronese et al., 2019).

We created a diagnostic framework of OA for use in Primary Health Care, we defined the criteria and risk signs for the early diagnosis of symptomatic knee OA (GOA), in which age, gender, body mass index and joint pain were very important identifying elements (Martel-Pelletier et al., 2019; Migliore et al., 2017).

The evolution of OA was addressed in some articles, with the identification of patients at high risk of progression using biomarkers and fragility indicators, the usefulness of the Kellgren-Lawrence Index and bone marrow lesions in predicting the radiological worsening of OA, and the importance of changes in the loss of the joint space in the development of OA (Edwards et al., 2016; Parsons et al., 2019).

Related to OA therapy, recommendations for guidelines to be used in research on medicinal products in OA and for clinical trials in hand OA, an analysis on discordant recommendations on the use of symptomatic slow-acting drugs for GOA, a review on complementary treatments for cartilage repair, a study on the treatment preference of a cohort of patients from 6 European countries, and an algorithm on the management of GOA, were published (Bruyère et al., 2016, 2019; Fuggle et al., 2020; Hiligsmann et al., 2020; Reginster et al., 2018).

Our team members also contributed for innovation in medical and surgical therapeutic approaches. Over the last decade, cellular and non-cellular autologous biologic therapies have been gaining momentum as treatment options for various conditions of the musculoskeletal system, particularly with regards to degenerative joint diseases, the most common being OA. Of the several biologic therapies currently in use, platelet-rich plasma concentrates (known as PRP concentrates), a type of non-cellular biologic therapeutic agent, have been among the most researched and utilized in musculoskeletal medicine (Caiado et al., 2020; Navani et al., 2017; Rendu and Brohard-Bohn, 2001).

When preparing a PRP concentrate, the liquid and solid fractions of a whole blood sample are separated in a test tube using plasmapheresis, through a single or two-phase centrifugation process. During the first centrifugation phase, or “soft

spin", plasma and platelets are separated from red blood cells and leukocytes. The second phase, or "hard spin", is performed in order to further concentrate and separate the platelet-rich and platelet-poor components. The final concentrate can then be injected in the site of injury, with the final goal of minimizing symptoms and enhancing the healing process (Caiado et al., 2020; Navani et al., 2017; Rendu and Brohard-Bohn, 2001).

In an attempt to keep up with the best international centers in the field, known internationally as Regenerative Medicine, as well as to be able to offer these treatments to its patients, Central Lisbon University Hospital Center set up its Interdisciplinary Regenerative Medicine Outpatient Clinic in 2018. Since then, the team has been highly involved in research projects regarding the role of cryopreserved PRP concentrates in the treatment of symptomatic mild to moderate hip and knee OA. Of note, in 2020 the team was awarded the 1st Annual Gofeld Scholarship Award by the World Academy of Pain Medicine United, for the development of an in-house technique for PRP preparation and cryopreservation.

Degenerative disc disease (DDD) is a prevalent problem in which, treatment and the optimized clinical outcome, became a never-ending story. that promotes research all around the world. However, on pursuing that purpose, are we all looking for the same goals? In a European multicentre study, including southern and northern countries, we found out that it diverges in between patients and spine surgeons, and this should be taken in consideration for future clinical research (Haefeli et al., 2008).

In the last two decades, one of the most relevant issues for DDD surgical treatment came out of knowing that arthrodesis is often associated with significant problems. On the other hand, Minimal invasive spine surgery (MISS) using motion technology devices keep claiming its superiority against rigid fixations systems. However, and apart from this, at the time, nothing has been said concerning postoperative disc height variation or psychosomatic profile evolution.

The results of our studies, using MISS to decompress the spine and apply interspinous devices in DDD (Pfirman 3 and 4) patients, pointed out that intervertebral disc height increase at the operated segment while preserving its motion (De Castro Guimaraes Consciencia, 2009) and improves the Oswestry Disability Index (ODI), quality of life (EQ-5D), pain (VASVisual Analogue Scale) and Distress and Risk Assessment Method (DRAM) (De Castro Guimaraes Consciencia, 2013).

Nowadays, one top item in DDD treatment and specifically in MISS is endoscopic spine surgery (ESS), considered at least equally effective and less aggressive

when compared to conventional open surgery. Even if motion technology devices are the main option in several clinical cases, some pathological situations still require fusion as an optimal solution. In such cases, ESS would be one of the most attractive as well as elegant available techniques. However, apart from the learning curve, there are significant obstacles to be overcome when selecting ESS to perform an interbody fusion, especially in specific spine locations, such as L5-S1.

The local anatomic features of the spine, with a protruding iliac crest, makes it hard to obtain an adequate, safe and clear access to perform the procedure. That's precisely why our group decided to certify the feasibility of trans iliac approach in treating DDD (Pfirman 4 and 5), at the L5-S1. This will be determined through a cadaveric study, while at a second stage, we will perform a prospective clinical evaluation comparing ESS interbody fusion with a conventional MISS technique.

A lot has been done so far, and a lot is still waiting for research in the field of DDD as well as its surgical treatment. That's one of the reasons why many experts still consider it a good asset for investment in future research. We share the same vision.

Osteoporosis

Under the scope of the EpiDoC cohort, the prevalence and individual burden of OP in Portugal have been studied. The prevalence of OP in Portuguese adults is 10.2%, higher in women (17.0%) than in men (2.6%) and increases with age. Almost half (40.0%) of Portuguese adults, 75 years and older have OP, and an OP diagnosis was associated with substantial physical function impairment but not with anxiety or depression symptoms (Branco et al., 2016). Also using EpiDoC data, it was documented that self-reported fragility fractures were highly prevalent among senior women (20.7%). This high prevalence was in stark contrast with the low rate of OP treatment (13.9%). Non-hip and non-vertebral fractures (i.e., lower leg, wrist, humerus, rib, clavicle, and elbow fractures) accounted for the majority of fragility fractures, and clinical risk factors independently associated with prevalent fragility fractures were increased age, obesity, and lower distal bone mineral density (BMD) (Rodrigues et al., 2018b).

The challenge to better identify seniors (people aged ≥65 years old) at high risk for a fragility fracture led to search for novel non-invasive biomarkers of bone fragility (Rodrigues et al., 2012a, 2012b) and fractures- Low serum levels of DKK2 predicted low-impact fractures, independent of BMD, and clinical risk factors for fracture. For every 1 standard deviation decrease in DKK2, fracture risk increased

by approximately 1.5-fold (Rodrigues et al., 2019). Also, in the context of the ESCEO and with other national authors, we studied the determinants of falls in Portuguese adults, low-energy fractures in diabetic patients and the usefulness of radiofrequency echographic multi-spectrometry for OP diagnosis and bone resistance assessment (Diez-Perez et al., 2019; Furtado et al., 2019; Marques et al., 2019a).

With regard to the treatment of OP, we have published a large national epidemiological study on therapeutic persistence with bisphosphonates, another on the role of calcium supplementation in healthy musculoskeletal ageing, another on the prevalence of Vitamin D deficiency and its predictors in the Portuguese population, and we have also developed an algorithm for the use of biochemical markers of bone turnover in the diagnosis, evaluation and follow-up of OP treatment (Duarte et al., 2020; Harvey et al., 2017; Lorentzon et al., 2019; Torre et al., 2019).

Finally, we participated in the multidisciplinary expert group that built the Portuguese recommendations for DXA request and indication to treat in the prevention of fragility fractures and also in the recommendations for the prevention, diagnosis and management of primary OP (update 2018) (Rodrigues et al, 2018).

Currently, innovation is far beyond the development of new drugs. Digital transformation is invading our practices and healthcare systems. It is transforming the health records, interprofessional communication and communication between professionals and patients, large registries with real world data, medical devices, support to clinical decisions and personalized health programs. We are now working in an information and communication technology (ICT) programme, "The Healthy Bone TV" application which is a multimodule, interactive, customizable app that delivers a home-based program including physical exercise, nutritional plan, OP and fragility fractures patient education, and treatment reminders to assist high-risk fragility fractures long-term self-management. Because ICT literacy in elderly is low, this solution is user friendly and blended with human contact. We are conducting a randomized controlled trial which aims to evaluate the effectiveness of this tool on OP treatment adherence among patients with a recent fragility fracture (Rodrigues and Canhão, 2019).

Spondyloarthritis

Axial SpA (axSpA) is a chronic disease that, though with slow changes over time, is associated with pain, impairment in spinal mobility and functional ability, a reduced quality of life, and an increase in morbidity and mortality. The social costs (individual and collective), tangible and intangible, are enormous. It is therefore

crucial to develop strategies not only to promote health, but also to prevent (ideally!) and early detect the disease. The identification of major outcomes and the best way to monitor progression is essential for its management. This helps to maintain the patients' independence and quality of life while simultaneously allowing to keep each individual as a valid and productive member of the society.

The authors have been working on both translational and clinical research in the field of ax SpA. A better understanding of the pathogenesis of the disease helps in the identification of new and more effective diagnostic and monitoring tools, as well as therapies.

We have described the clinical phenotypes of axSpA (Sepriano et al., 2020a). Genetic studies complemented this characterization, in the context of national and international consortia that allowed the analysis of a large number of patients, new biomarkers and new gene associations. The haplotype A*02/B27/C*02/DRB1*01/DQB1*05 confers susceptibility and A*02/B27/C*01/DRB1*08/DQB1*04 protection for axSpA functional and radiographic damage, showing that genes within the HLA region other than HLA-B27 might play some role in axSpA (Pimentel-Santos et al., 2013). After the identification of *ERAP1* and *IL23R* genes, an extensive evaluation of the association between single nucleotide polymorphisms (SNPs) in these genes and susceptibility and clinical manifestations to axSpA among a Portuguese population were performed (Pimentel-Santos et al., 2009). Using the Illumina Immunochip microarray, a large case-control study involving 10,619 individuals with axSpA (cases) and 15,145 controls we identified 13 risk loci and 12 additional axSpA-associated haplotypes at 11 loci. Through whole-genome microarray approach we identified and validated a gene expression signature from whole blood and identified strong candidate genes that may play roles in both the inflammatory and joint destruction in axSpA (Pimentel-Santos et al., 2011).

From the perspective of clinical and outcomes research, we have focused on improving our understanding of outcome measures used in axSpA, as on improving them and their use in daily clinical practice. The measurement of disease activity, spinal mobility and particularly of imaging outcomes has deserved our largest attention (Marques et al., 2019b; Ortolan et al., 2021; Ramiro et al., 2013, 2015a, 2015b, 2018). The widespread use of instruments also requires that they are made available in our Portuguese language (Cruz et al., 2017; Rodrigues-Manica et al., 2020). We have made important contributions to the insights into the evolution of structural damage over time and its relationship with other outcomes (Falcao et al., 2015; Ramiro et al., 2015c, 2019). In the specific area of musculoskeletal ultrasound

(MSU), our group contributed to aggregate significant objective data for a better definition of enthesopathy in SpA (Falcão et al., 2012). With our work we intended to open new horizons in order to understand the importance of other structural enthesis lesions not included in this definition, such as the entheseal bursa (Falcao et al., 2013); but also, the behaviour of lesions that were empirically considered as permanent structural damage, namely the entheseal cortical erosions. In the latter case, with the demonstration of the dynamic behaviour of entheseal erosions in SpA patients, we not only revolutionized the classical erosion concept, but also reinforced the importance of the new bone formation in the pathophysiologic process in SpA (De Miguel et al., 2011).

The use of cohorts with a long and structured follow-up of patients over time, together with proper statistical analyses, has allowed us to show for the first time that disease activity in axSpA leads to the progression of structural damage (Ramiro et al., 2014). We have further confirmed this important finding in another cohort (Sepriano et al., 2021a) and went one step further and identified that the suppression of disease activity through tumor necrosis factor inhibitors is associated with a lower progression of structural damage (Sepriano et al., 2021b). Disease progression, imaging abnormalities, their measurement and the link between inflammation and structural damage has been area to which we have made extensive contributions, so that such a link is at the moment no longer questioned (Dougados et al., 2017; Sepriano et al., 2019, 2020b). In general, we have identified and described relationships between multiple relevant outcomes in axSpA (Hirano et al., 2020, 2021; Nikiphorou et al., 2018, 2020; Rodrigues Manica et al., 2018).

Treatment of axSpA has been another focus of our group. We have all been involved in the 2016 ASAS-EULAR update of the management recommendations for axSpA (Van Der Heijde et al., 2017) to which we also contributed by systematically reviewing the literature (Regel et al., 2017; Sepriano et al., 2017). Additionally, and making use of our Portuguese registry, Reuma.pt, we have shown that co-medication with conventional synthetic disease-modifying antirheumatic drugs does not have an added value in patients with axSpA on TNFi (Sepriano et al., 2016). Also, in the same registry, we have reported that patients with axSpA with secondary failure to their first TNFi, compared to those with primary failure, have a better response to the second TNFi according to stringent outcomes (Manica et al., 2020). Reuma.pt data have also contributed to confirm the important role of the Ankylosing Spondylitis Disease Activity Score (ASDAS) criterion for high disease activity (>=2.1) for the identification of patients eligible for treatment with biologics (Marona et al., 2020).

Having identified relevant genetic, biologic and clinical markers and improved outcome assessment, we contribute for a better knowledge of the disease that for the application of strategies at an individual level striving for better outcomes, towards precision medicine (Van Vollenhoven et al., 2018).

We work in collaboration with national and international partners, including the Assessment of SpondyloArthritis international Society (ASAS), the European Alliance of Associations for Rheumatology (EULAR), the (IGAS) and have an important and active contribution to important international cohorts in SpA like DESIR (Dougados et al., 2011), SPACE (van den Berg et al., 2013), among others. These collaborations allow large epidemiological and genetic studies involving different populations. We are also conducting studies aiming at identifying molecular biomarkers of response to TNF inhibitors (the Bioefficacy study), to evaluate the influence of oral and gut microbiome in the response to biologic therapies (the MicroSpA study) and to understand the link between muscle properties and entheseal inflammation (MyoSpA study). The latter being a proposal for a novel pro-inflammatory mechanism, based on the interplay between muscle and entheses, and aiming at further exploring the link between inflammation and osteoproliferation. In an attempt to overcome the challenging measurement of the low structural damage progression in axSpA, our group has proposed low dose computed tomography (ldCT) as a promising alternative (de Bruin et al., 2018; De Koning et al., 2018). We are now undertaking a follow-up study (AXIOMA) that aims to fully validate the best scoring method in ldCT.

In the field of MSU we continue to develop projects related with entheseal lesions in SpA. Nevertheless, we extended our area of interest to other rheumatic diseases beyond SpA. We are currently giving the latest steps on a project using MSU in the diagnosis and follow-up of carpal tunnel syndrome; other in the evaluation of MUS synovitis and development of normality criteria in assessment of several joints – a national cross-sectional study-; and other in the evaluation of predictive factors for treatment response in patients with rotator cuff pathology. Last but not the least, we are developing a national MSU database to be used among rheumatologists who practice this technique, which allows the registration, the creation of reports and the crossing of its results with clinical data.

Sjogren's syndrome

Sjogren's syndrome (SjS) is one of the most frequent systemic rheumatic diseases and is an important cause of short and long-term disability. SjS is a

multifactorial disease, where a suspected infectious trigger (such as Epstein Barr virus), coupled with genetic and environmental aspects, initiates an autoimmune epithelitis primarily affecting the lacrimal and salivary glands. Systemic involvement occurs in about 50% of patients and may affect the skin, joints, lungs, kidneys, central and peripheral nervous systems and blood vessels. T and B cells infiltrate affected organs, where ectopic lymphoid structures may develop and contribute to immunoglobulin and auto-antibody production, glandular dysfunction and risk of lymphoma. Typical profiles of circulating B-cells have been described in SjS, such as a decrease in memory B-cell and increase transitional and naïve B-cells, and their utility as diagnostic items has been proposed.

We have studied the B and T lymphocyte circulating subsets in SjS, sicca patients, rheumatoid arthritis (RA) and healthy controls, confirming the already known profile in SjS (Barcelos et al., 2018). We also reported an increase in IL-21-producting CD4 and CD8 follicular T-cells in SjS.

We have studied lymphocyte subsets in SjS patients according to the EBV serologic profile (Barcelos et al., 2021a). In this study, CD8 follicular T cells were of particular interest because they may link a cytotoxic response to EBV with the pathogenesis of the disease. Our results also support that EBV may play a role in inducing B and T-cells towards an effector phenotype, typical of SjS. Interestingly, a correlation was found between disease activity, as evaluated by the ESSDAI, and the CD4 and CD8 IL-21 producing T-cells in SjS.

Afterwards, we have also analysed the relationship between lymphocyte subsets and the presence of anti-SSA antibodies in SjS patients (Barcelos et al., 2021b). We have confirmed a greater immune dysfunction in SSA+ patients, in whom IL21+ follicular CD4 and CD8 T-cells were significantly increased and correlated positively with systemic disease activity (ESSDAI). A negative correlation was also found between Tregs' numbers and the ESSDAI in SSA+ SjS patients.

We have also partnered with Ophthalmology specialists to explore a novel imaging tool to assess dry eye and keratitis, confocal microscopy (Cardigos et al., 2019). We have found greater degrees of corneal nerve tortuosity and decrease nerve density in SjS patients, which correlated with SjS systemic disease activity (Barcelos et al., 2021c). This non-invasive assessment method may be helpful to evaluate dry eye severity and response to treatment.

Finally, we have compared the 2002 AECG and the 2016 ACR/EULAR SjS classification criteria and explored the effect in their performance of the addition

of relevant lymphocyte subsets. Although already highly specific and sensitive, there seems to be an increase of their sensitivity and specificity when lymphocyte subsets are added to the mathematical model (paper in submission).

Overall, we have highlighted the differences in lymphocyte profile between SjS, RA, and healthy subjects, and demonstrated the feasibility of the study of lymphocyte profiles in a clinical setting, demonstrating its putative utility as a marker of disease activity in SjS, as well as its possible contribute to the optimization of diagnosis and classification of patients.

Currently, we are in the process of concluding the project "Lymphocyte subsets in the diagnosis of SjS". We expect that the analysis of available data will add information about the immunologic processes of patients with a mild phenotype reminiscent of SjS, some of them with lymphocyte profiles resemble those of SjS that are currently diagnosed as undifferentiated connective tissue disease. The disease burden in such patients is, in many instances, comparable to that of SjS, therefore pointing to unmet therapeutic needs in a vast group of patients that reach us in clinical practice.

We also intend to expand the study of the immune processes to newly diagnosed SjS patients and suspected cases not fulfilling classification criteria, through the study of relevant lymphocyte subsets and markers of immune activation, namely related to the Interferon pathway signature. Coupled with the study of lymphocyte subsets and immune activity of the disease, we aim to characterize glandular involvement, patient reported outcomes (PRO) and disease burden, with the purpose of developing a personalized clinical approach. Several objective tests and PRO are available and validated and can be added to the immunologic parameters to identify patients' subsets. Additionally, according to a binomium "disease activity" and "disease burden", the therapy could be tailored not only regarding pharmacologic intervention to suppress inflammation, but also to address issues like pain, dryness and depression. Concomitantly, the participation of other medical specialties and non-medical health professionals needs to be stimulated and cooperation protocols could be better applied with patient stratification.

We expect that the peripheral blood lymphocyte subset profiling could be useful in the diagnostic decision when facing a suspected SjS patient. Additionally, considering the correlation between some specific subsets, such as follicular T cells, and disease activity, lymphocyte profiling may be useful as a patient stratification tool for prognosis and therapeutic approach.

Juvenile Idiopathic Arthritis

JIA is the most common chronic inflammatory arthritis in childhood and is an important cause of short and long-term disability in children. JIA is a multifactorial disease and its onset and course is believed to be determined by genetic and environmental aspects. In a previous study from our group, with Portuguese patients with JIA, we have found an association between some single nucleotide polymorphisms and a worse prognosis of the disease (Mourão et al., 2015). The clinical features that were correlated with a worse prognosis were higher age at disease onset, higher score in visual analogic scale of the patient, any usage of corticosteroids and extra-articular manifestations of the disease.

Afterwards, we have also analysed the relationship between body mass index (BMI) and disease activity in patients with JIA and found an independent association between underweight and higher disease activity (Neto et al., 2021). The results suggested that active disease can impair child's weight gain and reinforce the relevance of the routine assessment of BMI as part of the management of JIA.

Pediatric rheumatology is a new subspecialty, recognized by *Ordem dos Médicos* in 2020, were we made significant contributions in the last 20 years. We have participated in almost all the clinical recommendations and guidelines issued by the SPR in the last 20 years (Santos et al., 2007, 2012, 2016).

Young people often struggle to self-manage chronic diseases during the transition from childhood to adulthood. In the global context, digital health technology has the potential to bridge the distance gap between all the key stakeholders involved in rheumatology health care. Recently, there has been a substantial increase in efforts to promote healthy lifestyles since childhood. Physical exercise, Mediterranean diet, good sleep, avoids smoking and alcohol abuse and psychological interventions are crucial for a healthy lifestyle.

Recent studies suggest exercise capacity is significantly impaired in a large proportion of children with JIA and other rheumatic diseases (Klepper S.E., 2008). These deficits are not limited to children with active inflammation. Moreover, the propensity of patients with paediatric rheumatic diseases to be hypoactive – often due to social self-isolation, overprotection, and fear and/or ignorance on the part of parents, teachers and health practitioners – can be detrimental to general disease symptoms and function.

Currently, we are carrying out the project "B-active: an integrative interactive platform for children with rheumatic diseases". Our aim is to develop an integrative and interactive virtual platform, in which patients with juvenile rheumatic diseases

can access an exercise plan according to their disease involvement, a nutritional plan adjusted to individual preferences and needs, and quick mindfulness programs, videos and stress management strategies according to a brief mental illness screening. We will also evaluate the effectiveness of this platform in acquiring healthier lifestyles.

We also pretend to develop training actions in schools, in order to increase the awareness of juvenile rheumatic diseases in the school community. We strongly believe that we have to put in practice interventions to make the children "sleep well, eat better, sit less and move more", not only to improve the disease symptoms but also to prevent future damage.

OTHERS DISEASES

Regarding FM we participated in the European group that published the EULAR recommendations for the management of FM. At national level, with several authors from different institutions, we produced papers on the life history of FM patients, the recognition of a FM personality, the relationship of social distress with pain modulation and also the correlation of widespread pain with the pain tolerance threshold under pressure of fibromyalgic tender points. In the therapeutic chapter, we have published three studies on the effects of functional respiratory training on pain and quality of sleep in patients with FM. We also participated in the translation and adaptation into Portuguese of the 'Start Back Screening Tool- SBST' questionnaire (Canaipa et al., 2017; Garrido et al., 2017; Macfarlane et al., 2017; Raimundo et al., 2017; Tomas-Carus et al., 2018).

Also, in the field of rheumatoid arthritis, its pharmacogenetics, therapies, predictors of response have constituted major achievements with international collaborations and publications on lead journals (Bergstra et al., 2018).

IMPACTS TO SCIENCE AND SOCIETY

Our research has been adding relevant insights to the current knowledge which contributes for general science and societal development. All provided information and the participation of our collaborators in different task forces promote the improvement of diagnostic and classification tools, the development of several

therapeutic guidelines that helps decision-making of physicians and different stakeholders. Society can reap the benefits of this successful research studies as the results have been converted into marketable and consumable products as new diagnostic tools, new therapeutic possibilities, and new services provided.

All these aspects are critical in our aging society in which new diagnostic tools and new therapeutic approaches may have an huge impact for an healthy aging and relevant for an efficient management of the available resources.

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RESPIRATORY DISEASES

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1. INTRODUCTION

We will focus on the contribution of our CHRC/CEDOC research group at NOVA Medical School regarding non-communicable respiratory diseases. Within this chapter, we will approach the research that has been developed since the last decades of the twentieth century. This research path began with the introduction of nitric oxide in Portugal[1][2] as a marker of inflammation in respiratory diseases[3] and continued with the Master Ship on respiratory diseases led by Professors Ramiro Ávila, António Rendas and Dr. José Rosado Pinto. During this period, several PhD students developed their work in respiratory diseases. We will discuss some research projects whose results were published and that were developed at the NOVA Medical School pathophysiology laboratory: "Association between bronchial reversibility and airway inflammation in patients with Chronic Obstructive Pulmonary Disease" [4]; the first environmental research study conducted in our Medical School – the "Saud'Ar Project" [5][6], and two other projects related to indoor air quality and health in different populations [7–10]. Other projects developed by PhD students in collaboration with our laboratory were "Airway obstruction and inflammation in preschool children" [11] and "Obstructive Lung Diseases in the elderly – the OLDER project" [12] – see Table 1. Finally, we will discuss a PhD project supported by NOVA Saúde on the mechanisms of nasal polyposis – a collaboration between NOVA Medical School and NOVA School of Science and Technology [13].

In the state of the art section, we will briefly review the burden of non-communicable diseases.

Year	Student	Degree	Research work
2002	Araújo-Gonçalves, P	Master in Respiratory Diseases	Markers of lung hyperinflation in asthma
2002	Bugalho, A	Master in Respiratory Diseases	Association between bronchial reversibility and airway inflammation in patients with Chronic Obstructive Pulmonary Disease
2012	Carreiro-Martins, P	PhD in Medicine	Mechanisms of bronchial inflammation resulting from exposition to environmental factors
2017	Belo, J	PhD in Biomedicine	Evaluation of institutionalized elderly people by spirometry and bronchial condensate from exhaled air: effects of environmental exposure
2020	Araújo-Martins, J	PhD in Medicine	Endogenous factors in the etiology of nasosinusal polyposis

Year	Student	Degree	Research work
2021	Leiria-Pinto, P	PhD in Medicine	Airway obstruction and inflammation in asthma, at preschool age
Ongoing	Gaspar-Marques	PhD sudent	Assessment of asthma, chronic obstructive pulmonary disease and overlap syndrome in the elderly

Table 1 – Academic degrees and respiratory research in collaboration with the pathophysiology laboratory/NOVA Medical School

2. STATE OF THE ART REVIEW

Chronic respiratory obstructive diseases: Burden, prevalence and risk factors.

Chronic respiratory diseases are diseases of the airways and other structures of the lungs. The most common are chronic obstructive pulmonary disease (COPD) and asthma. These diseases affect more than 600 million people worldwide and are a significant cause of disability, poor quality of life, and significant consumption of health care resources[14]. According to the Global Burden of Disease Study, chronic respiratory diseases are among the six leading causes of disability-adjusted life years (DALYs) in 2017 [15]. COPD is the third leading cause of death worldwide and is associated with higher morbidity and mortality due to COVID -19. Asthma is the second most relevant chronic respiratory disease in terms of DALYs. Chronic respiratory diseases are often underdiagnosed and undertreated, leading to an underestimation of their burden.

The prevalence of asthma and COPD varies widely depending on the survey method and diagnostic criteria. COPD is more common in smokers, in persons over 40 years of age, and men. The prevalence of COPD has been determined in various studies. In 2010, the global prevalence of COPD for the population aged 30 years or older was 11.7% (95% confidence interval: 8.4%-15.0%) [16]. Recently, a systematic review estimated that the global mean COPD prevalence for individuals aged ≥40 years was 13.1% (10.2-15.6%) [17]. In this study, Africa had a higher prevalence (13.9%; CI:12.0-15.9%). The prevalence in Europe was 12.4% (CI: 8.8-16.0%).

Epidemiological data suggest that asthma is more prevalent in children. According to the International Study of Asthma and Allergies in Childhood (ISAAC) Phase Three [18], the worldwide prevalence of current asthma in the 6-to 7-year-old and 13-to 14-year-old age groups was 11.7% and 14.1%, respectively. In adults, the global prevalence rates of physician-diagnosed asthma, clinical/treated asthma,

and wheezing in adults were 4.3%, 4.5%, and 8.6%, respectively, according to the World Health Survey[19]. In adults, the prevalence is highest in middle-aged people; however, mortality is more significant in the older age group.

Tobacco smoking is the most critical factor in the development of COPD. In addition to tobacco use, other environmental risk factors for COPD are known, such as occupational exposure. Several studies have shown that COPD is associated with workplace pollution and indoor air pollution from biomass fuels.

Asthma results from complex interactions between multiple environmental and genetic factors. Numerous factors have been associated with a higher prevalence of asthma, namely atopy, parental history of allergic respiratory disease, exposure to tobacco smoke and air pollution, low intake of fish and fruit, microbiome, and physical inactivity.

Portuguese data

Portuguese data on the actual prevalence of COPD in the Portuguese population is not yet available. In 2008, the Burden of Obstructive Lung Disease (BOLD) study [20] estimated a COPD prevalence of 14.2% in adults aged 40 years or older for the Lisbon region.

In the World Health Survey [19], the prevalence rates in Portuguese adults for "physician-diagnosed asthma", "clinical/treated asthma", and "wheezing in the last 12 months" were 7.8%, 7.8% and 8.7%, respectively.

The Portuguese National Asthma Survey (INAsma) [21], from 2010, a population-based cross-sectional telephone survey covering all municipalities in Portugal, showed a prevalence of "diagnosed asthma" and "current asthma" of 5.0% and 6.8%, respectively. The definitions used to diagnose asthma significantly impact prevalence estimates, mainly because asthma, like other chronic diseases, is often underdiagnosed and underreported.

Vulnerable ages

The early ages of life are critical for the development of asthma, and both chemical and biological pollutants can trigger the respiratory and allergic disease. Young children spend most of their time indoors, at home, in school, or in daycare centres and are particularly vulnerable to environmental exposures. This susceptibility results from their immunological immaturity, incomplete lung development and a higher exposure dose of inhaled substances due to their metabolic and ventilation rates [22].

Asthma is usually considered a disease more common in younger people, and for this reason, it may be underdiagnosed or misdiagnosed in older people. Nevertheless, several authors showed that asthma also occurs in older people and can overlap with COPD[23][24]. The coexistence of asthma and COPD is commonly referred to as asthma-COPD overlap (ACO)[12].

Among patients with chronic respiratory disease, the elderly may require significantly more nursing resources than younger patients. The elderly have additional comorbidities, more pronounced lung function and immunological deterioration, leading to a higher predisposition to respiratory infections.

Population ageing is a global problem, especially in developed countries. Age is often associated with respiratory symptoms, and COPD prevalence increases with age,[20]. In the elderly, asthma is more common in women [25]. Also, COPD may coexist with asthma and mask the latter, contributing to undertreatment. In epidemiological studies conducted in the United States and the United Kingdom, 17% to 19% of patients with obstructive airway disease reported having concurrent asthma and COPD [26] including more than 50% of patients with obstructive airway disease whose age was older than 50 years. Therefore, patients with a concurrent diagnosis of asthma and COPD are usually older.

The Global Alliance vs Chronic Respiratory Diseases (GARD)

GARD is a voluntary coalition of national and international organizations, institutions and agencies working to reduce the global burden of respiratory disease. It is supported by the World Health Organization (WHO). The GARD Portugal was implemented in September 2007 by the Portuguese Ministry of Health (MOH) [27]. Over the years, Portugal actively participated in various initiatives and led a GARD demonstration project to assess the burden of CRD at the primary health care level in Cape Verde [28]. Through the vision of "a world where all people can breathe freely" within the international GARD, the GARD -CPLP working group was established in 2017.

3. RELEVANT CONTRIBUTION FROM NOVA

The use of the fraction of exhaled nitric oxide (FENO) in the clinical setting was performed for the first time by our group in Portugal as part of a master's thesis on the relationship between bronchial reversibility and airway inflammation

in patients with COPD [4]. This study addressed the hypothesis that airway inflammation is different in COPD patients with and without reversible airway obstruction. Back in 2002, when the overlap of asthma and COPD (ACO) was not a hot topic, this hypothesis was confirmed because patients with reversible airway obstruction had higher levels of markers of eosinophilic inflammation, such as FENO, eosinophils and eosinophil cationic protein (ECP) in induced sputum, and IL -5 in blood and in induced sputum. On the other hand, COPD patients with fixed airway obstruction (FEV_1 <12% and 200 ml) showed a neutrophilic inflammatory pattern with a predominance of neutrophils, IL-8 and low FENO levels.

FENO was also used in the Saud'Ar project [3, 29]. In this study, 54 asthmatic school-aged children were studied to determine the association between air pollution and indoor air quality and the risk of respiratory disease. FENO was used as a marker of airway inflammation. Their mean age was 7.8±1.1 years. When comparing those who had wheezed in the six months prior to the study (n=27) with those who had not wheezed, statistical differences were observed for ΔFEV_1 (8% median versus 4.5%, p=0.0399) and FENO (23 ppb median versus 12 ppb, p= 0.0195). Children who required a bronchodilator in the last six months (n=19) and those who did not, show a statistically significant difference for FENO: 27 ppb median versus 11 ppb median; p=0.0001. When comparing children who required an unscheduled medical appointment in the six months before evaluation (n=9) and those who did not, there was also a significant difference for FENO: 28 ppb median versus 13 ppb median, p=0.0029. It was concluded that the presence of symptoms appeared to be better related to FENO than to spirometry. In the same project, an association between respiratory changes (FEV_1, ΔFEV and total exposure to air pollutants – PM_{10}, NO_2, benzene, toluene and ethylbenzene) was also observed in wheezing children, estimated by considering the concentration in the different microenvironments visited by the children [6].

Our laboratory was involved in two other projects dealing with indoor air quality on occupants' health: ENVIRH (Environment and health in children daycare centers) and GERIA (Geriatric study in Portugal on health effects of air quality in elderly care centres). In this chapter, we will focus on research on respiratory diseases.

In the ENVIRH project, we selected a population of susceptible children – those with recurrent wheezing – in whom an association between indoor CO_2 (a surrogate of indoor ventilation) and wheezing was observed[30]. In a subsample of this population of children younger than five years, using a surveillance system based on parental reports, it was also possible to characterise respiratory virus infections,

with influenza A (H3) being the most commonly detected. It has been suggested that this parental report system could complement the implanted system of the National Influenza Surveillance Program [31].

The impact of indoor air quality on respiratory health in older people was studied in our laboratory by a PhD student as part of the GERIA project (10). A total of 269 older people answered a breath health questionnaire, underwent spirometry, and 150 of them collected an exhaled condensate sample to analyse pH and nitrites. The study included an assessment of indoor chemical and microbiological contaminants. The median age of the participants was 84 years, and 70.6% were women. Spirometric data indicated the presence of airway obstruction in 14.5% of the sample. Median concentrations of air pollutants did not exceed existing norms, although elevated peak levels were observed. In a multivariable analysis, each 100 μg/m^3 increase in total volatile organic compounds was associated with the odds of respiratory tract infection in the preceding three months (OR =1.05; 95% CI: 1.00-1.09). $PM_{2.5}$ concentrations were inversely associated with Exhaled Breath Condensate (EBC) pH (β = -0.04, 95%: -0.06 to -0.01, for each increase of 10 μg/m^3) - a marker of airway inflammation. A direct and an inverse association was found between total bacteria and FEV_1/FVC and FVC, respectively.

Our group's contribution to the research on asthma in preschool children focused on factors associated with asthma control [11]. This study included 3 to 5-year-old children with asthma and healthy controls. According to the GINA criteria, a questionnaire was used to identify the potential risk factors for uncontrolled asthma (Global Initiative for Asthma). Lung function and bronchial reversibility were assessed by impulse oscillometry (IOS) and spirometry. Fifty-three patients (50%) had uncontrolled asthma. After adjustment, variables associated with increased risk of lack of control were as follows: more than three flare-ups in the last 12 months, moderate to severe rhinitis, relative variation in post-bronchodilator FVC and FEV_1. The AUC (Area Under the Curve) of the final models that included a variation in FVC or FEV_1 were 0.82 and 0.81, respectively. R5-20, R5-20% and AX z-score values of the healthy group were lower than those of the children with asthma. We concluded that clinical and functional parameters are associated with uncontrolled asthma in preschool children. To conduct this study, we validated the Portuguese from Portugal version of the Test for Respiratory and Asthma Control in Kids – TRACK questionnaire [32].

The OLDER (Obstructive Lung Diseases in Elders) study took place in Lisbon, Portugal. It was an observational study divided into three phases. In this study (Ventilatory Defects and Treatable Characteristics in Very Elderly Patients) [33], we report the results of phase I, which took place from April to December 2016,

with residents of 15 Lisbon elderly care centres (ECC) invited to participate. In Phase I, in addition to spirometry, fraction of exhaled nitric oxide (FENO) and atopy assessment, participants answered standardized questionnaires administered by a trained interviewer and took a blood sample. At this stage, each participant also performed peripheral pulse oximetry. To be eligible for the study, participants should be ≥65 years of age, have cognitive and collaborative abilities sufficient to perform spirometry, and have no contraindication to pulmonary function testing. Pulmonary, extrapulmonary, and behavioural treatable characteristics (TT) were assessed. Outcome variables were obstructive ventilatory defect and restrictive spirometric pattern. The included subjects (n=234) were predominantly female (72%), with a median age of 86 years (P25-P75: 82.6-90.0). At least one pulmonary TT was identified in 105 (44.9%) subjects. The most common extrapulmonary TT were: persistent systemic inflammation (47.0%), anaemia (34.4%), depression (32.5%), obesity (27.4), and poor quality of life (26.4%). Obstructive ventilatory disorder was associated with smoking only (OR 5.03; IC 95% 1.56-16.22). The restrictive spirometric pattern was associated with cognitive impairment (OR: 3.89; IC 95%: 1.55-9.79).

A high frequency of different TT was found. The novel association between a restrictive spirometric pattern and cognitive impairment highlights the urgency of clinical research in this vulnerable age group.

The last study analyzed in this book relates to the mechanisms of nasal polyposis [13]. This study suggests that the examination of polyp patients by rhinomanometry and PNIF (Peak Nasal Inspiratory Flow) can provide valuable and reproducible data. Several findings taken together indicate that polyp size is not the main determinant of nasal functional changes in these patients, which warrants further studies to verify whether PNIF changes reflect sinus inflammation or merely airway obstruction.

4. IMPLICATIONS FOR SCIENCE AND SOCIETY

The different studies in which we have been involved have contributed significantly to a better understanding of the prevalence, pathophysiology and inflammation of respiratory diseases. Our group is currently a national reference in respiratory disease research and is frequently invited to give scientific lectures and speak to general audiences. In recent years, members of our group have been regularly invited for interviews in newspapers, radio and television.

5. ONGOING RESEARCH

There are three funded but not yet completed projects:

- The first, Finnee – From spectra to formulas (PTDC/CCI- BIO /29702/2017), was developed following the OLDER project to search for specific biomarkers of respiratory disease using a metabolomic approach, and the methodology has already been published [34].This project is a collaboration between Oporto University, NOVA-ITQB and NOVA Medical School.
- The second project is "Epidemiology and functional assessment of chronic cough in Portugal (EPICOUGH.PT) – a cross-sectional study in primary care". It will be developed in the next two years after approval by the Ethics Committee.
- Finally, OSCAR: vOice Screening of CoronA virus is a one-year project aiming to search an algorithm on the sounds of SARS-Cov-2 (voice and cough) to help the scientific/health community to detect COVID -19. This is a consortium between NOVA Medical School, Fraunhofer Foundation and NOS innovation.

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PSYCHIATRIC DISORDERS AND MENTAL HEALTH

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I. INTRODUCTION

Based on sound epidemiological and clinical data, it is now recognized that psychiatric disorders and mental health problems have become the main cause of disability and one of the main causes of morbidity and premature death, especially in Western industrialized countries.

According to the Portuguese Directorate-General of Health data, in 2010 psychiatric disorders represented 11.75% of the global burden of disease in our Country, measured by the disability-adjusted life years (DALYs) and only preceded by brain/cardiovascular diseases, with an overall weight of 13.74%.

Scientific evidence of the specific contribution of psychiatric disorders to the global burden of disease has led governments and international institutions to devote increasing attention to the area of mental health, both concerning the burden on populations (morbidity, mortality, disabilities) and the provision of care (organizational model, access, liaison, diversity and improvement of care, quality, costs).

In the last decade, the impact of elements with a strong social inclination has also grown considerably, such as the advent of human rights and citizenship, the fight against stigma, the participation of patients and families in decision-making processes, the reorientation of services towards users' recovery, or the emergence of promotion and prevention.

All these elements created new challenges and needs for today's societies. Under their combined pressure, psychiatric services around the world have been changing their structure and practice, gradually getting closer to the populations, who in turn assume greater empowerment and participation in the organization and functioning of the services. From a global mental health perspective, although the "clinical disorder" remains the core element, it is now framed by other equally critical dimensions: well-being, subsyndromal psychological distress, prevention, promotion, rehabilitation, recovery and human rights. In Portugal, the reshaping of psychiatric services has been characterized in recent decades by a succession of advances and setbacks, alternating phases of significant transformation (embodied in mental health laws) with periods of reflux. For a long time, these issues prevented the system from meeting the needs of the population in more adequate ways.

NOVA Medical School, through the action of faculty staff of its Mental Health department, has been directly involved in the Portuguese mental health reform, encompassing domains such as service organization, legislative drafting, and post-graduate training and research.

II. STATE OF THE ART REVIEW

Social stigma, distance from other areas of medicine, and the centralization of care in psychiatric hospitals have meant that, for decades, mental health problems have been largely underestimated and outside the political priorities of governments.

The study "The Global Burden of Disease" was a turning point in this situation, showing that in Europe psychiatric disorders (depression, alcoholism, schizophrenia and bipolar disorder) were responsible for 40% of the years lived with disability, clearly standing out among the ten main general causes of disability. The launch of the World Health Organization, WHO Report 2001 "Mental Health: New Understanding, New Hope", together with the unstoppable emergence of human rights movements, were other milestones of a paradigm shift in mental health (https://www.who.int/whr/2001/en/).

In addition to their specific contribution to the global burden of disease, psychiatric disorders also have an indirect effect on increasing this burden, mediated by a complex interaction with other clinical conditions and dysfunctional lifestyles, e.g., cardiovascular and metabolic diseases, substance use, road and occupational accidents. The magnitude of this impact results not only from the high prevalence of psychiatric disorders, but also from the fact that a significant proportion of individuals start treatment late, or do not even have access to appropriate care.

Access to care has been a most in-depth studied area in recent years, with several of its determinants already identified: stigma and ignorance in the face of mental illness, scarcity of human and structural resources, low priority in terms of political options, disproportionately low budgeting considering the burden of disease involved, poor organization of services, with a concentration in old institutions and lack of coordination with primary health care. Due to the combined action of these determinants, there is a marked discrepancy between the number of people who need care and those who actually receive it. This 'treatment gap' may reach 50-70% for an annual epidemiological prevalence of 20 -30%, both in the United States and in Europe. The recognition of this major problem led the WHO to launch the 'Mental Health Gap Action Program', with the aim of helping countries to respond politically and technically, based on four complementary dimensions: information, policy and program development, advocacy and research (https://www.who.int/teams/mental-health-and-substance-use/mental-health-gap-action-programme).

Furthermore, the direct and indirect costs of psychiatric disorders, resulting from health care expenses and decreased productivity (e.g., unemployment,

absenteeism, sick leave, support for family relatives), have a huge economic impact on public budgets, reaching up to 20% of all health costs. This illustrates unequivocally the relevance that mental health policies must assume in the context of each country's general health policies.

The Lancet Series (firstly launched at 2007) offers an excellent overview of the new challenges and paradigms in mental health, namely in what concerns global mental health (https://www.thelancet.com/series/global-mental-health). In 2018, the 'Lancet Commission', taking the opportunity offered by the Sustainable Development Goals to enhance the scope of global mental health, proposed that "*the global mental agenda should be expanded from a focus on reducing the treatment gap to improving the mental health of whole populations and reducing the global burden of mental disorders by addressing gaps in prevention and quality of care. The Commission outlines a blueprint for action to promote mental wellbeing, prevent mental health problems, and enable recovery from mental disorders*". (https://www.thelancet.com/commissions/global-mental-health)

The situation that the world is currently going through is an excellent example of the challenges that societies face with regard to mental health. Indeed, the crisis we are experiencing during the COVID-19 pandemic will certainly have a significant impact on the mental health of the populations. Although it is too early to assess whether there has been a real increase in the prevalence of psychiatric disorders, there is already evidence of an increase in mental health problems (with a predominance of symptoms of anxiety, depression and insomnia). This came to show, on the one hand, the population's vulnerability to psychological distress in a crisis situation, and on the other hand, the need for countries to reinforce their responses, guided by the principles of proximity and continuity of care. Developing community services, promoting depression and suicide prevention programs, strengthening crisis intervention and support to health professionals, are all challenges that countries will have to continue to respond to (https://www.who.int/teams/mental-health-and-substance-use/covid-19).

III. RELEVANT CONTRIBUTIONS FROM NOVA MEDICAL SCHOOL

The contributions of NOVA Medical School's Mental Health Department stem from the clinical, teaching and research work of its members, among whom there are distinguished figures of Portuguese psychiatry and medicine.

Founder of the Mental Health Department, Eduardo Luís Cortesão made an enormous contribution not only to the Medical School, but also to psychiatry and mental health in general. An eminent clinician, analyst and pedagogue, he developed relevant concepts both for the theory and practice of Group Analysis, as well as for the connection between somatic medicine and psychiatry, namely regarding theories of adaptation to illness. This former dimension was later developed by José Caldas de Almeida in renal failure and dialysis, by José Machado Nunes in coronary heart disease and heart transplantation, and by Domingos Neto in the treatment of alcohol and drug dependence. Equally important were the implementation of a national mental health training plan for GPs, and the planning of a truly pioneering forensic psychiatry service at Hospital Prisional de S. João de Deus (Caxias), in cooperation with Henrique Rodrigues da Silva. As part of the psychiatric reform, Cortesão participated in the movement to reform the Hospital Psiquiátrico Miguel Bombarda and in creating an innovative community-based mental health service in Western Lisbon, to become the Mental Health Center of Lisboa-Oeiras.

The theme of mental health policies was developed in great depth by Caldas de Almeida during his role as Chairman of the Mental Health Department. He was responsible twice for the national coordination of mental health (1988-90, 2007-11), as well as chief of the Mental Health Unit at the Pan American Health Organization, PAHO (2000-5). He was responsible for decisive contributions in the elaboration of national mental health policies and plans, in the implementation of mental health services, in legislative production, as well as in the boost of epidemiological research and evaluation of services, in which he deeply involved his collaborators. Postgraduate training was also developed during this period, firstly with the launch of a master and then a PhD course in mental health, which resulted in several lines of research, and eventually with an international master course on mental health policies and services, in partnership with the WHO.

At the research level, and in addition to the various lines under study in the department, two projects had a major impact at the national level: the first national study on psychiatric epidemiology (1), and the 'Joint Action for Mental Health and Well-Being'.

In terms of general psychiatric morbidity, the 1st National Epidemiological Study of Mental Health highlighted a high annual prevalence of psychiatric disorders (22.9%). In comparison with countries within the World Mental Health Survey consortium, this is only surpassed by Brazil, the United States and Northern Ireland. The survey also revealed that a significant percentage of people with mental illness

remain without access to mental health care, and many of those who do have access still do not benefit from the most essential intervention models (psychosocial treatment and rehabilitation) (2). Our participation in this international project allowed the inclusion of Portuguese data in the international sample, fostering an extensive series of studies and publications, available at the project's website (https://www.hcp.med.harvard.edu/wmh/ publications.php).

The Joint Action for Mental Health and Well-Being addressed issues related to five main areas: a) promotion of mental health at the workplaces; b) promotion of mental health in schools; c) promoting action against depression and suicide and implementation of e-health approaches; d) developing community-based and socially inclusive mental health care for people with severe mental disorders; and e) promoting the integration of mental health in all policies. The scientific contributions of the project translated into a framework for action on mental health issues (3), later pursued by the European Union (EU) Compass project.

The forensic area, due to its connection with the organization of services and legislative production, has also been privileged in the Department's research. Within projects of the EU, studies were conducted on compulsory admissions (4), mentally ill offenders (5) and mental health care in prisons (6). This allowed a benchmarking with the situation in all the other European countries, that would prove very useful for the reshaping of forensic services in Portugal.

Another relevant line of research has been the assessment of the needs for care of patients with schizophrenia (7–9). This developed in parallel with the assessment of the quality of mental health services (10,11), with important contributions to the implementation of mental health plans and programs in the country.

Depression in the context of primary health care was also considered a central research topic, since it was closely linked to the clinical liaison practice established, from the very beginning, between the Department and health centers in the same catchment area.

The PREDICT-D study aimed to develop an algorithm to evaluate the risk of developing major depression in primary care attendees (12,13). Until then, strategies for prevention of depression were hindered by lack of evidence about the combined predictive effect of known risk factors. We studied a large sample from Europe and Chile, to construct a final predictive algorithm. This functions as well as similar risk algorithms for cardiovascular events and may be useful in the prevention of depression. This study in primary health care then extended to research on predictive algorithms for anxiety disorders (14) and alcoholism (15).

Ricardo Gusmão, a former member of our group, was also responsible for important inputs in the field of depression and suicide. This included collaborations within the "European Alliance Against Depression" (16), namely the OSPI Europe (Optimizing Suicide Prevention Programs and Their Implementation in Europe) approach (17).

Meanwhile, other relevant contributions took place, through Master and PhD dissertations, or small-scale research grants. These included needs assessment in forensic populations (18), implementation of integrated programmes for persons with schizophrenia and related disorders (19) and case management programmes (20), or challenges regarding adherence to treatment (21). The neuropsychiatric interface has also been an important line of research, particularly in the obsessive-compulsive area (22,23). Domingos Neto, another former member of our group, implemented a novel approach in the treatment of alcohol related disorders (24).

A major interest of ours regards families and chronic disorders. Since the first ENMESH (European Network for Mental Health Services Evaluation) conference in Amsterdam, 1994, we started to explore the impact of mental disorders on informal caregivers and families. On the one hand, there are negative components such as caregiver burden and psychological distress (anxiety, depression). On the other hand, positive aspects of caregiving must be acknowledged. In a first phase, our focus was on severe mental illness (schizophrenia and related disorders). International collaborations allowed us to explore for the first time how family burden, coping strategies and social support would evolve longitudinally, in large multinational samples (25,26) or confronting typical patterns in northern and southern European countries (27,28). We concurrently pursued the systematic validation of Portuguese translations of questionnaires for the assessment of caregiving experiences (29–32). This was a necessary step towards a valid and reliable evaluation of family psychoeducational interventions in schizophrenia, which we developed in Portugal (the Families of People with Psychosis Study, FAPS) (33), and within international networks that implemented groups for relatives (34) and behavioural family therapy interventions (35). Drawing on this experience, we were able to accommodate crucial technical elements in the context of ageing disorders, which leads us to a final topic.

In fact, for the last twenty years, there was a growing interest of the group in ageing and mental health, including neurocognitive disorders. This was expressed, for example, in a systematic review that contributed decisively to support depression and bipolar disorders as risk factors for developing dementia (36).

First, we analysed the prevalence of old age neuropsychiatric disorders in Portugal, as this information was not available from the World Mental Health Initiative Survey study (1). For that purpose, we counted on a collaboration with the Institute of Psychiatry, King's College London, and the 10/66 Dementia Research Group (37). Community prevalence rates were 3.7% (95% CI 2.8-5.0) for DSM-IV dementia, and 9.2% (95% CI 7.8–10.9) for 10/66 dementia, the discrepancy between these two rates possibly reflecting a potential underestimation of dementia prevalence using DSM-IV (38). Regarding late-life depression, prevalence rates were 4.4 (95% CI 3.5-5.6) for ICD-10 depression and 13.0 (95% CI 11.2-15.0) for subsyndromal depression (where evidence supports the preventive value of non-pharmacological interventions) (39).

A second focus has been on psychosocial research and person-centred care in dementia, in line with the most recent INTERDEM manifesto (40), and highlighting the importance of 'social health' in dementia (41,42). This led us to studying in-depth the complexity of family caregiver experiences, to better target family work with older age people. A small-scale project with valuable international collaborations (Universities of Santiago de Compostela, Spain; Penn State University, USA) allowed us to tackle interesting research questions: the Lisbon Families of Persons with Dementia (FAMIDEM) study. We were able to demonstrate that knowledge about dementia is not necessarily protective of family burden nor psychological distress (43), that secondary caregivers are not necessarily more free from anxiety or depression then those at the front line (so-called primary caregivers) (44), and that caregivers' sense of coherence (an health promotion construct, broadly related to mastery or resilience) may be an important protective variable in caregiving contexts (45). These results influenced a series of reviews on family caregiving in dementia, undertaken to highlight factors contributing to protection and vulnerability (46), systemic views on family dynamics (47) and practical aspects of family-sensitive clinical approaches (48).

Third, we were inevitably interested in old age mental health service research in our country (49). Building on previous collaborative findings on needs assessment (50) and quality of life (51), our group participated in the Actifcare (Access to Timely Formal Care in Dementia) initiative (52). This multicentre European project coordinated by the Maastricht University was supported by the EU Joint Programme – Neurodegenerative Disease Research (JPND). The consortium analysed access to and use of formal services in the community, as related to unmet needs for care. Qualitative explorations with patients, caregivers and staff highlighted specific

barriers and facilitators (53), other studies analysing relationships between unmet needs and quality of life, while no association was found between unmet needs and care costs (54). Eventually, best practice recommendations were issued and disseminated in each country (55). Of note, the Portuguese arm of the cohort study underlined difficulties regarding timely access and effective use of formal care in dementia, along with relevant unmet needs in our participants (56).

IV. IMPACTS TO SCIENCE AND SOCIETY

a) Mental Health Policies

The Mental Health department at NOVA Medical School, from where four national mental health coordinators emerged (Henrique Rodrigues da Silva, José Caldas de Almeida, Álvaro de Carvalho and Miguel Xavier), is closely linked to the history of mental health reform in Portugal during the last 3 decades.

It is important to note that political contributions have almost always been associated with research work on the organization, evaluation and monitoring of mental health services, conducted by the Department.

Between 1985 and 1991, within the Mental Health Services Directorate, important steps were taken to develop a decentralized and community-based model of care delivery – several national programs were developed, such as cooperation with Primary Care, deinstitutionalization, training of professionals, psychosocial rehabilitation and monitoring of clinical information (case registers).

In 1998-9 a new mental health legislation framework was published, covering the organization of mental health services and compulsory admission.

In 2006, as the Government recognized the existence of a wide range of mental health problems in the population, an insufficient supply of care and a growing gap in the organization of services as compared to similar European countries, a new mental health plan was designed. The launch of this National Mental Health Plan in 2008 made it possible to achieve, in the following four years, relevant advances in several areas: legislative, organizational, closure of psychiatric hospitals, creation of new services in general hospitals, long term care, training of professionals, promotion and intersectoral cooperation.

After several years in which the implementation of the plan was interrupted due to the financial adjustment period, an extension to 2020 was drawn up in 2017, of which the first author was the rapporteur.

In this re-launch phase of the National Mental Health Plan, we selected four main areas: Organization of Mental Health Services, Mental Health in Children and Adolescents, Primary Health Care, Mental Health Law/Forensic Services (https://saudemental.covid19.min-saude.pt/#).

Recently, because of the effective work carried out in the direction of the National Program, Portugal will benefit from a very significant investment from the 'EU-The Recovery and Resilience Facility', to complete several key elements of the mental health reform.

b) Professional training and medical education

Regarding mental health professionals' education, our group provided interesting insights on the nature and process of family interventions in mental disorders (57), namely those targeting "expressed emotion" by Leff and col. (58) and "behavioural family therapy" by Falloon and col. (59). NOVA Medical School has for some time been active in the dissemination of these approaches, by conducting mental health team training in these interventions, despite the challenges of implementation in the real world.

In what concerns the permanent curriculum at NOVA Medical School, we must consider undergraduate and postgraduate education contributions.

Regarding our teaching programmes of the Integrated Master of Medicine, we have focused on preparing the students to manage at a non-specialized level the most common mental health problems like anxiety and depression, recognizing the ubiquitous importance of mental health and its bio-psycho-social determinants. An additional focus is on clinical communication training, with a special emphasis on the emotional aspects of medical encounters, and the doctor-patient relationship. Specific topics include how to handle situations with overtalkative patients, aggressive or inadequate behaviours, or silence; delivering bad news; problems with adherence or the basic principles of motivational interviewing. At the same time, we have tried to foster the development of empathy skills in general (60,61) and more positive attitudes towards mental disorders (62).

The current curriculum includes a stepped approach to meet these objectives, starting with our structured collaborations in Curricular Units e.g., Introduction to Clinical Practice (2nd year), Medicine and Society (3rd year), followed by Medical Psychology and Behavioural Medicine (4th year) and Psychiatry (5th year). A final one-month rotation in Mental Health (6th year) provides a practical opportunity to contact directly with persons with severe mental illness, learning the basis of

working in multidisciplinary community or hospital-based psychiatry and mental health teams.

Regarding postgraduate teaching programs, besides running a local PhD program, NOVA Medical School has been involved in the development of several international MSc courses:

- the aforementioned and no longer ongoing International Master in Mental Health Policies and Services (coordinated by José Caldas de Almeida and Benedetto Sarraceno), in collaboration with the Department of Mental Health and Substance of the World Health Organization (WHO);
- the MSc in Mental Health Recovery and Social Inclusion (coordinated by the University of Hertfordshire, UK, and involving also schools from Portugal, Italy and Poland);
- the International MSc in Primary Care Mental Health, whose overall goal is to train the primary care and community workforce in mental health, improving and promoting mental health skills in primary care.

V. ONGOING RESEARCH

Our group at CEDOC, the Chronic Diseases Research Centre of NOVA Medical School, now works under the aegis of the Comprehensive Health Research Centre (CHRC). While José Caldas de Almeida and collaborators remain active at the Lisbon Institute of Global Mental Health (https://www.lisboninstitutegmh.org/), a new designation was coined for the NOVA Medical School group, led by us: the Mental Health, Psychiatry and Dementia Research Unit.

This is a transition phase, where we want to build on our experience on clinical and psychosocial assessment and interventions in psychiatric disorders throughout the life cycle, and epidemiological and health services research. As described, we coordinated or collaborated in multicentre studies funded by European and national funding agencies (e.g., adult and old age psychiatric epidemiology, prediction of depressive episodes, needs assessment in schizophrenia and dementia, family burden, psychoeducational interventions, forensic psychiatry, mental health in prisons, access to timely formal care in dementia). We maintain strong connections with research networks like INTERDEM (http://interdem.org/) or the 10/66 Dementia Research Group (https://1066.alzint.org/), alongside close collaborations with clinical services, affiliated or not with NOVA Medical School. The same applies to the

third sector, patient and family associations (e.g., Alzheimer Portugal). Therefore, we are strongly committed to pursue specialized research in our current areas of interest (Table 1).

Mental Health, Psychiatry and Dementia Unit at the CHRC
Main topics for currently ongoing research

- Clinical and psychosocial evaluation of psychiatric disorders and dementia, with a special focus on family aspects.
- Psychiatric epidemiology (psychiatric disorders and dementia, and their determinants).
- Needs and quality of life assessment of people with mental health problems and dementia, and their informal carers (e.g., general population, primary care, secondary care, tertiary care, prisons).
- Non-pharmacological interventions and person-centred care, including psychoeducation; multidisciplinary approaches to older people care, palliative geriatric medicine, prevention of mental disorders and mental health promotion; social health in dementia; information and communication technologies in dementia care.
- Evaluation of mental health policies, programs, and interventions (from local to national level).
- Stigma, attitudes toward psychiatry, recovery and social inclusion.
- Adherence to treatment.
- Implementation science related with the organization of mental health services.
- Communication, humanism and spirituality in health.

Table 1

Two examples may better illustrate the kind of research we are developing in dementia, both stemming from fruitful PhD projects nested in international collaborations. The first focuses on the quality of relationships in dyads of persons with dementia and their family caregivers, by using mixed-methods (quantitative and qualitative) approaches (63). The second is an example of health services research, with a special interest in communication and social health ("Dementia

in Primary Care: the Patient, the Carer and the Doctor in the Medical Encounter" – Bayer / NOVA Health Ageing 2018 grant). This ongoing project brings together researchers from different medical specialties and health science areas within NOVA University Lisbon and colleagues from the United Kingdom. Acknowledging that primary care services have an underestimated but highly relevant potential in dementia care (41,49,64), the idea germinated after a PhD project on the role and challenges of general practitioners and primary care teams overall in managing the great challenges posed by neurocognitive disorders (65).

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FROM THE IMMUNOLOGICAL PARADIGM OF PREGNANCY TO THE CHALLENGES OF PRESENT MATERNAL-FETAL MEDICINE

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1. INTRODUCTION

The main function of the Immune System is, admittedly, the protection of the organism against pathogens, such as bacteria, viruses, fungi, or parasites. In this way, the diverse immune players developed and differentiated throughout the evolution of the species, gaining the capacity to discriminate self from non-self patterns, antigens and molecules. After exposure to a foreign, non-self, antigen, the articulation between the different activation mechanisms of the immune response will be sequential and complementary, also leading to the generation of immune memory for the antigen in question. However, immune responses are supported by mechanisms of tolerance and suppression, which ensure the homeostasis of the responses and are of particular importance in the success of pregnancy.

During the gestation period, the pregnant woman will be exposed to paternal antigens expressed by the foetus, so the presence of tolerance mechanisms during pregnancy seems indisputable. Nonetheless, the maternal immune system must not neglect its protective functions towards infectious aggressions, since it must still ensure protection for both the mother and the foetus [1, 2].

Immunological tolerance represents the absence of rejection, allowing the coexistence of two organisms, assumed in a systemic way. However, the pregnant woman is not tolerant to foetal antigens, as suggest by studies in animal models, in which foetal tissues were transplanted outside the uterine area and were in fact rejected, without changing the viability of the embryos until the end of the pregnancy [3]. The existence of maternal T cells against paternal alloantigens [4] also corroborates these ideas, assuming, then, that their activation and activity are controlled by processes of temporal (and spatial) regulation imposed during pregnancy [3].

In fact, an "immunotolerant" mother would be susceptible to the possible attack of foetal immune cells that might cross the placental barrier, and we will still have to consider that the foetal immune system itself must be able to limit the action of maternal cells, capable of surpass the cocoon of foetal trophoblasts. Now, this complex network of regulatory elements has led to the alternative concept of tolerant symbiosis between mother and foetus [5, 6].

"Maternal-Foetal" refers to both mother and baby during the time before birth, which means obstetricians deal at least with two patients at the same time, or even more, in the case of multiples. Women outside the optimum childbearing age and those with chronic health conditions are more prone to adverse obstetric outcomes

and need highly specialized care during pregnancy and delivery, to minimize risks for themselves and their babies.

Though great advances have been achieved in the comprehension of the immunology of pregnancy, certain conditions like recurrent pregnancy loss, premature labour, foetal growth restriction, diabetes, and preeclampsia remain true challenges in maternal-foetal medicine practice.

2. STATE OF THE ART – REVIEW

2.1. The Immune Clock of Pregnancy

Although the role of tolerance processes is recognized in the establishment and maintenance of pregnancy, the idea that different immunological stages, sequentially established over the weeks of gestation, is being widely accepted, and comprises three major time points: the period of implantation, marked by a strong inflammatory component; the period of foetal growth, where Th2 responses prevail; and the final period of preparation for childbirth, where pro-inflammatory processes are once again gaining relevance (Figure 1).

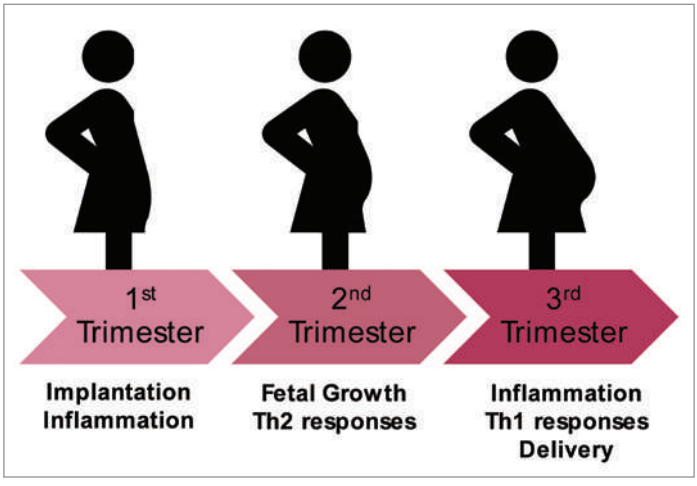

Figure 1 – Immune stages of Pregnancy.
Adapted from Mor and colleagues [2].

Assuming the concept of an immune clock in pregnancy, the Stanford University group observed that the signalling pathways responsible for promoting

immune responses against the foetus appear to be reduced in early pregnancy, but as the pregnancy progresses, immune patterns, especially adaptive immunity, begin to change, alternating between activation and immune suppression, in a remarkable effort of the mother's immune system to tolerate the foetus. At the same time, there seems to be a constant state of alert at the level of the mechanisms of the innate immune response that is maintained throughout pregnancy [7, 8].

The initial period of implantation of the embryo is characterized, therefore, by the presence of a pro-inflammatory environment, somehow similar to the one observed in processes of tissue damage or even to what occurs in the establishment of tumour micrometastases [2, 9]. Local inflammation, resulting from the release of cytokines (IL-6, IL-8, GM-CSF, CXCL1, CCL4, or TNF) by endometrial cells and infiltrating immune cells, appears to be essential to promote the expression of adhesion molecules in the surface of the endometrial epithelial cells, also promoting the elimination of the pre-existing mucin layer that compromises the adhesion of the blastocyst [10].

In the continuation of the initial implantation phase, it is also important to realize that the anatomy of the maternal-foetal interface, where physical barriers between mother and foetus develop, such as the placenta and amniotic sac, does not prevent the occurrence of several local and systemic interactions between the two immune systems [11]. Immediately after implantation, invasion of the endometrium by the foetal trophoblast begins. From a very early age, extravillous trophoblasts will contact locally with cells of the deciduous stroma, but also with NK cells (uterine NK cells, uNK), macrophages, γδ lymphocytes and regulatory T cells (Treg), already present in the endometrium, and which will interact with the progression of the trophoblastic invasion. Obviously, this whole environment is the result of a highly balanced and regulated crosstalk between the immune and the endocrine systems. The evidence is numerous, with reports for example on the role that progesterone and oestradiol, secreted by the placenta, assume in the differentiation, maturation and regulation of the function of dendritic cells (DCs) in the maternal-foetal interface [12-14] or the importance of hCG - Human chorionic gonadotropin, secreted by the trophoblast, as an chemotactic agent, also contributing for the conversion of non-regulatory T cells into Treg cells [15, 16].

From this moment on, pregnancy seems to be based on a deviation of the immune response towards a Th2 profile, with a strong anti-inflammatory component, to which regulated interactions between the maternal immune system and the foetal immune system also contribute [17]. Th2 cytokines produced at the level

of the maternal-foetal interface also contribute to the maintenance of pregnancy [18, 19]. However, it has been postulated that more than a deviation of the response to the Th2 profile, pregnancy assumes regulatory mechanisms that balance the production of cytokines [20]. The maintenance of maternal-foetal tolerance is thus recurrently associated with the regulatory effects of the Th2 response, but above all with mechanisms imposed by Treg cells [21-23].

The beginning of pregnancy is accompanied by an important rise in regulatory T cells, which increase both in the systemic circulation and in the uterus [24]. The clonal expansion of peripheral and uterine Treg cells, together with the presence of uterine NK cells (uNKs) and tolerogenic DCs, guarantees the tolerance of the foetus until delivery. Specifically, these mechanisms will be important to regulate cytotoxic T cells during pregnancy, in order to avoid local responses against foetal cells, maintaining the normal alertness against pathogens [25]. The increase and subsequent normalization of Treg cells during pregnancy is followed by the persistence of memory regulatory T cells after delivery, which will maintain tolerance to the foetal antigens of paternal origin. In a subsequent pregnancy, from the same male parent, memory Treg cells proliferate rapidly, managing the regulation processes of the new pregnancy [26].

Finally, it is necessary to move towards a new pro-inflammatory stage to start labour mechanisms. A new influx of immune cells into the myometrium is crucial, which will contribute to the contraction of the uterus, childbirth, and the separation of the placenta. Interestingly, one of the mechanisms described for this effect results from the secretion of surfactant A by the foetal lungs, which is a ligand of innate immunity receptors that activates the NF-κB factor signalling pathway [27]. Macrophages present in the amniotic fluid respond to these molecules and increase the production of cytokines such as IL-1β, crucial for the initiation of labour that is also associated with the release of prostaglandins [2, 27, 28].

Recognizing that innate immune cells (macrophages, but also neutrophils and mast cells) can thus mediate the delivery process by releasing pro-inflammatory factors, there is also growing evidence that changes in the function or quantity of adaptive immune cells, T and B-lymphocytes, can lead to term delivery or even premature birth processes. In addition, immune cells that bridge the innate response and the adaptive response, such as DCs or NKT cells, can contribute to the processes of inducing premature labour [29].

In conclusion, a balance between innate and adaptive immune cells is necessary to sustain pregnancy, and the physiological change in this balance will lead to

full-term delivery, but early or unregulated changes can lead to several pregnancy complications such as premature delivery [29].

2.2. The Biologic Clock

Fertility changes over the course of life. The expression "Biological Clock" generally refers to the psychological pressure felt by women related to the fact that it's usually harder to get pregnant later in life. Actually, there is extensive scientific evidence that the number and quality of both eggs and sperm decline as we age [30, 31]. Despite this evidence, the number of women who give birth at an older age is increasing [32]. Reasons for this postponement are related with cultural, social, and economic changes that took place in our society [33-35].

But this delay of motherhood has consequences for the mother and the newborn. Along with infertility and an increase use of assisted reproduction techniques, women of advanced age are at a significantly increased risk of gestational complications such as pregnancy loss, preterm labour, multiple pregnancies and those related to an increased prevalence of chronic diseases as diabetes, hypertension and autoimmune diseases [32, 36].

Research in these emergent areas of Maternal-Foetal Medicine is highly needed and there are a lot of opportunities for clinical and translational cooperation.

3. RELEVANT CONTRIBUTIONS FROM NOVA AND IMPACTS TO SCIENCE AND SOCIETY

3.1. Contributions in Reproductive Immunology

The obvious ethical implications of the approach to uterine tissues during pregnancy have hindered further studies in humans, or alternatively, have promoted the use of the characterization of circulating immune populations. Recognizing the scarcity of information on the approach of pregnancy imposed immune alterations, the Reproductive Immunology group at NOVA Medical School explored the evolution of several circulating immune populations and other related biomarkers in a cohort of women with uncomplicated pregnancies, followed from the third trimester of gestation up to six weeks after labour.

For this purpose, multiparametric flow-cytometry protocols were applied to peripheral blood samples collected from the pregnant women included in the study, in three time points: within weeks 32 and 36 of gestation, in labour day

and finally, six weeks after labour. The immunophenotyping strategy included the characterization of T and B cell subsets, from differentiation patterns (including distinct naïve or memory subsets) to recognized regulatory or regulatory-like phenotypes, but also the assessment of functional responses with the analysis of IFN-gamma, IL-17, or IL-10 secretion upon stimulation. Non-pregnant healthy women were recruited in parallel, to assure an age-matched control group.

First, we described the levels of circulation peripheral blood subsets, and besides minor variations in the naive/memory patterns for T-cells from late pregnancy to puerperium, we found that Treg cells with the $CD4^{Dim}CD25^{Hi}$ phenotype decreased significantly on the day of delivery in relation to the third trimester observations, with a significant increase in Treg populations after the puerperium [37]. Similarly, levels of Foxp3, the transcription factor that regulates Treg cells, appear to be decreased during pregnancy, and even during childbirth, possibly in response to hormonal modulation imposed by progesterone and oestradiol, restoring their expression levels throughout the puerperium period [37, 38].

For a long time, B cells have been somehow relegated to a secondary role in pregnancy, and even in other immune-related conditions, since research has focused attention mainly in immunoglobulins and their humoral functions. Following the sparse studies available, most of which exploring animal models [39, 40], the NMS group cohort brought new insights into this disregarded field. Not only we corroborated the existence a physiological lymphopenia of circulating B cells, which is observed from the middle of pregnancy onwards, but we also observed that throughout pregnancy, the B cell compartment undergoes important adaptations with a decrease in more differentiated subsets such as plasmablasts or memory cells, compared to the levels reported in non-pregnant women. Additionally, a recovery of circulating B cell occurs during the postpartum period, when we see also an important increase in the presence of transitional B-cells, also supporting the resuming of normal levels of B-cell lymphopoiesis [41-43].

Currently, regulatory B-cells have also been implicated in the process of maternal-foetal tolerance, for instance contributing to maintain DCs immaturity or supporting the expansion of Tregs. Bregs were able to control abortive processes in animal models with compromised immune tolerance, strengthening their relationship with the maintenance of immune balance in pregnancy [44]. In fact, their regulatory potential is under study for developing therapeutic approaches in obstetric pathologies [45]. Interestingly, the Portuguese cohort reported lower levels of IL-10 secreting B-cells during pregnancy and labour

day, suggesting also possible alterations in the regulatory functions of B-cells during pregnancy [41].

Moreover, when analysing the serum levels of B-cell associated markers, healthy pregnant women showed decreased levels of soluble CD23, a low affinity IgE receptor and marker of naïve B cell differentiation to memory B cells, which recovered postpartum [46, 47]. Additionally, in the third trimester of pregnancy there were also increased levels of BAFF, that reaches even higher values on the day of delivery and during the postpartum period [46]. BAFF is a B cell survival factor, extremely important to promote B-cell maturation and appears to be secreted by the placenta, to act as an essential element in embryo implantation and in maintaining pregnancy [48, 49].

The impact of different modes of delivery on maternal immune cells is also a subject under discussion in the scientific community. The study by Lima and collaborators addressed different immune populations in women with elective caesarean section, therefore without going into labour, and women with spontaneous vaginal delivery. The characterization of B and T populations showed only lower absolute counts of B cells and NKT cells in women with spontaneous vaginal delivery, with no changes in other populations such as Treg and Breg-like cells. Thus, labour does not seem to have a significant impact on circulating lymphocyte populations, at least compared to the final stage of the third trimester of pregnancy [50].

These findings may allow clinicians and researchers to recognize normal fluctuations in immune subsets and associated biomarkers, to better understand immune regulation during human pregnancy. In fact, recognizing the growing importance of other immune-related conditions in western countries, such as autoimmune and allergic diseases, a parallel study was also performed for atopic pregnant women, along the same gestational and postpartum periods, in an attempt to identify alterations associated with the underlying atopic or allergic background.

After recruiting both atopic pregnant and non-pregnant women, we were able to identify a distinctive immune profile in non-pregnant atopic women when compared to healthy counterparts (i.e., for the B-cell compartment, increased B cell counts, enlarged memory subsets, with less plasmablasts and transitional cells, or for the T-cells, an increase in regulatory T cells, possibly driven by therapeutics). However, the comparison of atopic and healthy pregnant women showed remarkable similarities, somehow reinforcing the idea of a pregnancy-imposed immune profile, for instance with reduced B cell counts, fewer memory cells and higher plasmablasts, but also with lower levels of Foxp3 expression in Tregs [42, 51].

Nonetheless, the levels of Foxp3 were not so low in atopic pregnant women (as observed in the comparison of the non-pregnant groups), thus suggesting that the atopic background can exert its influence, though to a lesser extent. In fact, we also observed slight increases in the production of IL-10 in atopic pregnant women, compared to healthy, somehow highlight interferences in the regulatory functions to be further clarified, as we addressed atopic women with controlled disease (asthma and/or rhinitis), and the effect of therapeutics must also be considered in the evaluation of the immune profiles [42, 51].

On the other hand, the changes observed postpartum reinforce the idea of a pregnancy-dependent-effect, probably hormone-driven, that loses strength afterwards. Not only cell subsets start to show more differences in atopic and not atopic women after puerperium, but also soluble biomarkers behave differently in these women, as we report for sCD23 in a recent study. Indeed, we observed different dynamics for serum levels of CD23: healthy women should decreased levels during pregnancy, which recover postpartum to pre-pregnancy levels; on the contrary, atopic women increase sCD23 levels during pregnancy (even compared to healthy pregnant), and still dramatically increased this marker in the postpartum period compared to non-pregnant atopic women [52].

Finally, it is well accepted that the maternal history of atopy is a relevant risk factor for the development of allergy in the offspring [53-57]. In fact, Liu and colleagues found that the history of maternal, but not paternal, atopy is associated with high levels of IgE in cord blood and with the occurrence of eczema [58]. The identification of risk factors for allergic diseases has been in our agenda for a while now. One of our first studies aimed to assess and compare the immunological status of steroid-naïve young children with three or more episodes of physician-confirmed wheeze, with and without clinical risk factors for developing subsequent asthma (i.e. parental asthma, personal history of eczema, wheezing without colds, among others). Wheezy children at a high risk of developing asthma showed lower absolute counts of Tregs and lower expression of IFN-γ and CTLA-4 after specific house dust mite stimulation, denoting and altered immune profile, which could be used as potential predictive factors for asthma in early life [59].

Nevertheless, although the transmission of a genetic background more prone to the development of a Th2 profile should be highlighted, together with the possible impact of environmental factors, the immunomodulation observed during pregnancy can also affect the development of the foetal immune system [56, 57].

In this regard, we followed the babies from atopic and non-atopic mothers in our cohort, in the first months of life, and identified that imbalances in maternal B-cells and associated markers could be related to the occurrence of allergic manifestations. Indeed, atopic women whose babies developed early allergic manifestations, showed an increased presence of circulating transitional B-cells in late pregnancy, suggesting that these cells may play a role in the regulation of the shift from Th2 to Th1 responses that occur in new-borns [42]. Similarly, maternal serum levels of free λ light chains, and even serum levels of IgG, were identified as possible predictive factors for allergic manifestations in babies born to atopic women [52]. The use of these biomarkers is now being validated in a larger cohort with extended follow-up of the children up to the age of two years. In brief, the possibility to identify early risk markers for allergy, may represent the first step towards the establishment of a prevention strategy for individuals more likely to develop these pathologies.

Considering the myriad of functions assumed by distinct immune subsets and molecules, in addition to their indisputable role in the immunoprotection of the foetus, their fluctuation in throughout pregnancy, and even in the postpartum period, can effectively be considered for a better understanding of changes in the course of autoimmune or allergic diseases during pregnancy and in the puerperium. Moreover, the recognition of normal profiles and evolution patterns may be incredibly helpful to identify new strategies for the diagnosis and treatment of pregnancy-associated disturbances, as well as the mechanisms of maternal responses to vaccination and infection.

3.2. Contributions in Maternal-foetal Medicine

NOVA collaborators are engaged in clinical research in some emerging fields of maternal-foetal medicine.

Pregnancy loss is a common event, occurring in 15% of all clinically recognized pregnancies. When recurrent, it can be devastating. An investigation carried out by us, revealed a great impact of recurrent miscarriage (RM) on a couple's lives and showed the existence of important gender differences in grief, attitudes and sexuality following this loss [60].

Other studies conducted by our group clarified the role of hereditary and acquired prothrombotic states in RM [61, 62]. Antiphospholipid antibodies may be present in patients with autoimmune diseases and in women with pregnancy losses. In a cohort of 157 pregnancies complicated with Systemic Lupus Erythematosus

followed by us, the main risk factors identified for gestational loss were maternal age and the presence of antiphospholipid antibodies [63]. Along with recurrent miscarriage and foetal death, women with Antiphospholipid Syndrome (APS) may suffer from foetal growth restriction, pre-eclampsia, premature delivery, and thrombosis. Conventional treatment with aspirin and low molecular weight heparin combined with close maternal-foetal surveillance can change these outcomes [64, 65]. In order to identify the most effective treatment strategy in high-risk primary APS pregnancies, our group was enrolled in a multicentre study carried out in 20 European tertiary centres; results showed that the effect of additional treatments, namely hydroxychloroquine, combined with conventional therapy were found to be safe and linked to a significantly higher live birth rate [66].

Premature birth, defined as a delivery before 37 weeks of gestation, is one of the great obstetric syndromes and its incidence seems to be rising, especially in higher income countries. It can have devastating consequences, particularly in very early gestational ages, as it encompasses severe neonatal morbidity and mortality. Despite its importance, current knowledge in premature birth is still limited.

One crucial field of research pertains to accurate prediction of preterm birth, as it could allow for implementation of preventive strategies (such as administration of tocolytics) or mitigation strategies (such as foetal lung maturation and neuroprotection), aiming to decrease the incidence of this syndrome and to improve its outcomes. The study of contractions through several methodologies has been proposed as a possible strategy in this setting, although current methods present multiple limitations [67]. Electromyography (EHG) has emerged as a very promising tool in this setting. A research conducted by our group in collaboration with the Department of Electrical Engineering and Computers from Faculdade de Ciências e Tecnologia (FCT) shown that EHG can automatically detect uterine contractions [67]; that it is possible to cluster these contractions according to their characteristics, allowing for risk stratification [68], and to visually present them in an intuitive and clinically meaningful way [69]. This line of translational research is an exciting and promising new field that can potentially accurately predict premature birth.

The incidence of pregnancies with more than one foetus has risen dramatically over the last decades. The likelihood of having twins or multiples increases with the use of fertility treatments, which are more common in older women. The risks with multiples include premature birth, gestational diabetes, preeclampsia (PE) and problems related with delivery.

To clarify some of these issues, studies regarding twin pregnancies were conducted by NOVA collaborators. Changes in BMI mothers of dichorionic twins and the outcomes of twin pregnancies with gestational diabetes where studied in several investigations [70-72]. Hypertensive disease in twin pregnancy was also the focus of our research and, recently, twin pregnancies were shown to have a nine-time higher risk for preterm preeclampsia in when compared to singletons [73-74]. The effect of maternal and pregnancy characteristics on the risk of PE was also calculated; results showed that screening for preterm PE using just maternal factors has a very high detection rate (99-100%) but at the expense of an extremely high screening positive rate (97-99%) [75]. Therefore, a model combining maternal characteristics with biophysical and biochemical markers was developed. The performance of this screening model for preterm PE was very good with a detection rate for PE before 32 weeks of 100%, at any cut-off used [76]. The safety of labour induction and the optimal time of delivery for uncomplicated twin gestations, and the perinatal outcome of triplets and monochorionic twins conceived by assisted reproduction were also investigated by our group [77-79].

Gestational Diabetes (GD) has been steadily increasing since the 1990s. This is partly due to changes in its diagnostic criteria, but mostly because of an increased populational incidence of its known risk factors, such as an advanced maternal age, higher body mass index and ethnic demography. In GD, there is an association between maternal hyperglycaemia and adverse perinatal outcomes and minimizing negative results implies optimizing metabolic control. Vigilance is mostly based on capillary glycemia. Notwithstanding, glycaemic indicators can provide different, additional information. The traditional marker in Diabetes Mellitus is HbA1c. Its clinical utility in GD, however, is controversial, especially because in addition to its known and transverse limitations (such as interpretation in hemoglobinopathies), it is affected by iron deficiency states (frequent during pregnancy), and it reflects the average concentration of glycemia over a long period of time (whilst pregnancy is a very dynamic state) [80].

Recently, NOVA investigations showed that glycated albumin and fructosamine, two short term non-traditional glycaemic markers, besides from providing additional information to Hba1c, when used separately perform better that the traditional biomarker in predicting neonatal birthweight and large-for-date babies in pregnant women with GD. They further showed that, in a prospective cohort of 85 pregnant women with GD and their new-borns, glycated albumin and fructosamine better discriminated mothers of infants with and without perinatal complications than HbA1c [81, 82].

4. ONGOING RESEARCH

Despite the outstanding developments in Reproductive Immunology achieved in the past decades, there is still much to explore in this field. The importance of several immune populations for a successful implantation was previously highlighted, as immune cells seem to be involved in both tissue remodelling and angiogenic processes that support this critical step. In fact, there is a growing evidence on changes in the different uterine immune populations and their recruitment in infertile women [83, 84]. A greater understanding of the uterine environment favourable to implantation has allowed the development of promising strategies to combat infertility. In women with a manifest reduction in uterine inflammation levels, pre-implantation uterine biopsies (endometrial scratching) appear to lead to an increase in uterine receptivity. This invasive procedure has the potential to increase the secretion of pro-inflammatory cytokines, promote the recruitment and differentiation of monocytes in dendritic cells and macrophages, in an activation cycle that ultimately allows an increase in the expression of adhesion molecules [2, 85].

Several studies demonstrate that the more prevalent lymphocyte population in the endometrium are uNK cells, functionally and phenotypically distinct from peripheral NK cells (pNK), typically presenting with a strong expression of CD56 and without CD16 [86-88].

Though their origin is still controversial, recognizing the possibility of its recruitment from peripheral blood to the uterus, where they may undergo maturation and differentiation processes, or, alternatively, be populations that differentiate from CD34 + hematopoietic cells present in the uterine mucosa [89, 90], the observation of reduced levels of uNK cells may be related to an increase in apoptotic mechanisms and a reduction in the remodelling of uterine arteries at the beginning of pregnancy, with potential implications for the implantation process [91].

A detailed characterization of local immune populations, such as uNK cells, seems therefore essential to improve the understanding of the physiological mechanisms of pregnancy, but also to analyse the impact of different immune elements on the pathogenesis of gynaecological and obstetric diseases, such as endometriosis, and its implication in different types of infertility.

Similar to what happens with peripheral NK cells, several receptors have been described in uNK cells, that are able to respond to molecules expressed by trophoblast cells, namely MHC molecules. However, limited information is available on the repertoire of NK receptors and their levels of expression in fertile and infertile

women, and mostly arise from immunohistochemical procedures, that are limited in multiparametric analysis. Thus, we have an ongoing study, using multicolour flow cytometry addressing a deep characterization of endometrial biopsies in fertile and infertile women (i.e. women with recurrent idiopathic pregnancy loss and women with recurrent implantation failure).

On the other hand, assuming that B-cell responses are regulated along pregnancy, according to the previously described, we aim to explore the context of maternal immunization, which has increased over the past decade, as an approach to protect the health of the pregnant woman, her foetus, and the infant, later. The recent resurgence of pertussis and diphtheria has been reported in several industrialized countries with a high associated morbidity and mortality, occurring primarily in young infants [92]. These events have resulted in policy changes for vaccines aimed to protect both mothers and their infants during the first months of life, and many health authorities are now recommending certain immunizations during pregnancy. In Portugal, it is actually recommended that pregnant women be vaccinated with a combined vaccine against pertussis, tetanus and diphtheria, ideally at week 32, considering the optimal timing for this specific vaccination is between weeks 27 and 36 of gestation.

Overall, it is of utmost relevance to better understand how B cells and their related immune players, such as the recently described follicular T cells, behave during a normal pregnancy, at first, to identify alterations resulting and even predicting bad pregnancy outcomes or further atopic conditions in the progeny. Furthermore, a deep understanding of these immune mechanisms can enlighten what we know so far about the efficacy of immunization strategies in this particular period for the maternal immune system, being useful for the improvement of safety and efficiency of maternal vaccination programs. Thus, it is also our goal to continue the characterization of the immune dynamics of the B-cell compartment during normal pregnancy, and how does it impact the normal immunization processes in healthy pregnant women.

With COVID-19 pandemic, obstetricians and immunologists faced new challenges. Translational research in NOVA on this subject is ongoing. In a collaborative effort, researchers are studying the impact of SARS-CoV-2 in maternal and foetal outcomes, including maternal blood, cord blood and placental evaluation regarding inflammation, presence of the virus and its receptors. COVID-19 raised other important questions that are also under investigation, namely the presence of antibodies in human milk after vaccination in breastfeeding women.

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FIGHTING CANCER IN ALL FRONTS

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INTRODUCTION

We all know someone whose life has been deeply impacted by cancer. This is one of the leading causes of death worldwide and a disease that inflicts tremendous suffering in patients and their families [1]. In 2020, the estimated new cancer cases exceeded 19 million and there were almost 10 million deaths caused by cancer [2]. These numbers already represent one in six deaths globally, but the cases and deaths continue to rise as the population grows and life expectancy increases. Low-income countries still have less access to early diagnosis and advanced treatments, but social and economic development also impacts negatively on some of the risk factors. COVID-19 is likely to make this scenario even worse as the pandemic is contributing for a reduction in cancer screening and diagnoses as well as a delay in some treatment programs, we may face higher death rates in following years, due to late detection and treatment of cancer cases.

Cancer is now globally recognised as a major challenge that needs to be tackled, leading governments and international organisations to make commitments to develop prevention and control programmes. Examples of those commitments are the World Health Organization Report on Cancer [1], the 2030 United Nations Agenda for Sustainable Development [3] and the Mission on Cancer from the Horizon Europe Framework Programme [4]. Progress has been incremental and slow in recent years, but the momentum seems to be shifting towards more robust action.

At NOVA University we are also very aware of this major health problem and we are involved in fighting cancer in many fronts. These includes developing fundamental research aimed at identifying the cellular and molecular mechanisms that cause cancer, lead to its progression and are responsible for treatment-resistance, and collaboration with oncologists in reference cancer hospitals to develop more translational research focused on improving diagnostic/prognostics tools and develop novel therapeutic approaches. Additionally, we are involved in international consortia to develop and implement targeted public health policies, aiming at improving cancer management, particularly in low-income countries. We highlight here two of the many areas of where NOVA is very actively contributing to help patients with this terrible disease and hopefully reducing cancer-related death rates. The first one, focused on breast cancer, aims to provide doctors with tools to be more precise in the selection of the best possible treatment for each person. The second example deals with integrated strategies for early detection of cervical cancer in hard-to-reach female populations.

LOOKING FOR BIOMARKERS AND NOVEL THERAPIES FOR BREAST CANCER

Breast cancer is the most common type of cancer, representing 11.7% of cases worldwide in 2020 [2]. The treatment commonly used in patients with locally advanced breast cancer is Neoadjuvant chemotherapy (NACT). This means that anti-cancer drugs are given to patients for a period of time before surgery, which potentially leads to a reduction of the tumour size and allows a more conservative surgery of the breast tissue. However, for reasons that are mostly unknown, more than 50% of patients do not respond to this treatment, and their disease often progress to more severe forms. In order to direct this "non-responder" patients more quickly to alternative or complementary therapies, it is very important to find reliable biomarkers predictive of the response to conventional treatment, avoiding their exposure to high doses of toxicity without a clear benefit.

NOVA Medical School researchers have demonstrated that for NACT to be successful, it is essential that a specific type of immune cells, the cytotoxic T lymphocytes (CTLs), are present in the breast tumour microenvironment and express high levels of the HLA-DR molecule [5]. Thus, it was proposed that CTLs expressing high levels of HLA-DR can function as a biomarker for the response to NACT and it was suggested that its evaluation in a tumour biopsy can be a very useful tool in the therapeutic decision process. To confirm the predictive, and eventually long-term prognostic, value of this biomarker, a validation study was conducted in an independent cohort of patients with breast cancer, anticipating its implementation in clinical practice. The biopsies analysed confirmed the initial results and allowed the establishment of a robust model of the probability of a patient to respond or not to NACT, applicable to all breast cancer subtypes. We believe that the model that has been have created will be a valuable tool to promote more informed therapeutic decisions, hence improving the quality of health care in breast cancer.

The correlation between CTLs expressing high HLA-DR and a better prognosis also prompted the exploration of the therapeutic value of the presence of this cell type in the tumour microenvironment. To investigate such possibility, an *in vitro* co-culture system was established by combining on breast cancer cell lines with patient immune cells [6]. This experimental set-up was instrumental in demonstrating that CTLs that express HLA-DR at high levels do have indeed anti-tumour properties, since they have the capacity to fight and decrease the viability of the breast cancer cell lines. These in vitro results are in line with the clinical observations and point to

a potential development of new therapies. Our preliminary results showed that we can manipulate, *ex vivo,* blood from NACT "non-responders", which has low numbers of CTLs expressing HLA-DR, at the baseline, modifying their phenotype towards a phenotype more similar to the CTLs of NACT "responders". Therefore, increasing their ability to reduce the viability of tumour cells and supporting the idea that it is possible to upsurge the numbers of CTLs expressing high levels of HLA-DR, consequently increasing their cytotoxic ability. Another approach would be the isolation of these immune cells that seem to be particularly aggressive to breast tumours, multiply their numbers ex vivo, and reintroduce them in the patients to fight the tumour. These possibilities are still at the early research stage and they could be challenging. Nevertheless, this research highlights the importance of investigating all the promising biomarkers and therapies in the laboratory to find the ones that may be used as tools to help oncologists to cure cancer patients.

EARLY DETECTION OF CERVICAL CANCER IN HARD-TO-REACH POPULATIONS

Cervical cancer is caused by Human papillomavirus infection (HPV) in more than 90% of the cases. In 2020, over 500,000 women were diagnosed with cervical cancer, with around 85% of the global burden occurring in ow- and middle-income countries [7]. Cervical cancer is one of the most detectable in pre-malignant stages and preventable cancers. Nevertheless, over 300,000 women die from this disease every year. Without further action to prevent or screen for cervical cancer, or to improve its treatment, a 25% increase in the number of new cases is predicted in the next 10 years. Prevention can be achieved through the implementation of public health interventions such as education, vaccination, screening programs and management of pre-malignant lesions [7]. It is known that in high-income countries, such interventions have reduced the cervical cancer incidence and mortality rates by at least 80%. However, those prevention programmes are not made accessible to every woman around the world, increasing the risk of cervical cancer in populations considered hard-to-reach.

Researchers from NOVA National School of Public Health participate in a Horizon 2020 funded project, ELEVATE, which involves a consortium with institutions from 8 different countries [8]. It assembles a multidisciplinary team, including medical doctors, public health researchers, economists, biological engineers, and

technology developers, with the aim of developing a new form of HPV test and an approach to improve cervical cancer screening in hard-to-reach populations. The test, based on microfluidics, molecular methods and electronics, will detect the presence of HPV, will combine self-sampling with a new low-cost, portable measurement device and will be validated in dedicated screening trials. The main target of the project are women in Europe and Latin America who have never been screened or are not regularly screened and who have a higher risk of developing cervical cancer. These women are often from minority groups, in disadvantaged socioeconomic situation, with migrant background, with lower health literacy or living in isolated areas and face multiple barriers in accessing preventive care.

The ELEVATE HPV test will be designed to be performed at the point of care and to yield easy-to-understand results on site, resulting in increased continuity of care and efficient follow-up processes. The new strategy and test will be validated in pilot studies with hard-to-reach populations in Belgium, Brazil, Ecuador, and Portugal, to determine the feasibility, user and health provider acceptability, costs, logistics, and population compliance of self-sampling and the rapid HPV testing device. The NOVA researchers will be responsible for profiling and identifying who are the hard-to-reach and most-at-risk populations, for addressing the social/contextual barriers that women face in cervical cancer screening, and for exploring what are the best strategies to reach those women, from several key stakeholders' perspectives. NOVA researchers will also contribute to design and implement an innovative intervention to pilot the new HPV self-sampling test and device with migrant socially vulnerable communities in diverse settings and will evaluate it in terms of communities' acceptability and cost-efficiency. The analysis of the social context and barriers will be considered when designing and developing the new HPV testing device, but this knowledge will also allow for optimised screening initiatives that will enhance participation among hard-to-reach women. Ultimately, this information will contribute for further refinement of national health strategies for cancer prevention and optimisation of existing cancer control strategies in Europe and Latin-America.

FROM MOLECULES TO SOCIETY AND BACK

Improving the outcomes of a disease as complex and heterogeneous as cancer requires a multi-level approach. Knowing more about the molecules that cause cancer only helps patients if the knowledge can be translated into useful

diagnostics and therapeutical tools to be applied by oncologists and other health professionals, and if all patients have access to those technologies and doctors in the healthcare system. On the other hand, the implementation of public health strategies is much more effective when the social and economic determinants of the disease are taken into account and when such knowledge drives advances in medical research and technology. At NOVA we are approaching cancer and other diseases at all those levels (Figure 1), aiming to address unmet medical needs such as untreatable breast cancers and insufficient diagnosis of cervical cancer by bringing together scientists from different disciplines, collaborating with hospitals and other stakeholders of the healthcare system, and participating in patient cantered purposeful international networks.

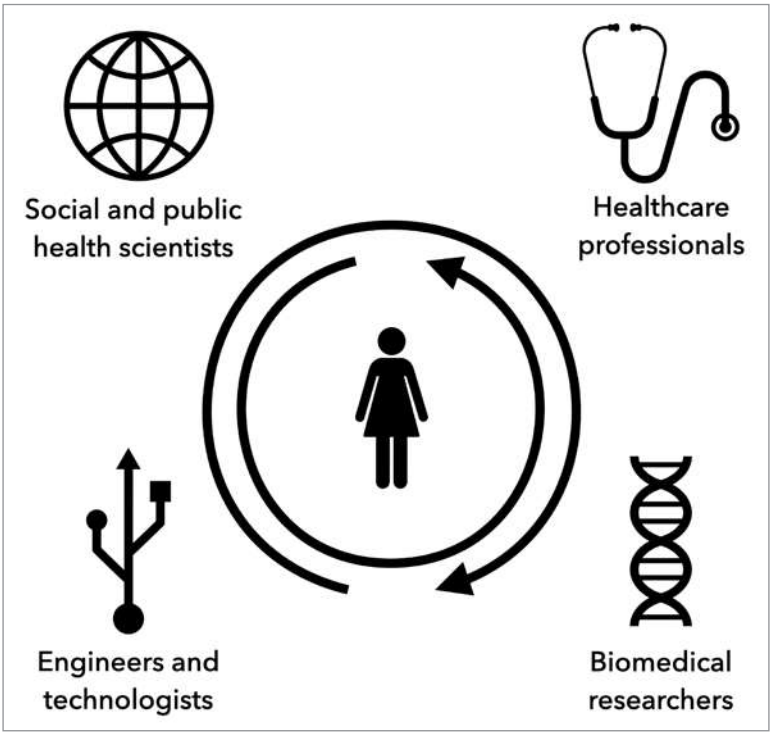

Figure 1 – NOVA health research on cancer is focused on the patients and approaches the unmet medical needs from multiple perspectives. Brings together social and public health scientists, doctors and other health care professionals engineers for different areas and clinical and biomedical researchers.

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CARCINOGENESIS – A CHESS GAME WITH VARIOUS GENETIC PIECES

José Rueff

Cancer is essentially a genetic disease, meaning that cancer can be caused by changes in our genes namely those that control how our cells divede. Those changes are mutational events, or as more recently accepted to be a more correct designation, pathogenic DNA variants.

Those pathogenic variants that trigger and promote cancer can be inherited and are present in germ cells (eggs and sperm). These variants are designated germline changes and found in every cell of the offspring. Cancers arising from germline mutations are events leading to rare cancers, rarely representing more than 10% of human cancers. A reason for this can be found in the fact that a cancer to 'succeed' may need not one but some 7 to 11 different mutations in different genes.

In another hand, cancer-causing genetic changes can also be acquired and accumulate during one's lifetime, as the result of errors that occur as cells divide (1×10^{-10} mutations/bp/cell division), or from exposure to environmental carcinogenic substances that damage DNA. Genetic changes that occur after conception are called somatic or acquired changes. Such DNA lesions can be taken as stochastic effects and as such may not be dose dependent. They depend on the effects of chemical nucleophilic agents in our environment to which man is exposed mainly through airborne or foodborne sources.

The other important source of DNA damage is ionizing radiation, may it be accidental radiation or diagnostic and treatment one. An important guide are the figures of conventional vs. CT effective doses when the latter gained in the last decades a much higher use: CT doses for chest diagnostic purposes are of the order of 7mSv compared to 0.1 mSv for conventional X ray. All together the estimated accumulated dose in a lifetime of 80 years can reach 800 mSv.

SOMATIC MUTATIONS – THE FIRST THREATS OF THE CHESS GAME OF CANCER

The International Agency for Research on Cancer (IARC) of WHO, identified and classified more than 500 agents in Groups 1 (carcinogens to human), 2A (probably carcinogenic to humans) and 2B (possibly carcinogenic to humans), depending on the weight of evidence for each Group and thus representing potential threats for man as carcinogens. Some 88% of those potential agents classified by IARC are DNA-damaging agents, i.e. they carry an unmistakable mutagenic or clastogenic activity.

Those primarily DNA lesions leading to the fixation of a mutation are the first damage in DNA, as if they would be a first movement of a pawn in a chess tray.

But the primarily event of DNA damage is far from fully explain alone the cancer process although it is amongst the first primarily events leading to cancer. Indeed, by quoting Benjamin Franklin: "Life is a kind of chess, with struggle, competition, good and ill events". The same in the cancer course where good and ill events take place all along the cancer process.

Nonetheless, mutations in about 1% of human genes (a total of about 290 genes) are reported to contribute to a cancer.

One can trace back the genetic origin of cancer to the early work of Theodore Boveri "Zur Frage der Entstehung maligner Tumouren" [*Concerning the Origin of Malignant Tumours*] firstly published in 1914 by Boveri and thereafter translated to English by his wife in 1929 (1).

Besides predicting in his work of 1914, the existence of tumour suppressor mechanisms, Boveri's monograph was perhaps the first to suggest that hereditary factors (genes) are linearly arranged along chromosomes. At its very centre though, the text of Boveri draws for the first time the attention to the genetic basis of cancer: "This primordial cell of a tumour (...) contains, as a result of an abnormal process, a definite and wrongly combined chromosome-complex. (...) This is ... the cause of the tendency to rapid cell proliferation, which is passed on to all the descendants of the primordial cell"

FROM BOVERI TO AMES – HOW PAWNS ARE CAUGHT, AND THE GAME BEGINS

During the 1970's and even in the 1980's, one could still hear far too often from distinguished cancer researchers that the observation of carcinogens as mutagens was not a proof of mechanism. Indeed, carcinogenesis is not only the fate of mutations or DNA lesions. Rooks, bishops or knights are key pieces in the chess which determine the fate of the game. Might they play the role of DNA repair mechanisms, or act as the tumour microenvironment of initiated cells not to mention the immunological surveillance and the hormonal *status* may be paramount in dictating the proliferation of the initiated cell.

As in chess, the initial DNA mutations as the exit of unprotected pawns, do not constitute *per se* the phenomenon that determines the loss of control of cell

proliferation. Those mutations are protected by rooks, bishops or knights which play mainly the role of DNA protecting mechanisms.

And even so, the biological behaviour of the initial tumour is not *per se* a hallmark of poor prognosis. A tumour is an agglomerate of cells, many of them constituting oligoclones with genetic micro heterogeneity that may be drug resistant and prone to highly metastatic behaviour. Or otherwise, a cancer is a thousand diseases with a multitude of oligoclones with the appearance of only one clone masquerading as only one disease.

A key role in the demonstration that carcinogens are mutagens, at least many them, was the development of the Ames assay by Bruce Ames.

The early episodes with the Ames test in our laboratory were on human serum lipoproteins (2). Why these circulating molecules? Simply to try to understand how a chemical that enters the body through the gut, or the respiratory tract or skin could travel to the target cells far apart and/or even before being metabolized in the liver. Indeed, many if not most, putative carcinogens are lipophilic (e.g. PAHs) and the reasonable carriers in circulation would be lipoproteins. With not so much success we could nonetheless show that VLDL (very low-density lipoproteins) are mutagenic in some individuals, something which led us to think that they could be carriers of environmental mutagens to which those individuals were exposed (2, 3). Data by others also pointed to a possible role of serum lipoproteins as preferential carriers of absorbed lipophilic carcinogens/mutagens in the body.

HOW TO TRACK LEVELS OF MUTAGENS IN OUR BODY – PREVENT A CHECK TO THE CHESS KING

Another of our works with the Ames assay and again having man, not experimental systems, as the centre of the research, was to assess the urinary excretion of mutagenic compounds in workers exposed to mineral oils and iron oxide particles. this work was carried out by concentrating urine in XAD-2 resins' columns to make it possible to be tested and used as biomonitoring assay of exposure.

The study was conducted in a main shipbuilding shipyard nearby Lisbon, with strict follow-up of the medical occupational staff of the company. The idea behind was not only to study those workers to assess their exposure, but mainly to evaluate if the Ames' assay could distinguish different putative levels in excreted mutagenic compounds in different working areas where workers were exposed.

And it did. Indeed, workers exposed to both mineral oils and iron oxide particles showed significantly higher levels of urinary mutagenicity as compared to the group of age matched workers exposed only to mineral oils. Since both groups included smokers, the effect of smoking was also assessed and it turned out that smoking tends to act synergistically in urinary mutagenicity with exposure to mineral oils and iron oxide particles, although it has a smaller effect than iron oxide particles in enhancing mutagenicity in urine of workers exposed to mineral oils.

To assess differences in patterns of chemicals excreted in the urine of the studied workers, a thin layer chromatography of urine concentrates was performed, a method which showed that sharp differences in chromatographic separation of compounds excreted by workers only exposed to mineral oils or exposed to mineral oils and iron oxide particles could be clearly demonstrated (5, 6).

IMPERFECT DNA REPAIR OR THE KING THREATENED WITH CAPTURE

If the safeguards of DNA may be mutated or functioning inaccurately not only by germline mutated DNA repair genes (e.g. *BRCA1/BRCA2)* but also by variants susceptible to lead to an improper repair, DNA can be said to be in check leading to an initiated cancer cell.

Our contributions to DNA repair variants have been concentrated mainly in thyroid and breast cancers.

Significant associations with thyroid cancer risk were observed for *CCNH* rs2230641, *MSH6* rs1042821, *XPC* rs2228001 and *XRCC3* rs861539. The association of *XRCC3* rs861539 with DTC (differentiated thyroid cancer, DTC) susceptibility had been frequently reported in prior studies and was confirmed in this work. Additional genotype-disease associations were observed upon stratification according to histological, gender or age criteria.

The aim in our studies in thyroid cancer was to evaluate the potential contribution of multiple SNPs, across different DNA repair pathways to DTC susceptibility. Also, for many of these SNPs (e.g. BER, MMR), it was the first time they were evaluated in the context of DTC susceptibility.

When investigating the joint effect of multiple SNPs, a clear-cut gene-dosage effect between the number of risk genotypes and DTC risk was observed, together with a high number of significant results on paired SNP analysis.

The involvement of polymorphic variants or SNPs across different DNA repair pathways (*XRCC3* rs861539 of the HR pathway, *CCNH* rs2230641 and *XPC* rs2228001 of the NER pathway and *MSH6* rs1042821 of the MMR pathway) in DTC susceptibility is expected: several DNA damaging agents, able to induce different lesions repairable by corresponding different pathways, have been proposed to contribute to DTC and even IR, the best-established risk factor for DTC, induces a complex pattern of DNA lesions – including single- and double-strand breaks, oxidative lesions (e.g., 8-oxoG), DNA-protein crosslinks (DPCs) and clustered DNA lesions – that require different pathways (and their crosstalk) for successful repair.

Moreover, many of these DNA repair proteins, besides their canonical actions in a specific DNA repair pathway, also play additional roles on cellular processes such as signalling for cell cycle arrest and apoptosis.

The observation of additional genotype-disease associations upon stratification (e.g. *XRCC3* rs861539, *NBN* rs1805794, *XPC* rs2228001, *ERCC5* rs2227869 and *MUTYH* rs3219489 in PTC; *MSH6* rs1042821, *MLH3* rs175080 and *XRCC2* rs3218536 in FTC; *XRCC3* rs861539, *MSH6* rs1042821, *XPC* rs2228001, *CCNH* rs2230641, *ERCC5* rs2227869 and *ERCC5* rs17655 in women; *XPC* rs2228001 and *XRCC5* rs2440 in younger patients; *XRCC3* rs861539, *CCNH* rs2230641, *ERCC6* rs2228529 and *RAD51* rs1801321 in older patients) suggests the existence of histotype, gender and age-specific SNP effects on DTC susceptibility.

To further clarify the role of DNA repair SNPs in DTC susceptibility, we analysed 36 SNPs in 27 DNA repair genes in a population of 106 DTCs and corresponding controls with the aim of interpreting joint data from previously studied isolated SNPs in DNA repair genes.

Significant associations with DTC susceptibility were observed for *XRCC3* rs861539, *XPC* rs2228001, *CCNH* rs2230641, *MSH6* rs1042821 and *ERCC5* rs2227869 and for a haplotype block on chromosome 5q. From 595 SNP-SNP combinations tested and 114 showing relevance, 15 significant SNP combinations ($p < 0.01$) were detected on paired SNP analysis, most of which involving *CCNH* rs2230641 and mismatch repair variants. Overall, a gene-dosage effect between the number of risk genotypes and DTC predisposition was observed.

The cumulative increase in DTC risk that was observed for increasing number of risk alleles as well as the high number of SNP pairs presenting significant findings are suggestive of additive (or even multiplicative) effects of different SNPs on DNA repair activity and, hence, cancer risk. This is biologically plausible since the

different DNA repair proteins physically and functionally interact with each other, within the same or different DNA repair pathways. Moreover, it establishes ground for a polygenic approach to risk assessment (e.g. through genetic risk scores) in DTC, a multifactorial disease that likely develops because of the interaction of multiple genetic and environmental factors (7, 8, 9, 10, 11)

Similarly, in studies we carried out in breast cancer it could be demonstrated that DNA repair genes' variants may be associated with cancer susceptibility.

As an example, using unconditional logistic regression we found that *MLH3* (L844P, G>A) polymorphism GA (Leu/Pro) and AA (Pro/Pro) genotypes were associated with a decreased risk: OR = 0.65 (0.45-0.95) (p = 0.03) and OR = 0.62 (0.41-0.94) (p = 0.03), respectively.

Analysis of two-way SNP interaction effects on breast cancer revealed two potential associations to breast cancer susceptibility: *MSH3* Ala1045Thr/*MSH6* Gly39Glu – AA/TC [OR = 0.43 (0.21-0.83), p = 0.01] associated with a decreased risk; and *MSH4* Ala97Thr/*MLH3* Leu844Pro – AG/AA [OR = 2.35 (1.23-4.49), p = 0.01], GG/AA [OR = 2.11 (1.12-3,98), p = 0.02], and GG/AG [adjusted OR = 1.88 (1.12-3.15), p = 0.02] all associated with an increased risk for breast cancer.

Still the combination of *XRCC1* rs25487 and *MUTYH* rs3219489 revealed a significant correlation for combination between heterozygous and homozygous variant (G/A – C/C), showing a differential risk for women carrying this combination [OR 0.321; 95% CI: 0.106 – 0.970; p = 0.044]. (11,12, 13, 14, 15, 16).

WHEN CHEMOTHERAPEUTICS MAY NOT BE THE WAY TO CHECK THE KING

When cancer prevention cannot be achieved and worse, cancer therapeutics cannot achieve success the dynamics of expression of cancer genes may represent the culprit of failure. Indeed, cancer drug resistance represents 90% of treatment failure in metastatic cancers.

Our contributions to the understanding of cancer drug resistance have concentrated in leukaemia and breast cancer cells.

In chronic myeloid leukaemia (CML) patients' *ABCG2* gene expression levels correlated with *ABCB1* and *ABCC1, and* interestingly there seems to exist *a* correlation between efflux genes and the influx gene *SLC22A1* which supports the hypothesis that absolute bioavailability may also be influenced by the balance between efflux

and influx transport and most of these transporters were also found over-expressed in the majority of resistant CML cell lines.

Efflux pumps of the ABC (ATP-binding cassette) transporters' family are subject to microRNA-mediated gene regulation. As a matter of fact, it appears that ABC transporters are regulated by a circuitry of microRNA-guided networks that mediate altered drug transport and cell survival upon defy by cancer drugs. There is increasing evidence that microRNAs are crucially involved in co-ordinating and fine-tuning this complex network of proteins mediating increased drug efflux and cell survival.

MicroRNAs play, therefore, an important epigenetic role in controlling the levels of expression of ABC transporters' genes, being thus connected with drug distribution as well as with drug resistance.

In another line of study to analyse mechanisms of cancer drug resistance in breast cancer cells we could demonstrate in KCR cells (doxorubicin-resistant, expressing ABCB1) that after 16 weeks of doxorubicin withdrawal, a decrease of ABCB1 activity and expression occurred. By analysing the expression of 1008 microRNAs before and after doxorubicin withdrawal and determining the signature of 23 microRNAs, 13 underexpressed and 10 overexpressed, three pathways were identified as relevant in cancer drug resistance (17, 18, 19, 20, 21)

CASTLING TO MOVE THE CHESS KING TO A SAFER POSITION

Carcinogenesis is still short of straightforward mechanistic pathways, despite the longstanding advances achieved all along the last decades. In spite of the fact that somatic mutations have long been taken as important clues to the understanding of the cancer process involving cancer hallmark genes collectively representing driver events in cancer and accordingly having led to the identification of a wealth of potential carcinogenic agents, one is not still in a stage of attributing to somatic mutations the main causes of driver events leading to cancer.

Preventing exposure to environmental carcinogens/mutagens would be the gold standard of cancer prevention. A sort of castling the king to avoid an unavoidable cancer. But in the chess game of carcinogenesis this move of the king cannot prevent the appearance of driver mutations and not even passenger ones that can turn out to occupy the function of diver mutations.

Indeed, germline variants may occur in the same hallmark genes whose somatic pathogenic variants have been inherited leading to cancer in an inversely

age-dependent way. Or otherwise, environmental carcinogen-dependent cancers may display a higher load of somatic mutations following an age-dependent trend, whereas the occurrence of germline mutations tend to lead to early-onset cancers.

Germline mutation load which has recently became a possibly main mechanism explaining different susceptibilities to cancer in different populations is paving the way to a new paradigm in understanding genetic predispositions to cancer. We are much too involved in this new paradigm, hoping to scratch a new move in this chess game of cancer.

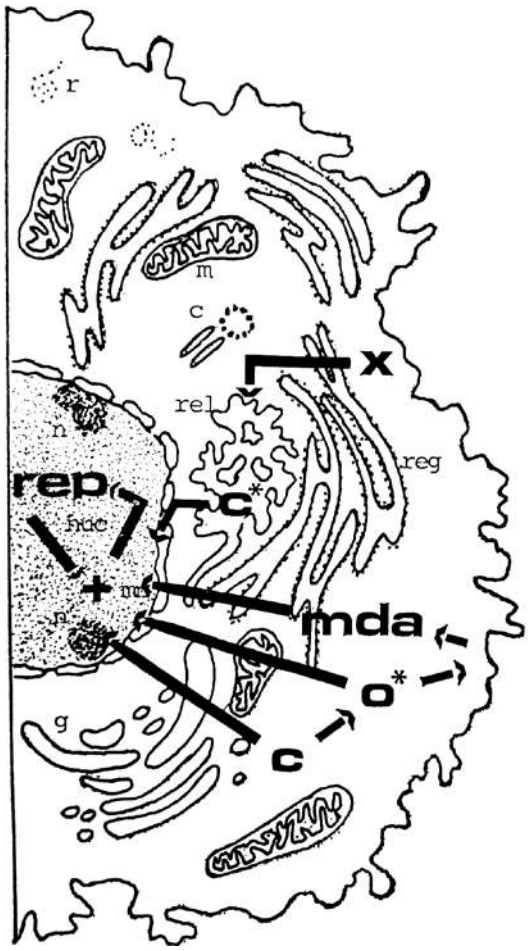

Figura – Cellular scheme of DNA damage by carcinogens, or as if a chess table whereby a DNA lesion is established as a king in check. An environmental carcinogen [**c**] may directly react with DNA leading to the fixation of a mutation or a lesion, like DNA breakage that may

be correctly repaired [**rep**], or subject to inadequate repair with subsequent fixation of a lesion leading to cancer. Environmental carcinogens [**c**] are more often than not subject to metabolic transformation by cytochromes P450 located in the smooth endoplasmic reticulum [rel] leading to ultimate carcinogens able to react with DNA [**c***]. Cytochromes P450 can be induced by a number of xenobiotics [**X**] which increase their putative conversion to a DNA-reactive molecule. Additionally, other pieces of the game may act as oxygen radical species [**O***] that directly or through resulting molecules [**mda**] may also damage DNA.

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CHAPTER V

PUBLIC AND GLOBAL HEALTH

A

GLOBALIZATION AND HEALTH

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Globalization is commonly described as the process of interaction and integration among people, companies and governments worldwide. It entails increased movement of capital, goods, services, people, ideas, technology and data, resulting in economic, social and cultural inter-connectedness and interdependence. This is a centuries old process that has been facilitated and accelerated by improved transportation experienced in the 19th and 20th centuries and by increased connectivity in the 20th and 21st centuries. In 2000, the International Monetary Fund (IMF) identified the four key aspects of globalization as trade and transactions, capital and investments movements, migration of people and dissemination of knowledge and culture.[1,2]

The determinants of health are commonly organized under general categories such as Health genetics, environment, behaviors and social and economic environments, and health services, through prevention, treatment and cure, and rehabilitation.[3]

This paper discusses the impact that four key aspects of globalization had or may have had on the health of the population through changes in the major determinants of health. In some cases, these changes may have positively influenced health, in others they may have had a negative impact.

GLOBAL HEALTH, GLOBALIZATION AND HEALTH

Growing out from medicine, public health and international health, global health is a newly branch of health sciences, with significant input from the international organizations such as the Organization for Economic Co-operation and Development (OECD), the World Bank, and especially, from the World Health Organization (WHO).[4-6] The main differences between global health and its ground sciences are essentially three. Firstly, global health deals with medical and health issues with global impact; secondly, the main task of global health is to seek global solutions to the issues with global health impact; and finally, the ultimate goal of global health is to use academic research and science to promote health for all, and to improve health equity and reduce health disparities globally. Thus, the target of global health is the population of all countries, and although global health research and practice can be conducted locally, it involves all sectors beyond medical and health systems, in an interdisciplinary way.[6,7]

Being a branch of medical and health sciences, global health aims to analyze spatial-temporal patterns of a medical and/or health issue across the globe in order

to increase the understanding of that problem and to assess its global impact. Global health intends to investigate the determinants associated with medical and health issues that are known to have global impact. Finally – and perhaps most importantly – global health addresses evidence-based global solutions, including strategies, frameworks, governances, policies, regulations and laws.[6-8]

As proposed by Chen et al. global health should have three basic functions. The first one should be to generate new knowledge and theories about global health issues, influential factors, and develop global solutions. The second function should be to distribute the knowledge through education, training, publication and other forms of knowledge sharing. Finally, the last function should be to apply the global health knowledge, theories, and intervention strategies into practice to solve global health problems.[7-10]

In its global health strategy 2008-13, the UK Government refers to global health as "*health issues where the determinants circumvent, undermine or are oblivious to the territorial boundaries of states, and are thus beyond the capacity of individual countries to address through domestic institutions. Global health is focused on people across the whole planet rather than the concerns of particular nations. Global health recognizes that health is determined by problems, issues and concerns that transcend national boundaries*".[11] Although this definition includes important ideas, it is perhaps too complicated and not focused on outcomes. On that regard, Macfarlane et al. propose a much shorter and useful definition of global health, as being the "*worldwide improvement of health, reduction of disparities, and protection against global threats that disregard national borders*". [12]

As much as globalization influences countries' policy-making in several domains, countries continue to influence—or try to influence—global policy processes, including in relation to health. Understanding global health requires a nuanced understanding of domestic policy-making within national government systems, understanding where health intersects with foreign affairs, trade, environment, development and security agendas and drawing on international relations theory, public policy, political science and social and economic environments.[13]

GLOBAL CAPITAL AND INVESTMENTS

The relatively free flow of international capital and rapid spread of technology and connectivity has led to the rapid industrialization and urbanization of

many formerly low-income countries which, in the process, have moved into well-established middle or high-income countries. Brazil, China, Korea, India, Vietnam, South Africa are good examples of such change. Companies moved from industrialized countries in the United States and Western Europe and relocated to East and South Asia, Africa, South America and the Caribbean.

Figure 1 – Billions of women were empowered with jobs in the formal economy. Photo: Companies moved from industrialized countries to low and middle-income countries. (https://www.google.com/search?q=apparel+brands+produced+in+bangladesh&sxsrf=ALeKk02Kc88wi4dbe12IUq7g-WgR8HbGXg:1618853847592&source=lnms&tbm=isch&sa=X&ved=2ahUKEwivpp3E7IrwAhXIz4UKHWedCeoQ_AUoAXoECAEQAw&biw=1189&bih=957#imgrc=sV8HJOzw9Wmu4M)

New technologies led to rapid increases in productivity in agriculture, industry and services. As a result, new jobs were created and billions of people moved from agriculture-based rural communities to cities to serve in newly created industry and services. Billions of women were empowered through jobs in the formal economy.

Global investors have invested in health insurance and health service companies around the World. Even in Portugal, two out of the three major health insurance and private hospital chains are currently owned by foreign capital ventures.

High-end pharmaceutical companies that specialize in new products in the industry have undergone a process of concentration. Ten companies in the European Union and the United States control around 50 percent of the World's market for innovative products. This concentration may mean that these companies focus on research and development of drugs that will benefit people who can pay, namely middle-age and older people in high income countries, while foregoing research on drugs that could solve the health problems of people living in low-income countries but would not be able to pay for those drugs.

On the other hand, the generic pharmaceutical market became global and is now an important playing field for middle-income countries such as Brazil, India and South Africa, where this market represents a significant share of exports from those countries.

The rapid worldwide industrialization has created enormous wealth and opportunities. Billions of people have risen out of poverty and hunger, as documented in the literature related to the achievement of the Millennium Development Goals. Life expectancy increased by 5 years between 2000 and 2015.[14] Child mortality was reduced by 53% between 1990 and 2015.[15] Maternal mortality went down by 44% percent between 1990 and 2015.[16] Many more girls were enrolled in school in 2015 compared to 2000.

Unfortunately, the creation of global wealth has not been accompanied by an equitable distribution of wealth. Capital has accumulated among those in the 1% higher income group and the gap between the higher and lower income groups has widened.[17]

As people moved from rural areas to big cities, they often ended up in shanty towns with poor sanitary conditions. Yet, they have gained access to education, health and other services from which they could hardly benefit while living in their equally squalid rural villages.

Industrialization and urbanization also led to increased motorization of low- and middle-income countries. The problem lies on the fact that roads are often in poor state, cars are generally very old and without adequate working safety devices, drivers are poorly trained and authorities lack the capacity to enforce security regulations This has led to a sharp increase in the incidence of motor vehicle accidents and the related prevalence of disability.

Low- and middle-income countries where these industries established themselves often lack adequate legislation or the capacity to enforce social and environmental responsibility. As a result, many of the industries ended up

contributing in a significant way to pollution, greenhouse gases and climate change. Also, work conditions in such enterprises do not offer in developing countries the same social benefits and conditions that they offer in the countries of origin.

We have no basis to judge whether foreign investment in health insurance and health care services have had a positive or negative impact on the quality or efficiency of health care.

GLOBAL TRADE AND TRANSACTIONS

Globalization has led to a sharp increase in trade and economic and financial exchanges. These exchanges have allowed people in Colombia, Mexico, Peru and Vietnam to sell their production of coffee avocados, asparagus and rice around the World. Between 1950 and 2008 world exports increased 27-fold.[18]

Figure 2 – Billions of people have risen out of poverty
Photo: High end agriculture in Peru, ex. asparagus crops. (https://www.google.com/search?q=asparagus+exports+from+Peru&sxsrf=ALeKk02m3Osqahh75jc-_v9r-egRj5uskQ:1618853985356&source=lnms&tbm=isch&sa=X&ved=2ahUKEwjd0PWF7YrwAhVU5uAKHWuOB4AQ_AUoAXoECAEQAw&biw=1189&bih=957#imgrc=NhXmgtGw5AjkLM)

On the bright side, this acceleration of economic exchanges has led to strong global economic growth and industrial development, creating much economic wealth and improved living standards. Worldwide, billions of people have risen out of poverty and hunger. Health gains have been tremendous, as can be shown in terms of the improvement of life expectancy around the World.

On the negative side, economic growth and industrial productivity may have had, in many instances, negative impacts on the environment through deforestation, destruction of ecosystems, and loss of biodiversity which, in the long term, will affect health negatively.

The increased movement of goods, namely the export of livestock, has also facilitated the transmission of some zoonosis around the world, such as the spread of mad cow disease in 1994-96.

As described in the previous section, increased industrialization in low- and middle-income countries often lacks the relevant social and environmental responsibility concerns and investments, leading to pollution, greenhouse gases and clime change which affect health negatively.

MOVEMENT OF PEOPLE

Tourists

According to the United Nations World Tourism Organization, in 2019, there were more than 1.4 billion of international tourist arrivals. More than half the tourists arrived in Europe, followed by Asia and Pacific. Lower income countries in the Middle East and Africa and the Americas received relatively fewer tourists.

Tourism has created millions of jobs and is a source of wealth creation in the receiving countries. On average, tourism contributes directly to 4.4% of GDP, 6.9% of employment and 21.5% of service related exports to OECD countries.[19] Unfortunately, tourists are often shy to travel to low-income countries due to perceived health and security related risks. This has certainly improved the livelihoods and health of those involved in the tourist industry.

On the other hand, the large circulation flow of people around the World facilitates the transmission of communicable diseases across continents and countries, and has been a key driver of the transmission during pandemics. In 2020, the spread of COVID-19 from China to Italy, and then around the World, is the most recent example of such phenomenon. History is full of other examples

in which movement of people led to epidemics to cross borders and taking major proportions: the Spanish Flu in the early 20th century, cholera in Haiti in 2011, plague, yellow fever, *Meningococcus* epidemic in Mecca during Haj, SARS, Ebola epidemic in 2014, spread of multi-drug resistant forms of Tuberculosis (TB) from Russia, South America to Africa.[20]

Figure 3 – Large circulation of people around the world facilitated transmission of communicable diseases.
Photo of cruise line stranded by COVID (https://www.google.com/search?q=cruise+ship+stranded+by+COVID&sxsrf=ALeKk00bM8w3J9LhYLXM9bsGiP5qgjn2Cw:1618854067368&source=lnms&tbm=isch&sa=X&ved=2ahUKEwj0kYOt7YrwAhWxA2MBHepfBYoQ_AUoAnoECAEQBA&biw=1189&bih=957#imgrc=xwHBULWGeEnKnM)

Migrants

The UN Migration Report estimates the number of international migrants at around 272 million globally, about 3.5 percent of the population, with nearly two thirds being labor migrants and 74 percent aged 20-64.[21]

Remittances from migrants are a significant part of the economy for the receiving countries, namely for India, China and Mexico who are the largest beneficiaries.

"Conventional wisdom" frequently associates migration with the spread of diseases and to an unfair burden on health and social services in the host country.

The opposite is true. There is a healthy migrant effect that is well known in the scientific literature.[22-24] Migrants tend to be younger, more entrepreneurial and healthier when compared to natives and to the population in the host country. They also tend to use health and social services less than the population in the host country, at least during their first five years.[23,24]

Professionals

It is estimated that six percent of the World's medical doctors and four percent of registered nurses work outside their countries of origin. The market for health care professionals is now rather global. Many nurses in US hospitals come from the Philippines and a significant number of doctors in the United Kingdom National Health Service come from Sub-Saharan Africa and South Asia. The United States, United Kingdom, Canada, Ireland, Australia and New Zealand, are large importers. Ghana, Malawi, Zambia and Zimbabwe India, Pakistan and Bangla Dash, the Philippines, are significant exporters. Movement of health professionals in Europe is also on the rise, due to the European Union system of academic equivalence and work mobility policies.

Many have complained about the brain drain from low income to high income countries. Poor African countries that export health care personnel often lack human resources to deal with their major health programs such as immunization, malaria and HIV/AIDS control programs. Yet, in economic terms, the remittances of health professional migrants are significantly higher than what their country of origin has invested in their education and training. This is why some of these low-income countries such as the Philippines and Ghana have developed nursing training as an export niche.

Health tourism

Hundreds of thousands of people travel from one country to another for medical care. In the Americas, Canada, Costa Rica, Mexico and Panama are the most popular destinations. In Asia, Japan, India, Singapore, South Korea and Thailand attract most clients. In the Middle East, Abu Dhabi, Dubai, Oman and Israel offer attractive packages.[25] In Europe, the Czech Republic, France, Germany, Spain, and the United Kingdom rank highest in as destinations. Each country seems to develop their own specialty niche on the basis of advanced facilities, skilled doctors, low cost of treatment and regulatory framework, or lack of it. Some countries specialize in heart surgery, others in plastic surgery, hair transplant, assisted reproduction, or organ transplant.[26]

In Europe, France, the United Kingdom, Denmark, Poland and Luxembourg are the major sources of patients seeking care in another country. The United Kingdom, Germany, Spain, Portugal, Ireland and the Czech Republic are the most popular destinations. [27]

This flow of patients has advantages. Patients can often forego long waiting-lists and get good care at a lower price. On the other hand, treatment in a foreign country normally is a one-shot process, with no continuity of care.[28] Also, the regulatory framework in some receiving countries does not offer adequate protection against medical malpractice when the treatment does not succeed or results in death or disability. Even if it does in theory, when things go wrong, legal action at a distance is difficult and expensive. Finally, in the case of organ transplant and assisted reproduction, donors and surrogates may not have been adequately screened for health problems, and may not be protected by adequate legislation in terms of rights and obligations.

DISSEMINATION OF KNOWLEDGE AND CULTURE

Increased movements of people, improved information technology and increased connectivity has led to cultural globalization. Laptops, tablets and hand-held devices, internet and social media have allowed people in a rural community in Vietnam to share their habits and beliefs with people in a Dublin neighborhood, and vice versa. Books, movies and music are available instantaneously around the world.

Is this good or bad for health? It is both. On the positive side, knowledge about healthy lifestyle is now universal. More exercise and physical fitness are popular everywhere. Healthy food is trendy around the World. Unfortunately, these behaviors are often adopted by more affluent and educated people. People in lower income groups may not be able to afford the healthy lifestyle or be less willing to invest in the present to gain health benefits in the future.

On the negative side, fast food with too much salt, sugar and saturated fats have now been adopted widely in low- and middle-income countries, leading to high incidence of overweight, obesity and the rapid increase in the incidence of cancer and cardiovascular diseases. As a result, low- and middle- income countries end up with the double burden of disease, that of communicable and non-communicable diseases. Chronic and degenerative diseases (CDD) affect people in low-income

countries at younger ages and age specific CDD mortality rates are higher in those countries than in higher income countries.

The dissemination of behaviors and beliefs around the world, and specific marketing strategies adopted by the relevant industry, have also led to a significant increase in the consumption of tobacco and alcohol in low- and middle-income countries, mostly among younger people, women and lower-income groups, resulting in increased incidence and prevalence of cancer, accidents and injuries, as well substance abuse and dependence.

Figure 4 – Consumption of tobacco is rising in low- and middle-income countries, mostly among younger people
Photo: https://www.google.com/search?q=Tobacco+advertising+in+developing+countries&sxsrf=ALeKk01ADKv1fRijJ04hJgleRr4s-otegw:1618854151991&source=lnms&tbm=isch&sa=X&ved=2ahUKEwjYo7DV7YrwAhWR2BQKHU5zB4oQ_AUoAXoECAIQAw&biw=1189&bih=957

CONCLUSION

It is undeniable that globalization has brought enormous and rapid changes to the world. In an unprecedented way, millions of people have seen their lives change for better: people live longer, more people they have access to housing, health

services and education than ever before, technology and scientific progress made people closer and healthier. However, these progresses were not equal in all countries. Major gaps still remain and, some of them were deepened during the recent economic or pandemic crises. In that sense, global health can be a crucial part of the global response to address those issues: health inequity, social and economic inequalities, inadequate health systems, lack cooperation in global response to health crisis, to mention just few.

Globalization has posed a number of challenges to countries world-wide, and some of those issues are directly or indirectly related to health and well-being of the populations. The recent pandemic crisis lays as the perfect example of how a coordinated global health response is needed to protect the daily economic and social habits of people around the world. The increasing demand for innovative tools to prevent diseases must be accompanied by a global health perspective on fair distribution of those scientific and technological discoveries. The same applies to well-known medicines and medical devices.

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B

TRAVEL MEDICINE

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INTRODUCTION

Travel Medicine is the field of medical knowledge that aims at preventing travel related diseases, preserving the health status of the traveler and diagnosing and treating medical conditions acquired during the journey. In doing so, travel medicine also contributes to the prevention of introduction of transmissible diseases both at the origin and destination regions of the traveler.

The body of knowledge necessary to practice travel medicine basically derives from geographical medicine, infectious diseases/tropical medicine and public health. Data on the epidemiological distribution of infectious diseases affecting specific areas of the planet is used to estimate the risk of acquiring them. In addition, this data is also used to recommend preventive interventions such as vaccines or prophylactic drugs or instructing the traveler on behaviors that reduce the risk of exposure. Pretravel advice is then adjusted to the clinical characteristics of the individual traveler to obtain the best possible risk-benefit ratio for the optimal prescription of prophylactic interventions, for the elaboration of an adequate travel pharmacy and to indicate the need for medical follow-up after the journey.

STATE OF THE ART REVIEW

Travelling seems to be part of human nature. The concept of travelling includes, however, many different types, motivations, and modalities of travelling that influence the dangers and impact on the risk of health problems during or after the journey. Famous travelers give detailed accounts of their extensive, long and often dangerous endeavors in the classical literature. Instances of these authors include Herodotus, Giovanni da Pian del Carpine, Marco Polo, Ibn Battuta, Ibn Khaldu and Chang-Chun.

Following the European, American and Asiatic industrial revolution and global expansion, geographical exploration of the planet increased vastly, motivated by economical demands and scientific curiosity. Tropical areas presented a peculiar challenging and dangerous environment that was described by explorers such as David Livinsgstone, Henry Morton Stanley, Serpa Pinto, Hermenegildo Capelo e Roberto Ivens in Africa and many other travelers to tropical America and Asia.

In the 19th century, as more and more wealthy individuals start to travel for recreative motifs, the concept of tourism starts to gain momentum. An interesting

definition of the difference between a tourist and a traveler is given by Paul Bowles (himself an American traveler): "Whereas the tourist generally hurries back home at the end of a few weeks or months, the traveler, belonging no more to one place than to the next, moves slowly, over periods of years, from one part of the earth to another"(*in* "The Sheltering Sky").

The number of travelers worldwide increased exponentially during the last 50 years. The United Nations World Tourism Organization estimates that internationally there were just 25 million tourist arrivals in 1950. In 2018 this number was 1.4 billion international arrivals per year, a 56-fold increase. This growth is mainly related to the offer of cheap intercontinental flights, that became available with the development of big airlines cruisers, starting in 1970 with the Boeing 747, soon followed by other such aircrafts built by Lockheed and Airbus, that contributed to massify international travel for recreational motifs. Tourists tend to do shorter journeys but due to the easiness to travel and the possibility of fast airborne transportation they may be unaware of the consequences of the very fast changes in climatic conditions, poor standards of sanitation and long-haul flight related problems such as jetlag and deep vein thrombosis.

This massification of tourism results in considerable pressure on the environment and social structures of destinations areas. Awareness on the negative consequences of this kind of unprecedented enormous number of individuals crossing international borders has motivated the development of the concepts of responsible and sustainable tourism (International Centre for Responsible Tourism (at https://responsibletourismpartnership.org/icrt/). We believe that it is the moral obligation of the travel medicine practitioner to increase awareness on this topic.

The increasing clinical complexity and variety of the travelers, including those under specific risks (older age, preexisting chronic conditions, including immunosuppressing disorders, children and pregnant women) and the changing patterns of the journey itself (longer duration of travel, visiting multiple destinations, travelling off-the-track and to areas with social instability and low access to medical care) also contribute to the need for the practitioner to produce an individually tailored comprehensive advice for a given traveler. Presently, travel medicine has to respond to the specific and peculiar needs of very diverse travelling individuals (Table 1).

Many of these travelers will have a better awareness of the risks, a better preparation for the journey and a strong motivation. However, they will also frequently travel for longer periods, to more remote areas, and sometimes with a reduced budget. These factors can contribute to a limited access to good standard health care and

lead to a tendency to incorporate local habits and beliefs, such as feeding habits, sexual practices, or the use of low-quality transportation. When compared to tourists, many of these travelers will be therefore exposed to a higher risk of communicable (including uncommon parasitic diseases), non-communicable diseases and accidents. In many instances these travelers will learn from experience: the role of the travel medicine practitioner is to optimize and decrease the cost of this apprenticeship.

NGO workers	People visiting friends and relatives
Missionaries	Adventure travelers
Militaries	Radical travelers
Diplomats	Backpackers
Journalists	Surfers
Workers	Trekkers
Explorers	Dark tourism (thanatourism)

Table 1 – Types of travelers

Although travel medicine is generally not considered a medical specialty, its practice can be quite challenging. Factors such as the instable epidemiological scenarios of infectious diseases (outbreaks, emergence or re-emergence of pathogens), the evolving patterns of drug resistance (for instance in malaria, enteric infections or sexually transmitted infections) and the introduction of new vaccines, implies that the travel medicine practitioner needs to be constantly updated on those factors in order to avoid excessive or insufficient prescriptions and advices.

Access to good quality and updated information is paramount for the correct practice of travel medicine. Presently this information is available on several specialized internet sites (Table 2). The practitioner has to be minimally acquainted with them to be able to, as close to the real scenario at destination as possible, establish the correct risk for the traveler.

WHO Travel Advice	http://www.who.int/ith/en/
CDC Travelers' Health	http://wwwnc.cdc.gov/travel/
Health Canada Travel and Tourism	https://travel.gc.ca/
Fit For Travel	http://www.fitfortravel.nhs.uk
Safe Travel	http://www.safetravel.ch
Santé Voyages	http://www.astrium.com/

Table 2 – Commonly used internet sources of information in travel medicine for the general public and health professionals

RELEVANT CONTRIBUTIONS FROM NOVA

As for several other Institutes of Tropical Medicine in Europe, the prevention and clinical management of diseases affecting travelers to hot climates such as colons, missionaries, military and administrative personnel, as well as the indigenous population, was a priority since the creation of the IHMT in 1902.

At the IHMT travel counselling was offered for migrants to the Portuguese colonies including Angola, Mozambique, Guinea-Bissau, São Tomé e Príncipe, Cape Verde, Timor and small territories in India (Goa, Damão, Díu) and China (Macao). Collective pretravel counselling sessions were held in the "Sala dos Colonos" of the IHMT. Information and instruction were given on measures such as personal hygiene, clothing, nutrition, house building, water-born and vector-borne disease prevention and general aspects and tips on how to survive in the tropics. The attendants were recommended a complete clinical evaluation before travelling. The sessions were followed by vaccination, mainly against yellow fever and cholera.

During the expansion towards the hinterland of the new territories, training and education of health workers was also offered at the IHMT in the form of short and long courses on Tropical medicine. Progressive acquisition of knowledge on at that time largely unknow etiology, transmission, diagnosis and treatment of the dangers for the health in the tropics, coupled with the development of clinical laboratory techniques for the correct diagnosis of these new and exotic diseases, allowed progress in patient management. Research on sleeping sickness, malaria, leprosy, schistosomiasis, filariasis and several other endemic or epidemic diseases allowed significant progress on the partial (and in a few instances total) control of many of these pathologies in affected areas.

The independence of Portuguese African colonies occurred in 1975. Travel consultation continued to be offered to Portuguese diplomats, military, and teaching personnel allocated to perform usually long stays in all former colonies. Vaccination was stopped at the IHMT and had to be performed in specific premises of the Ministry of Health. For official governmental assignments, a document certifying the good health and mental fitness of the candidate to work in the tropics was issued. Travelers were usually medically evaluated at the end of the mission to screen for long term consequences of cryptic infections. This aspect became progressively more and more important since the health situation deteriorated progressively in the Portuguese Speaking African Countries (PALOPS) after the breakdown of health systems associated with war or political and economic instability that followed

independence and control was lost for several endemic diseases; epidemic outbreaks were also identified for arbovirus diseases, malaria, cholera and sexually transmitted infections (including HIV infection) in most of those countries.

Again, as in several other Institutes of Tropical Medicine in Europe, the IHMT adapted and evolved to meet this demand of a considerable number of long-term travelers to the PALOPS (including Portuguese migrants to those countries), but also to the increasing numbers of tourists to worldwide destinations.

In 1998, a Travel Medicine Clinic conform to modern international standards was created by members of the Tropical Clinic Teaching and Research Unit at the IHMT. This clinic offered pretravel advice, on-site vaccination and trans and post-travel consultation, including on-site laboratory diagnosis using state-of-the-art techniques for imported diseases, such as malaria, dengue and other arboviral diseases, travelers' diarrhea and uncommon parasitic diseases (i.e. schistosomiasis, human African trypanosomiasis, Chagas disease, filariasis, among others). This Travel Clinic became the largest in the country, reaching a volume of 10,000 consultations per year in the second half of the first decade of the 21st century. Progressively, the vaccination offer included basically all travel-related vaccine-preventable diseases (Table 3).

Yellow fever	Meningococcal disease
Hepatitis A	Rabies
Typhoid fever	Tetanus/Diphtheria
Cholera	Hepatitis B
Japanese encephalitis	

Table 3 – Vaccines available at the IHMT Travel Clinic

Concomitantly, a course on Travel Medicine (2 ECTS) was created by the Tropical Clinic Teaching and Research Unit. This course, that proposes a comprehensive approach to the body of knowledge needed for the practice of good quality Travel Medicine, was the first to be offered in Portugal. The course includes practical training at the Travel Clinic, bridging theoretical and practical aspects. Since its inception this course has contributed to the qualification of a high number of health professionals (mainly medical doctors) for the correct practice of Travel Medicine, thus impacting on the preservation of the health of a significant number of citizens.

Furthermore, the scientific potential of the Travel Clinic has been exploited mainly by academic works performed by MSc and PhD students of the IHMT, that

have resulted in interesting dissertations and thesis works and publications, with unique and relevant data for the Portuguese Travel Medicine context.

The full potential of the impact on health and scientific output of the quite unique offer of the IHMT Travel Clinic is jeopardized by the fact that it is not inserted in the National Health Service (SNS), meaning that the cost of the provided service, although very efficient, is too expensive for a significant part of the Portuguese population, including migrants. These constitute an important group of travelers, i.e. those visiting friends and relatives, that are at a higher risk of acquiring infections during their journey back to their country of origin.

Members of the Tropical Clinic Teaching and Research Unit decisively contributed to the creation of the Portuguese Society of Travel Medicine (SPMV), launched in 2015. This Society maintains a mailing list that helps stimulate discussion on emerging aspects of travel medicine among its members. On the SPMV website (www.spmv.pt.) a repository of Portuguese publications on Travel Medicine topics can be found.

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C

TRANSMISSIBLE DISEASES AND INFECTION

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INTRODUCTION

Deaths caused by transmissible diseases have been declining steadily since the turn of the 20th century (1, 2). In 1918, Sir William Osler, observed that pneumonia had overtaken tuberculosis as the leading cause of death in Europe and described pneumonia as the "*Captain of the men of death*", and this designation is still valid today. In the first half of the 20th century, the implementation of better sanitation and hygiene, the discovery of antibiotics, the implementation of universal childhood vaccination and better hospital organization lead to a dramatic fall of the crude death rate from transmissible infectious diseases overall. However, three transmissible diseases continue to be ranked in the top ten causes of death worldwide in 2019 by the World Health Organization. They are lower respiratory infections (3.0 million deaths), diarrheal diseases (1.4 million deaths), and tuberculosis (1.3 million deaths). HIV/AIDS, which was previously on the list, has dropped from the global list of the top ten causes of death (0,56 million deaths in 2019 compared with 1.5 million in 2000), but it is still a leading cause of death in low-income countries. Lower respiratory infections (including pneumonia) and diarrheal diseases are caused by a variety of infectious agents (3). Another infectious disease, malaria, accounts for a top cause of death in low-income countries. Furthermore, there has been a steady and fast spread of infections caused by multidrug resistant (MDR) bacteria (Figure 1). Resistance rates to many classes of antibiotics are increasing worldwide and estimates point towards that antimicrobial resistance (AMR) could cause no less than 10 million deaths a year by 2050 (4).

Although sepsis incidence and mortality seem to be decreasing worldwide, in 2017 the estimated incidence of sepsis in the world was 48.9 million (95% uncertainty interval [UI] 38.9–62.9) with a sepsis-related death of 11.0 million (10.1–12.0), that represented a total of 19.7% (18.2–21.4) of all global deaths (Figure 2) (5). But the distribution of sepsis incidence and mortality varies markedly across regions with the highest burden in low- and middle-income countries. More than a century later, pulmonary infections, namely pneumonia, are very frequent infectious diseases, either in the community or in the hospital setting. Although infection is a frequent disease its definite diagnosis remains difficult. We should always keep in mind that there is no pathophysiological aspect that is pathognomonic of infection/sepsis and the diagnosis of infection results from the intersection of three vectors (systemic manifestations, manifestations of organ dysfunction, and microbiological documentation). However, the microbiologic confirmation of infection is obtained

only after 2-3 days of empiric antibiotic therapy and in around half of the cases documentation is never achieved. This uncertainty is at least in part responsible for the overuse and misuse of antibiotics in the community and in hospital, and this practice is probably the main drive for antibiotic resistance (6, 7). As result, antibiotics are commonly prescribed in patients without a definitive diagnosis of pneumonia, in particular in the presence of severe infections, since a delay in treatment is associated with worse outcomes, a strategy that, without proper guidelines and monitoring, can contribute to increase AMR (8, 9).

STATE OF THE ART REVIEW

Infectious agents

The antimicrobial-resistant ESKAPE (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa*, and Enterobacter species) group of pathogens alongside with drug resistant *Neisseria gonorrhea*, *Clostridium difficile*, *Mycobacterium tuberculosis* and *Candida* spp., represent the worse microbial threats to human health as limits the therapeutic options and pose a considerable menace to clinical patient care since they became difficult-to-treat. For this reason, the development of new antibiotics has been categorized as a priority 1 for research and development of novel drugs by the World Health Organization to answer to the challenge of Sustainable Development Goal 3: Good health and well-being (10). Concurrently, transmissible viral infections either person-to person or vector-borne, are increasing worldwide and impose heavy health and economic burdens by their effects on both humans and animals as seen by the recent SARS-CoV-2/COVID-19 pandemics. If the HIV/AIDS and hepatitis pandemics are slowly decreasing their incidence globally due to efficient pharmacological control measures, globalization, human migration, climate change is steadily altering the distribution of Neglected Tropical Diseases (NTDs) and transmitting vectors, including blood feeding arthropods such as mosquitoes, ticks and flies. Some zoonotic viruses occur worldwide, in a variety of ecological settings. Others are found only in limited ecologic and geographic foci. Important worldwide zoonotic viruses include influenza viruses, SARS coronavirus, hantaviruses, flavivirus, arenavirus, among other viral families and genera with global impact in human health (Table 1) (11, 12). Others are still limited and contained in Africa and the Middle East, including Rift Valley fever virus, Marburg and Ebola filoviruses, monkeypox virus,

and Alkhurma virus, but the rapid ecological change that is occurring worldwide due to climate change and globalization, additional zoonotic viruses will emerge with potential to create new pandemics with very limited efficacious treatments available. Likewise, diseases that were previously relegated to distant and tropical parts of the world are becoming more common globally as people bring them back from their travels as well as global warming and climate changing is bringing their vectors to traditionally more tempered locations (Figure 1). Malaria, for many years with endemic transmission in Southern Europe and Mediterranean basin, still is the deadliest vector-borne transmissible disease in tropical countries globally. Trypanosoma spp. infections (Chagas disease in the Americas and human African sleeping sickness), that occurs when a person is bitten by the tsetse fly (Glossina genus) and Leishmaniasis, common in Asia, the Middle East, North Africa, Southern Europe, Central and South America, spread by infected sand flies, are parasitic diseases that now are recurrently diagnosed in regions where they were totally absent in recent years. Also, with relevant impact on public health in tropical regions are the helminthic diseases such as cysticercosis, ascariasis and the schistosomiasis that are more and more commonly diagnosed in travelers world-wide. As prioritizes WHO in 2020, the revitalization of efforts to tackle communicable diseases is one of the ten priority global health issues to track during this decade, with focus on the control and prevention of current and future pandemics (Figure 1) (13).

Biomarkers

All the above-described limitations in the diagnosis of infection, as well as the lack of a gold standard test to diagnose infection, led investigators and clinicians to use biomarkers, in particular those from the inflammatory cascade, as surrogate markers of infection (14). As a result, biomarkers became very popular in clinical practice since they may improve the diagnosis of infection as well as the assessment of response to antibiotics and in the antibiotic stewardship programs.

A biomarker is defined as a biological characteristic, objectively measured (i.e., with acceptable accuracy and reproducibility) and used as an indicator for a physiological or pathological process, or of the activity of a medicine. Before being used clinically, biomarkers need to be evaluated in a three-stage process: analytical validation, qualification, and utilization (14).

Biomarkers can be divided in two categories: prognostic and predictive biomarkers. In sepsis and infection, >250 biomarkers have been evaluated, and the great majority to assess prognosis. But why should we use a biomarker merely to

determine whether a patient has a higher risk of death when the currently available interventions are not able to modify the prognosis? Only a small number of biomarkers have been assessed as predictive biomarkers to give additional clinical information that can be categorized as triage, diagnosis, risk stratification, monitoring clinical course and antibiotic stewardship (15). A good predictive biomarker of infection should be absent if the patient is not infected, should appear concomitantly with and ideally preceding the clinical manifestations of infection, and disappear with successful therapy or remain elevated if infection is refractory to treatment (15, 16).

Among all the potential biomarkers, C-reactive protein (CRP) and procalcitonin (PCT) are the more extensively studied and used in clinical practice. Before using a biomarker in the clinical decision process at the bedside it is fundamental to have a good knowledge of the strengths, limitations and confounders.

Serum CRP is the prototype of the acute phase proteins being exclusively synthetized in the liver mediated by cytokines, namely interleukin 6. Its secretion starts 4-6 hrs after an inflammatory insult, its concentration doubles every 8 hrs with a peak at 36–50 hrs. As a result, CRP levels depend on the intensity of the inflammatory insult and the rate of synthesis. After the removal of the inflammatory stimulus CRP decline follows a first-order elimination kinetics with a half-life of 19 hrs (17). Its synthesis is not influenced by immunosuppression (steroids or neutropenia) (18, 19) nor influenced by renal failure or renal replacement therapy (RRT) (20). Thus, CRP is an accurate biomarker of infection in patients with renal dysfunction. Overall CRP presents a good sensitivity (68–92%) and reasonable specificity (40-67%) for bacterial infection (21, 22). The immunoturbidometric assays are reliable, stable, reproducible, with rapid turnaround time, cheap (<4€ in Europe) with an adequate limit of detection (0.3–5 mg/L) for infection management.

Ventilator-associated lower respiratory tract infections

Although mechanical ventilation is a potentially lifesaving intervention, it is associated with significant risks and complications such as ventilator-associated lower respiratory tract infections (VA-LRTI), including ventilator-associated pneumonia (VAP) and ventilator-associated tracheobronchitis (VAT).

VAP is one of the most common health care-associated infections and accounts for 25% to 42% of all infections that occur in intensive care units (ICUs). It can hit 10% to 25% of all ICU patients, being associated with longer duration of mechanical ventilation, longer ICU and hospital stays, increased healthcare costs

and increased morbidity and mortality (23). VAP mortality rates range from 20% to 70% with healthcare costs of $20,000 to $40,000 per patient, making it a primary focus for risk-reduction strategies (24). In recent years preventive measures have been implemented and disseminated to reduce both its incidence and its consequences and VAP has become a mean for tracking quality of care for ventilated patients.

VAT was first described in the early 2000s, as an intermediate process between lower respiratory tract colonization and VAP (25). Several studies reported increased duration of mechanical ventilation and length of ICU stay in VAT patients compared with those with no VA-LRTI, but these effects were uncertain.

Other data suggest that VAT might be a separate entity to pneumonia that independently contributes to increased length of stay in the ICU and longer duration of mechanical ventilation.

RELEVANT CONTRIBUTION FROM NOVA

Infectious agents

Grounded on a strong and internationally recognized biomedical research, with solid clinical application coming from the life sciences research centers of the NOVA organic units, several major contributions and scientific landmarks have been recorded at NOVA on the area of transmissible diseases and infection. The molecular epidemiology, phylogeographic and evolutionary studies on HIV-1 and 2, setting their evolutionary origin in central Africa, with strong connections with Angola and the relevant studies on the acquisition of antiretroviral resistance that menace HIV control are scientific landmarks alongside with the molecular diagnosis and epidemiology of tuberculosis and drug resistant tuberculosis in Portugal and worldwide (26-28). In tight conjunction with the HIV epidemics, the reemergence of tuberculosis after the 1980's decade of the XXth Century, especially with multi-drug resistant forms, caused severe impacts in the TB control programs worldwide. In Portugal, the chronic high incidence of tuberculosis, fueled by an uncontrolled dissemination of the HIV infection in the last two decades of the XXth Century, lead to severe multi and extremely drug resistant TB outbreaks, mainly in Lisboa and Porto, that forced a strong response from the health system. It was on this reality that the Instituto de Higiene e Medicina Tropical of NOVA (NOVA-IHMT) and its teams of specialists in mycobacteriology, came out in support of the National Health System and the

Tuberculosis Control Program, bringing to Portugal, evaluating, developing and making available to the main hospitals in Greater Lisbon and later on to all Portugal, new tools and molecular tests that allowed the early and rapid laboratory diagnosis of *M. tuberculosis* and M/XDRTB, directly from respiratory samples of suspected TB and MDR-TB cases between 1990 and 2010 (29). Noteworthy, the detection and characterization of one of the most infectious and M/XDRTB strains in the world, the Lisboa Strain (27). Complementing from a public health perspective, the evolution of the unsuccessful treatment rate in the Portuguese pulmonary TB patients during this period was monitored and evaluated by Escola Nacional de Saúde Publica (NOVA-ENSP) among other important studies on space-time clustering and temporal trends of hospitalizations due to pulmonary tuberculosis (30, 31).

Simultaneously with the outburst of bacterial drug resistant strains, the development of molecular biology tools to early detect and characterize the resistance mechanism of these infectious agents prompt many NOVA researchers teams, being ITQB-NOVA pivotal in the deployment of microbial molecular genetics research in Portugal, to contribute with very important findings such as the characterization of the main b-lactamase resistance mechanism in *Staphylococcus aureus* and other staphylococci and the characterizations of highly infectious and transmissible drug strains of this pathogen such as the Iberian clone (32). Likewise, outbreaks of difficult-to-treat gram negative pathogens received the attention of NOVA's researchers that characterized the mechanism of infection and resistance, as well as new therapeutic and control options, with relevance to multidrug resistant bacteria in hospitals and *Legionella pneumophila* in the hospital and in the community (6, 8, 9, 33). Concerning public health emergencies, NOVA has been in the forefront of the struggle against imported Dengue in the Madeira islands and the study and control of arboviruses in the Community of the Portuguese Speaking countries – CPLP (34), alongside with the long-lasting studies on malaria and trypanosomiasis (35, 36), HIV, hepatitis, SARS-CoV2, among other viral infections in a host-viral interaction (37). Important to stress also other less known epidemies and emergencies under moderate control, to which NOVA has contributed significantly, such as leishmaniasis in southern Europe and Portugal (38), the rise of drug resistance in Clostridium spp. (39), Cryptosporidium in immunosuppressed patients (40) and Leptospirosis in Portugal (41). All of these infectious agents became life-threatening with emergent antimicrobial resistant forms and the early identification of an infection by these agents and their resistance profile is crucial for successful outcomes.

Infection prediction

Unfortunately, not that many studies assessed serial measurements of biomarkers before the diagnosis of infections, independently of the infectious agent.

NOVA's Medical School (NOVA-NMS) research groups performed the first study assessing the course of a biomarker before diagnosis of infection, showing that a steady increase or a persistently elevated CRP level were associated with a high risk of infection, namely VAP (42). Similarly, in the BioVAP study CRP and PCT kinetics were prospectively assessed in 108 patients under mechanical ventilation for non-infectious reasons, demonstrating that a patient with an average increase of CRP of 1 mg/dL/day had a 62% greater chance of having a VAP when compared to a patient with no CRP increase (43). The same was not true for PCT kinetics. More recently from a population-based bloodstream infection (BSI) database, we showed that CRP and plasma albumin (PA) concentrations began to change inversely some days before CA-BSI diagnosis, CRP increasing by day −3.1 and PA decreasing by day −1.3 (44).

In other words, these studies showed that serial measurements of biomarkers, namely CRP, could be useful when infection is not present. Assessing biomarker kinetics, persistent elevation or progressive and continuous rise, are associated with increased risk of infection.

Diagnosis – value of a single measurement

Obtain a rapid and precise diagnosis of infections, namely pneumonia, specially differentiating between viral and bacterial etiologies, as well as between infectious and non-infectious chest x-rays infiltrates, could help in the reduction of unnecessary antibiotic prescription. In the last 20 years, the value of several biomarkers has been studied in the diagnosis of respiratory infections frequently with conflicting results.

In HAP, CRP presented a good diagnostic accuracy for VAP (AUC of 0.92) (45) and a recent study in VA-LRTI evaluated the ability CRP and PCT to differentiate between VAT and VAP (46). Although VAT presented lower values in comparison with VAP, there was a large overlap of both biomarkers. In addition, the level of both biomarkers was not significantly different according to the bacterial etiology as well as the presence or absence of MDR bacteria.

With all the limitations of a single measurement, biomarkers can give to the clinician additional helpful information of the presence or absence of an infection but should not be used as a stand-alone tool. The information coming from the

microbiology laboratory is crucial to ascertain the identification of the infectious agents and possible drug resistance mechanisms and guide therapeutics (6).

Assessment of clinical course

Currently, in respiratory infections, after initiation of antibiotic therapy, the assessment of clinical course and of resolution of the infection relies on the monitoring of the same criteria used for diagnosis. However, some begin to improve late in the clinical course and others their course can be influenced by common interventions in ICU not related to the infection itself (e.g. mechanical ventilation, steroids, antipyretics or beta-blockers). Consequently, there have been a search for reliable biomarkers to assist in the assessment of clinical course and prognosis of patients with pneumonia.

Again, NOVA-NMS contributed with the evaluation of dynamic variations of biomarkers (kinetics), namely the relative variations of CRP (CRP-ratio), as biomarker trend in the assessment of clinical course of patients with pneumonia, both CAP, VAP and BSI. A sharp decrease in CRP-ratio could be used as surrogate marker of infection resolution whereas a persistently elevated or an increasing CRP-ratio suggests that infection is refractory to therapy (47-50). In severe CAP, CRP monitoring was evaluated as a surrogate marker of clinical course (47) and, at day 3, a CRP >50% of the initial value was associated with poor outcome. In a previous study, in patients with microbiological documented VAP, we had also demonstrated that at day 4 a CRP >60% of the initial value was a marker of poor outcome, as serial measurements of CRP-ratio response to therapy evidenced 4 patterns: fast response, slow response, nonresponse and biphasic response (48, 49, 51). In severe CAP, patients with the patterns of fast and slow response had significant lower mortality than patients with nonresponse pattern (51). Also, in cancer patients with healthcare-associated pneumonia (HCAP), CRP course was significantly different between survivors and nonsurvivors as early as 48h of antibiotic therapy. Patients with nonresponse or a biphasic response pattern presented higher ICU mortality rates, reaching 70%, when compared with those with fast response and patients treated with adequate antibiotic therapy presented faster decrease of CRP (52). The kinetics of PCT, PCT-ratio, mid-region fragment of pro-adrenomedullin, temperature and white cell count and Clinical Pulmonary Infection score were not useful in the assessment of VAP course (53). Finally, CRP and PA kinetics in community-acquired bloodstream infection (CA-BSI) patients' showed that serial CRP measurements at D1 and D4 after CA-BSI is clinically useful to identify patients with poor outcome

and that individual patterns of CRP-ratio response were useful in predicting short or long-term mortality (50).

In summary, the kinetics of biomarkers, namely of PCT and CRP, seem to be a complementary useful tool in the assessment of pneumonia response to therapy, either HCAP, CAP or VAP, as well as community and ICU-acquired BSI. Even though the guidelines do not formally recommend the use of any biomarker, CRP kinetics was recognized as potentially useful for this purpose (54, 55)

Ventilator-associated lower respiratory tract infections

Focusing on the VA-LRTI the TAVeM project (Traqueobronquitis Asociada Ventilación Mecánica) was implemented with active participation of NOVA-NMS, with the following main aims:

- Perform an international survey to know the practice and rates of VAT
- Measure the incidence of VAT, and establish its effects on patient outcomes and the effect of appropriate antibiotic treatment on progression from VAT to VAP
- Assess the role of biomarkers in diagnosis of VA-LRTI
- Study other VA-LRTI outcomes as ARDS, shock, immunosuppression, COPD

Concerning the first objective a total of 288 ICUs from 16 countries answered the survey. The majority of respondents (n = 228; 79.2%) reported making the diagnosis of VAT based on clinical and microbiological criteria and 40 (13.9%) by clinical criteria alone. We found that approximately half (50.3%) of the respondents agreed that patients should receive antibiotics for the treatment of VAT. Out of all respondents, 269 (93.4%) assume that a VAT episode increases ICU length of stay. Additionally, half of the physicians considered that VAT increases the risk of mortality. In other words, it seems that VAT has a high incidence and that is could be a risk factor for VAP and mortality (56).

Later was performed a large multicentre, prospective, observational study in 114 ICU in Spain, France, Portugal, Brazil, Argentina, Ecuador, Bolivia, and Colombia for 10 months to establish the incidence and outcomes of VAT. We included 2960 under invasive mechanical ventilation of whom 689 (23%) developed VA-LRTI. The incidence of VAT and that of VAP at baseline were similar (320 [11%; 10.2 of 1000 mechanically ventilated days] vs 369 [12%; 8.8 of 1000 mechanically ventilated days], p=0.48). Of the 320 patients with VAT, 39 patients have progressed to pneumonia. Significantly more patients with VAP died (146 [40%] of 369) than those with VAT (93 [29%] of 320) or absence of VA-LRTI (673 [30%] of 2271, p<0.0001). Median

time to discharge from the ICU for survivors was significantly longer in the VAT (21 days [IQR 15–34]) and VAP (22 [13–36]) groups than in the group with no VA-LRTI (12 [8–20]; hazard ratio 1.65 [95% CI 1.38–1.97], p<0.0001) (57).

In other preplanned studies in the TAVeM project, it was found that although PCT and CRP presented lower values in VAT as compared to VAP, with a marked overlap of both biomarkers values not allowing adequate discrimination (58), that even after controlling for relevant confounders, no association was found between occurrence of VA-LRTI and ICU mortality in patients with ARDS (59). Contrary to our hypothesis the incidence of VA-LRTI was significantly lower in immunocompromised patients, but it was associated with significantly higher mortality rate (and MDR pathogens were more frequently found in immunocompromised patients) (60). Finally, also contrary to our thought's patients with VA-LRTI and cardiovascular failure did not show an association to a higher ICU survival with appropriate antibiotic treatment; but, in patients without cardiovascular failure, appropriate antibiotic treatment conferred a survival benefit for patients only with VAP (61).

IMPACTS TO SCIENCE AND SOCIETY

Accurate and timely laboratory diagnosis of infections, based on solid scientific evidence, is crucial to control infectious diseases in the clinical and public health context. From the XIXth century clinical practice, based on the identification of the patient's distinctive characteristics, signs and symptoms, without much scientific or laboratory support, to the XXIth century practice, strongly grounded in diagnostic complementary exams supported by science and technology, we are now in the era of evidence-based preventive medicine focused in the early identification and even anticipation the infectious disease, that is, diagnosing prior to its own manifestation.

NOVA has significantly contributed to the progress of the laboratory and clinical diagnosis of infections producing solid scientific knowledge that allowed: i) to identify molecular biomarkers associated with the disease onset and progression; ii) improve laboratory methods, increasing their sensitivity and specificity; and iii) making the methods more effective, faster and reliable, improving outcomes and allowing the prediction, monitoring and control of outbreaks, epidemic events and public health emergencies, strengthening epidemiological surveillance in a global and one health perspective – One Global Health (Figure 1).

We are "at a state where the line between the normal and the pathological became a *numerical abstraction*!" by Jeremy A Greene (in Prescribing by Numbers). If used with caution, biomarkers are very useful, and we should use biomarkers whenever we need additional information at the bedside. The ideal biomarker is not yet available, but it is now clear that serial determinations are more informative as we showed in several of our studies; biomarkers are useful in several clinical settings, but should NEVER be used as a stand-alone test, but always in conjunction with a complete clinical evaluation and with a perfect knowledge of the biology, interferences, strengths and limitations of the biomarker.

As result of NOVA's integrated contributions to biomedical sciences, an algorithm to guide antimicrobial stewardship in critically ill patients based on the clinical course, kinetics of biomarkers and duration of antibiotic therapy was proposed (Figure 3) (62). Our integrative approach of antimicrobial stewardship helps clinical decision making at the bedside in a structured way since it integrates all data considered important and critical to consider stopping antibiotics earlier (6, 8, 62). This integrated approach could potentially contribute to decrease antibiotic consumption and pressure leading to decrease MDR rates, less toxicity, decrease microbiota impact, less costs.

ONGOING RESEARCH

NOVA research in biomedical and medical sciences is deeply aligned with the "One Health" strategy that proposes a holistic vision for Global Health and highlights the importance of understanding the interactions between the domains of human, animal and environmental health (Figure 1). This strategy promotes multidisciplinary collaboration and inter- and cross-sectoral cooperation at local, national and international levels in order to achieve quality health for people, animals and the environment (63).

Among the most prominent threats to Global Health related to the "One Health" concept are animal-borne diseases (zoonosis) transmitted by vectors and resistance to antimicrobials. Zoonosis are responsible for approximately 70-75% of infectious diseases emerging in humans as previously described, with particular emphasis on malaria, leishmaniasis, leptospirosis, arboviruses and more recently SARS-CoV2/COVID-19 (11-13, 64). It is crucial to gather the best scientific knowledge to allow the early detection, the interruption of transmission and the

best pharmacological options to combat and control these infections and NOVA continues to invest in the study of: mechanisms of infection and resistance to antimicrobials; global dissemination trends and molecular epidemiology of pathogens, new diagnostic tools for early detection, universal health coverage and literacy in health promoting the control of infectious diseases.

In addition to the relevance of science and innovation in the laboratory diagnosis and universal access to health care, issues related to cost-effectiveness in health, prevention of antibiotic prescription, resistance to antimicrobials, weight of chronic diseases, the response to public health emergencies, early diagnosis and, consequently, the prevention of care and the reduction of infectious disease burden, especially in situations of economic and social constraints, continues to be our driving force.

Regarding the focus on infection, we are currently starting with several projects some in collaboration with the Center for Clinical Epidemiology and Research Unit of Clinical Epidemiology, OUH Odense University Hospital:

1. Assess the value of a new biomarker (pancreatic stone protein) in the prediction of infection in critically ill patients (in particular under invasive mechanical ventilation)
2. Study artificial intelligence tools in big data to design algorithms to identify patients with infections as well as those with high risk of treatment failure and por outcome
3. In patients with high risk of infection (immunosuppressed or neutropenic), assess the usefulness of a POC device at home to measure sequentially biomarkers as an early predictor of infection

Concerning VA-LRTI we are currently with a project on COVID19 patients under mechanical ventilation:

1. We conducted a prospective observational study to evaluate the incidence of VA-LRTI in COVID19 patients during the first wave in Europe (in comparison with influenza and no pneumonia) (65)
2. Assess the rate of bacterial and viral co-infection at admission
3. Study the incidence of COVID19 associated pulmonary Aspergillosis
4. Assess the impact of corticosteroids on VA-LRTI (VAT and VAP) outcomes

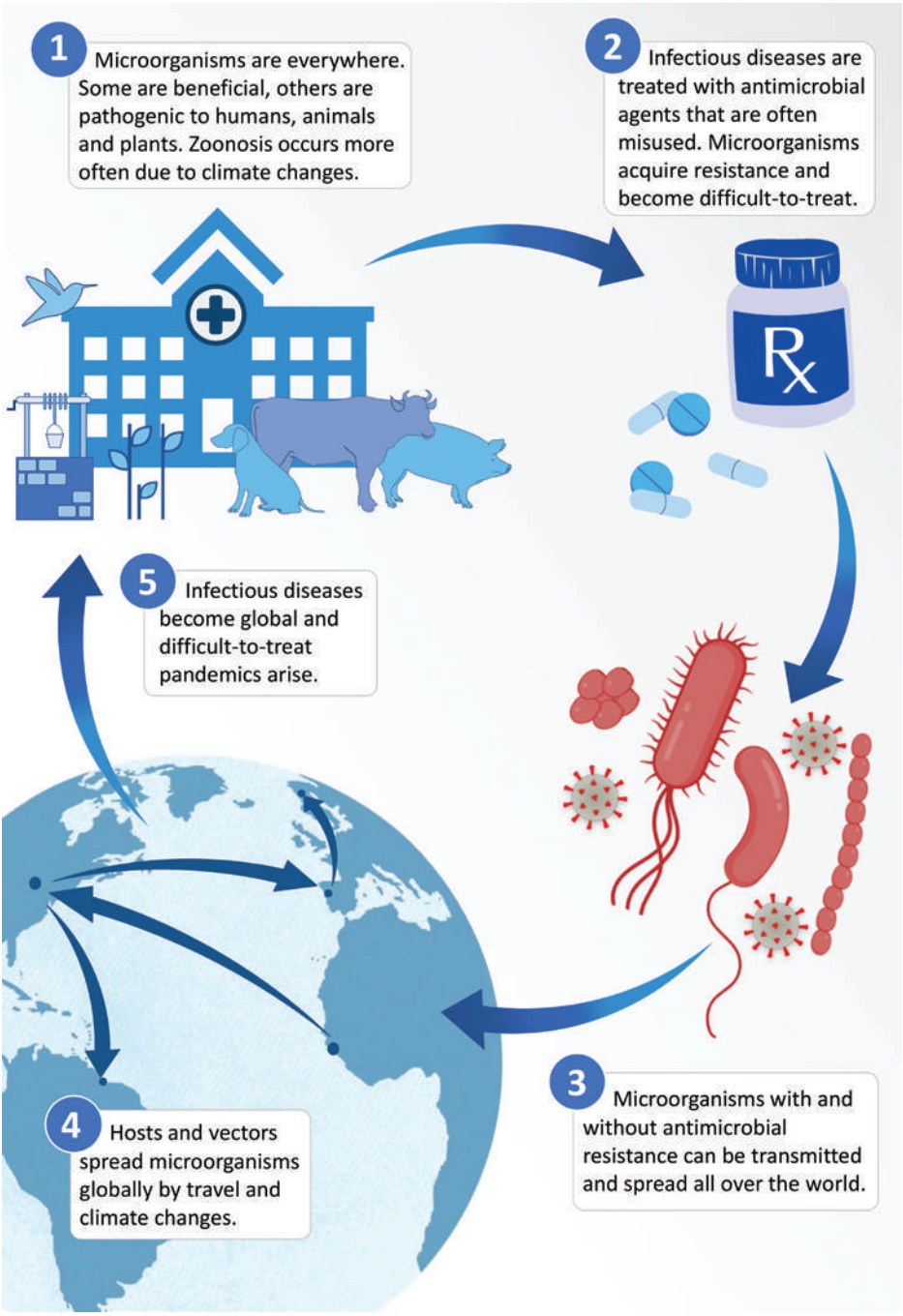


Figure 1 – The One Global Health dynamics of infectious desiases– From zoonosis to globalization of difficult-to-treat pandemics (63,64)

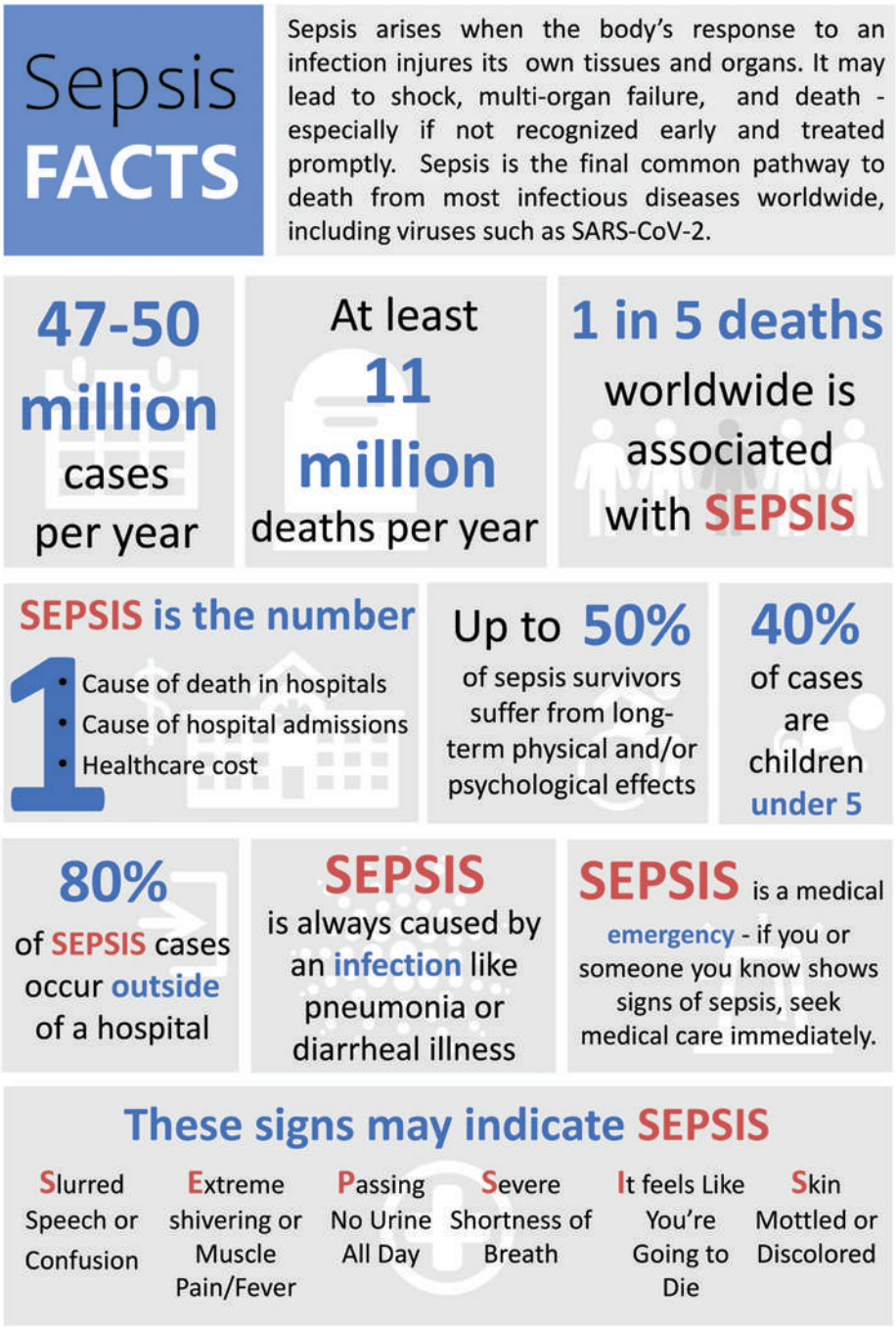
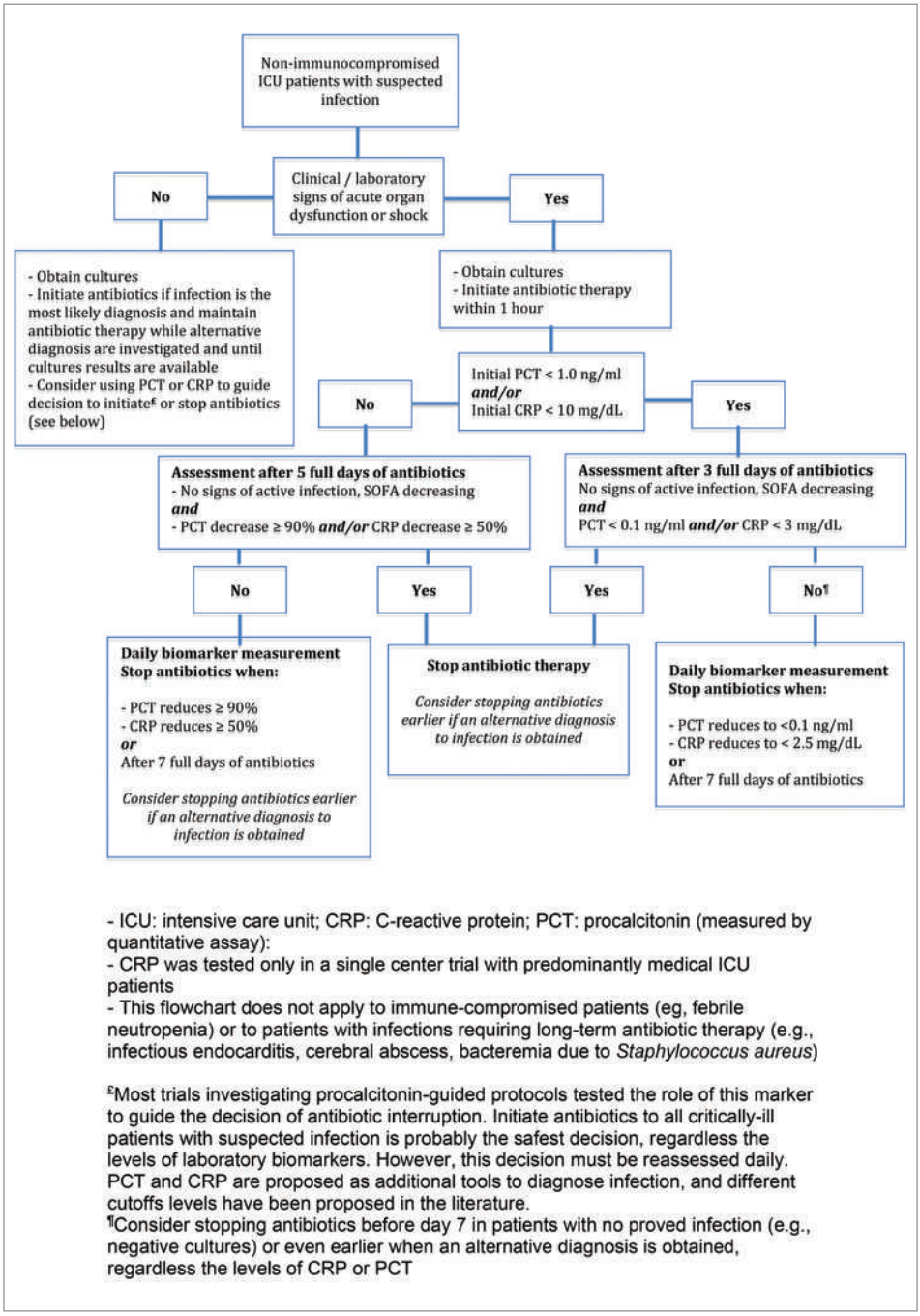


Figure 2 – Sepsis facts and signs – Adapted from (5) combined with the World Sepsis Day, an initiative by the Global Sepsis Alliance – https://www.worldsepsisday.org/



- ICU: intensive care unit; CRP: C-reactive protein; PCT: procalcitonin (measured by quantitative assay):
- CRP was tested only in a single center trial with predominantly medical ICU patients
- This flowchart does not apply to immune-compromised patients (eg, febrile neutropenia) or to patients with infections requiring long-term antibiotic therapy (e.g., infectious endocarditis, cerebral abscess, bacteremia due to *Staphylococcus aureus*)

£Most trials investigating procalcitonin-guided protocols tested the role of this marker to guide the decision of antibiotic interruption. Initiate antibiotics to all critically-ill patients with suspected infection is probably the safest decision, regardless the levels of laboratory biomarkers. However, this decision must be reassessed daily. PCT and CRP are proposed as additional tools to diagnose infection, and different cutoffs levels have been proposed in the literature.

¶Consider stopping antibiotics before day 7 in patients with no proved infection (e.g., negative cultures) or even earlier when an alternative diagnosis is obtained, regardless the levels of CRP or PCT

Figure 3 – Use of biomarkers to guide antimicrobial therapy in critically ill patients – an integrative approach (62).

Viral group	Comments
California serogroup viruses	California encephalitis, Jamestown Canyon, Keystone, La Crosse, Snowshoe hare and Trivittatus viruses with relevant outbreaks in the USA
Chikungunya virus	With primates as reservoir between human outbreaks with human-to-human vector-mediated transmission occurring. Thousands of cases in recent years in the Indian Ocean and Africa, particularly Kenya, Reunion, and India.
Coronavirus	Common cause of mild to moderate respiratory infections, among this group there are virus that cause severe forms of atypical pneumonia such as the Middle East Respiratory Syndrome (MERS) and the Severe Acute Respiratory Syndrome (SARS), eg. the virus that causes COVID-19 (SARS-CoV2), responsible for the 2019 and 2021 pandemic.
Crimean–Congo haemorrhagic fever virus	Emerging/re-emerging in eastern Europe, the Balkans, and Turkey, with evidence of a spread western Europe. Important in Central Asia and, partly, in sub-Saharan Africa.
Ebolavirus and Marburg virus	Wildlife serves as the virus reservoir between outbreaks mainly in the Democratic Republic of the Congo, Angola and Central Africa, with recent outbreaks and importation to USA, Europe and other regions. Ebola Reston variant in Philippines.
Hantaviruses	Bayou, Dobrava, Hantaan, Puumala, Saaremaa and Seoul viruses cause hemorrhagic fever with renal syndromes (HFRS) in USA and Western Europe, Russia and East Asia. Andes, Sin Nombre and Laguna Negra viruses cause hantavirus pulmonary syndrome (HPS) in South America.
Hendra virus	Menangle, Murray Valley encephalitis and Kunjin viruses, are still limited to Australia are a novel emerging pathogen with significant environmental reservoirs.
Influenza viruses	Not-strictly zoonotic, animal hosts are substrates for the development of novel influenza strains such as the avian H5N1 influenza, that requires close contact with infected animal hosts, with high lethality in Indonesia, Egypt, Vietnam, and China in 2004 or the H1N1 influenza virus initially zoonotic that easily became pandemic after human-to-human transmission. Global distribution.
Japanese encephalitis virus	Frequent in East and Southeast Asia. Expansion to Western Pacific and Central Asia highlight risks and significant mortality in the future.
Lassa virus and otherArenaviridae	Causing viral haemorrhagic fevers are Guanarito virus (Venezuela), Machupo virus (Bolivia), Sabia virus (Brazil), Junin virus (Argentina), Lujo virus (southern Africa). Americas and Africa with increasing global presence.
Nipah and Chandipura virus	Human-to-human transmission with important outbreaks of zoonotic origin (bats) and reservoir (pigs). Limited cases with major mortality in Bangladesh and India.
Oropouche virus.	Emerging in Brazil in recent years with sloths as the main hosts; vector-borne, clinically similar to dengue.

Viral group	Comments
Rabies and lyssaviruses	Although rare in Europe and USA nowadays with few existing cases imported, extremely rare in the USA [has more than 30 000 deaths annually in India, China, Pakistan, Bangladesh, and Myanmar. Lyssaviruses include Duvenhage virus (Africa, bat-related cases), Mokola virus (Africa), and Australian and European bat lyssaviruses Rift Valley fever virus, with outbreak in South Africa, Yemen, Saudi Arabia Sudan, Kenya, Somalia, and Tanzania.
Sindbis virus	Traditionally linked with Egypt this vector-borne disease is nowdays common in Southern Africa, East Africa, Arabic region, the Philippines and parts of Australia.
Tick-borne encephalitis	With thousands of cases in Russia and central–northern Europe. Incidence on the rise, possibly owing to climate changes.Venezuelan equine encephalitis virus with outbreaks in south-america.
Flavivirus (eg. West Nile virus, yellow fever virus, dengue, zika)	Zoonotically sustained massive outbreaks of west-nile virus in North America in recent years as well as in Romania, Russia, southern Europe, Mediterranean basin, and Arabic countries. WHO estimates more than 200 000 annual cases of yellow-fever worldwide, with 30 000 deaths, with global vaccination campaigns. Both the sylvatic and intermediate cycles of disease transmission are zoonotic. Being vector born diseases have their dissemination spread along with the global warming and the geographical expansion of the vectors.

Table 1 – Most important zoonotic viruses with global impact in human health. [Adapted from 11, 12].

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MIGRATION AND HEALTH – A PRIORITY IN THE GLOBAL HEALTH AGENDA

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INTRODUCTION

In an increasingly globalized world, migration and human mobility have great influence in shaping global health, which has put the topic of migration and health on the international and national agendas.

In population-based statistics, a diverse set of definitions and terms are used across countries – e.g. 'migrants', 'immigrants', 'foreign born', 'second generation', 'ethnic minority'. For ease of reading, in this chapter we will use the International Organization for Migration's definition of migrant as "a person who moves away from his or her place of usual residence, whether within a country or across an international border, temporarily or permanently, and for a variety of reasons".

There has been a significant increase in the number of migrants worldwide, including in Europe. In 2019, over 82 million international migrants lived in Europe, an increase of nearly 10% since 2015[1]. In Portugal, between 2018 and 2019 there was an increase of 22.9% of regular migrants living in the country. The foreign-born population represented 10.8% of the total resident population in 2019, mostly from Brazil, Portuguese-speaking African countries, Eastern Europe, China and a variety of other countries[2]. Migration has increasing in scale but also in complexity. Over the past decades migratory routes have multiplied, with most countries being simultaneously destination, transit and origin countries, and migrant populations have diversified, moving for a wider range of reasons and in disparate conditions. Changes in the gendered patterns of mobility towards a "feminization of migration" have also been observed, with women increasingly migrating on their own, often to enhance economic opportunities through employment or education, besides for marriage or family reunification.

Health is a crucial factor for migrants' integration in destination countries. Migration can bring valuable economic, social and cultural benefits for migrants and communities in host societies, but for a positive migration experience it is important that migrant populations are and remain healthy (1). In this sense, improving migrants' health and ensuring their access to quality health care are essential aspects for their integration and social inclusion.

Migrants in general tend to report a good health status, however health inequities persist as migrants are frequently amongst some of the most socially

[1] International Organization for Migration. World Migration Report 2020. Geneva: IOM; 2019.

[2] OECD. Foreign-born population (indicator); 2021. doi: 10.1787/5a368e1b-en

vulnerable and disadvantaged populations and often remain out of reach of health services (1). Migration brings relevant challenges to public health in terms of tackling social determinants of health, designing and implementing effective health policies, and enhancing the health system' responsiveness in a context of diversity (2).

The global and national commitments for improving health outcomes and well-being of all populations, ensuring that no communities are left behind, call for effective development of migrant-sensitive policies and strategies that are evidence-based.

STATE OF THE ART REVIEW

Considerable research has been devoted to examining health indicators among migrant populations and health-illness processes during their stay in destination countries. Nevertheless, many of the migration-related health risks and challenges result also from factors outside the jurisdiction of the migrant-receiving nations (3). In fact, the broadly documented interrelated and multilevel determinants that influence health operate alongside the conditions under which migration occurs and in the different contexts of origin, transit, destination and return (Figure 1). As such, there is growing awareness of efforts that are not only national in scope but also address the complex relation between health and migration at the global level (4).

The dynamic migration flows and the increasing heterogeneity of the migrant populations translate into disparate health needs that extend across cultural backgrounds, migratory experiences, and demographic characteristics. In addition, the factors associated with vulnerability, risk of illness, and adverse health outcomes are not equally distributed across migrant groups (3).

Research has shown that migrants are generally in good health at arrival in the destination country when compared to the host population – the so called 'healthy-migrant effect' (1). However, this apparent health advantage diminishes as migrants' health status tends to deteriorate over time in the host country. Upon arrival migrants face a new context with differences in the physical and social environment, clash of cultures and lifestyles, language barriers, and discrepancies in administrative and legal systems that can lead to physical, psychological, and social problems (1). Some migrants face social and economic hardships and legal restrictions on their rights, especially associated with irregular status. The resulting

lack of social protection and social exclusion contributes to individuals' reduced opportunities for access to information, social and health services, as well as limited capacity and resources for health protection (1,5).

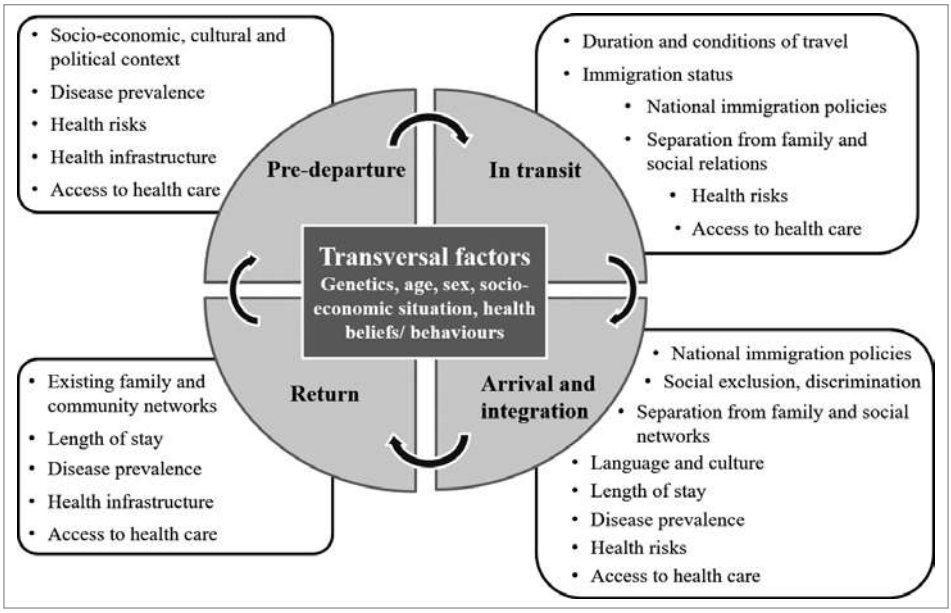

Figure 1 – Health determinants throughout the migration process.
Adapted from: IOM. Social Determinants of Migrant Health; 2021
[https://www.iom.int/social-determinants-migrant-health]

Traditional approaches to health and migration have mainly dealt with communicable diseases of public health significance, like HIV and TB (1). Migrants, particularly those from highly endemic countries, have been considered a disproportionately vulnerable population to HIV infection. But increasing evidence shows that in Europe a significant proportion of migrants who are HIV positive have acquired infection after they arrived in the region (1). This can be explained by the socioeconomic vulnerability in host countries and individuals' limited capacity for adopting preventive measures, along with separation from partners and social isolation that have been found to pave the way for risky sexual behaviours such as multiple partnerships and unprotected sex.

Although attention has been traditionally drawn to infectious diseases, the effects of sustained migration on the longer-term epidemiology of chronic non-communicable diseases has led some migrant-receiving nations widening their

focus to these health problems (1). This includes focusing on the impact of lifestyles and behaviours on health, as well as on the demand on health promotion and the use of health services for disease prevention and management among migrant populations. The literature has documented that, within the process of acculturation, new health-related norms, values and lifestyles are adopted (i.e. diet, physical inactivity, alcohol consumption, substances use and smoking), resulting in increased morbidity rates, e.g. for obesity and cardiovascular disease, and mortality rates for diabetes mellitus, ischaemic heart disease and stroke (1).

The fact that in many countries almost half of all migrants are women and mostly of reproductive age has making sexual and reproductive health another priority. Research has shown that some migrant women report lower knowledge and use of contraceptive methods compared to their native counterparts, resulting in higher risk of unintended pregnancies and induced abortions (1). Migrant women are found to be generally more prone to poor pregnancy-related outcomes, such as later initiation of antenatal care, higher rate of complications during childbirth, increased risk for preterm birth, neonatal and maternal mortality, and congenital malformations, and higher incidence of postpartum depression. In addition, migrant women are particularly exposed to gender-based violence during migration, that causes serious harm to physical and mental health (1).

In addition, increasing attention has been given to the impact of migration on mental health. Along with exposure to risk factors throughout the migration process, as humanitarian and conflict situations in origin countries or adverse travel conditions, there may be additional psychological burden when settling into a new country and facing uncertainty and insecurity about socioeconomic conditions and separation from family and social networks of support (1). Mental health problems, such as post-traumatic stress disorder (PTSD), depression and anxiety, tend to be higher among some migrants compared to host populations. Migration was also found to be a risk factor for children's mental condition, particularly unaccompanied minors who experience higher rates of depression and symptoms of PTSD compared with other migrant groups (1).

Access and utilization of health services are crucial for attaining a good health status. However, in many destination countries it has been evidenced that migrants often face obstacles in accessing health services which can result in underuse (6). Some of those obstacles include unawareness of migrants' health rights and services available, especially among recently arrived migrants, economic constraints to afford costs of care, as well as language barriers, culture-related constraints in

provider-patient relationship, and structural factors linked to services functioning (6,7). The irregular status and the fear of associated consequences, such as deportation, can also be a barrier, even in countries that assure legally the access to health care regardless of the immigration status (6).

In the current context of the COVID-19 pandemic, the available evidence shows that social and health inequalities have aggravated, with a disproportionate impact on migrants, especially in Southern European countries (8). In addition, the constrained access to health services at expense of COVID-19 care, that resulted in reduction or delay in screening, diagnosis and treatment, including among those with chronic diseases, potentially affected migrant populations even more.

RELEVANT CONTRIBUTIONS FROM NOVA

In line with the worldwide awareness of migration and health as a global societal challenge, the research agenda has been set to address the lack of evidence and support health policies and strategies (9). NOVA has long been dedicated to advance knowledge on this field, namely concerning the health status, determinants and needs of migrant populations.

As shown in an analysis of data from the 5th National Health Survey, migrants tend to report a better health status and less frequently chronic diseases (e.g. diabetes, asthma, hypertension) than national populations (10). Additional analyses indicated that 86.6% of migrants living in the country for less than 6 years report more frequently good/very good health status than those living longer (57.4%) (Figure 2). On the other hand, throughout length of stay, the proportion of migrants reporting good/very good health status, as well as no chronic diseases and mental health tends to decrease and to get closer to that of the national population. Indeed, the same trend has been found for diabetes, hypertension and associated behavioural factors. Data indicate a reduced consumption of fruits and vegetables, which is associated with a greater likelihood of being overweight, as well as an increased alcohol consumption and physical inactivity among migrants staying longer in Portugal (11,12).

Research aimed to understand the determinants of health disparities among migrants has demonstrated the key role of multiple and interrelated individual, social and structural factors. In a study with 1375 migrants conducted in some of the most deprived areas in Lisbon and Tagus Valley (the region with the highest

concentration of immigrants in Portugal), migrants with lower education and lower income were less likely to report good health (12). Similarly, higher risk of experiencing poor mental health outcomes, including psychological distress and depression, were found in female migrants, those unemployed, socially isolated, with lower income and high job insecurity (13).

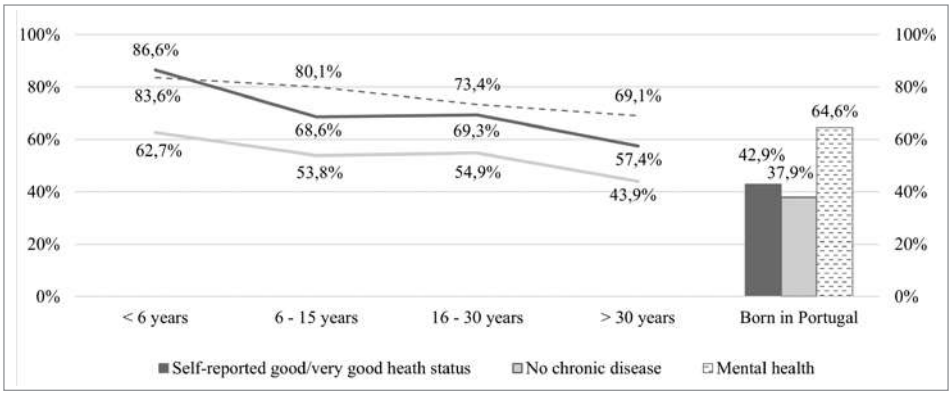

Figure 2 – Self-reported health of migrants over length of stay and nationals.

In a participatory biobehavioural HIV survey developed by our team with a sample of Sub-Saharan African migrants in great Lisbon, HIV infection was over five times higher among participants with primary or no education and almost three times higher among those with insufficient income (14). But further analyses, in a joint collaboration with a research team from the Institute of Tropical Medicine in Antwerp, showed that, besides partnering and relational contexts, migrants are mobile and engage in sexual risk behaviours with different partners in the countries of residence and while travelling, which increases risk of post-migration HIV-acquisition (15). The project *Genomics, socio-behavioral and clinical data to prevent HIV transmission in migrants: an innovative approach*, promoted by the Institute of Hygiene and Tropical Medicine (IHMT), showed that HIV-1 molecular epidemiology in migrants suggests high levels of connectivity with their country of origin (16). Research has also provided data on the burden of other infectious diseases among migrants, such as Tuberculosis (14). Overall, these projects intend to contribute to a holistic model to guide infectious diseases prevention and control policies.

Sexual and reproductive health of migrant populations has also been a much-explored area. A qualitative study with African and Brazilian women in Portugal reinforced other research showing that migrant women are more likely

to face socio-economic difficulties and lack of social support that may intensify during pregnancy and postpartum (17). The migratory process can be a source of difficult experiences, often resulting in post-traumatic stress conditions, with high incidence of mental illnesses such as anxiety or depression, especially among women in post-partum period.

Disparities in access to and use of health services have been found to persist. A study aimed to compare migrant and native populations in five European Union countries, including Portugal, demonstrated that migrants tend to use less the preventive care such as colorectal cancer screening, mammography, and cervical cancer screening (18,19). Low uptake of STIs screening has also been reported, contributing to underdiagnosis of infections, delayed diagnosis and treatment (14).

It is worthy to mention that, in Portugal, efforts were made over the past years to adopt inclusive policies and regulations promoting migrants' access to health services. Nevertheless, evidence indicates that migrants may experience difficulties in exercising their rights and further efforts are needed to improve health services responsiveness. A study conducted by our team to explore the patterns of health care seeking in a community-based sample of migrants showed that those undocumented are less likely to have sought health care for the first time at a primary health care service and tend to postpone the use of health services for emergency situations, which indicates that these groups may not be aware of their health rights and the services available, but may also prioritize concerns related to the adaptation to a new context, such as housing, job and social protection, over preventive care (20). Qualitative studies on migrants' perceptions and experiences about health services evidenced other barriers such as office workers and health professionals' unawareness and misinformation about migrants' health rights, as well as structural and functioning characteristics of the services, like restricted working hours, highly bureaucratic procedures and long waiting time that tend to affect more those with less labour protection and flexible work schedules, as many migrants (21).

Just as determinants of migrants' health are complex, so are the solutions to tackle them and promote migrant populations' health. NOVA has been extensively committed to produce relevant and useful scientific knowledge that, by deeply involving the populations in the research process, potentially contributes to create evidence-based policies, strategies and interventions that respond to real health needs. But developing meaningful research on migrants' health has several conceptual, methodological and practical challenges. NOVA has developing cutting-edge research focused on overcoming those challenges, trying to address questions

such as 'How to research most vulnerable, understudied and underserved subgroups?' and 'How to further unveil their real needs and understand the contexts that increase vulnerability?'.

Most vulnerable populations, including those socially disadvantaged as some migrant groups, are frequently missed in conventional surveillance efforts, are understudied and underrepresented in research findings, and remain out of reach of health programs for prevention, diagnosis and treatment. Overall, the frequent inability in reaching most vulnerable populations and obtaining their perspectives about the phenomenon under study makes the gap between the knowledge produced and the populations' real needs complex to overcome. This has called for a different paradigm of doing research, integrating several actors, methods and disciplines. In this context, several projects at NOVA adopt a participatory approach, involving community members, representatives of governmental and nongovernmental organizations, health professionals, policy makers and academics in the process of research. This allows incorporating stakeholders' different perspectives and experiences in defining the key research questions and the most appropriate methodological approaches, in designing and implementing research and interventions, and in disseminating results, as adopted in many research experiences worldwide (22).

In addition, health research, strongly influenced by the biomedical perspective, has been much based on quantitative approaches focused on examining the risk factors and associated determinants, and mainly driven by the researchers' point of view. Research has been developed at NOVA gathering and integrating quantitative and qualitative knowledge. The need for understanding more comprehensively the complex, multifactorial and multidimensional nature of health problems in a way that goes beyond quantitative descriptions has reinforced the relevance of mixed methods.

NOVA has also consolidating a track-record of international and multicentric research, within competitive funding programmes, such as European Union's Horizon 2020. Several projects combine insights and knowledge from the social and behavioural, the biological and medical, and the applied technologies and engineering sciences, gathering partners from several European and third countries (see example of ELEVATE described in *Ongoing Research*).

But if global challenges require global solutions, these can only be effective if they are contextually relevant and implemented locally. An endeavour of NOVA health research has been, not only to generate context-specific evidence, but also to co-design, implement and evaluate evidence-based solutions, and understand what

works, how it works, why and for whom, in real-world settings. In this sense, NOVA has been strongly engaged in implementation research. This integrated approach links research and action to optimize multisectoral and context-congruent public health responses for promotion of migrants' health and stakeholders' responsiveness (one example is the project on health literacy and NCDs prevention among migrants, described in *Ongoing Research*).

In another approach dedicated to the evaluation of complex integrated care interventions, our research team has been working, in collaboration with primary care health services, in the evaluation of Social Prescribing (SP). SP is a pioneer complex intervention aimed to enhance the provision of integrated care by linking health care services to the tertiary sector, to effectively reduce the impact of social determinants of health and promote health, well-being and social integration.

In the current context of the COVID-19 pandemic, while the situation of many migrant populations was highlighted in technical reports and in the media, the evidence on how migrant populations are experiencing the pandemic and its effects on daily life was clearly missing. The survey "Migrant populations and COVID-19: Perceptions about the impact of the pandemic", promoted by the National School of Public Health, analysed the perceived impact of the pandemic on migrants' living conditions, health status and access to health services. The findings showed that, in a total sample of 1,091 migrants, more than a half considered that his/her financial and work situation have worsened during the pandemic and over a quarter experienced feelings of agitation, anxiety or sadness almost all days, most frequently women and undocumented migrants. In addition, the access to health services during the pandemic period has got worse for 43.5% of participants and a half considered not to have the information they needed to take care of their health. This knowledge along with a panel of experts allowed to elaborate recommendations for effective strategies and practices that, with the involvement of health authorities, health institutions, civil society and citizens, could help mitigate the social and health effects of the pandemic and also promote a more adequate and equitable access to health information and services. Another project focused on the impacts of the pandemic was *Response to the Covid-19 pandemic in the context of social inequalities in health: a cross-sectional study among the native and immigrant population of Amadora*, developed by Institute of Hygiene and Tropical Medicine, aimed to analyse the socioeconomic dynamics, apprehensions and difficulties in accessing health care among 420 families, of which 217 were migrants. Preliminary results also reinforced that migrant families have been disproportionately affected

by the pandemic when compared to native ones, experiencing more unemployment, lower income, difficulties in paying rent, provision of credit or current expenses, and greater difficulties in accessing health care.

Motivated by the will of further addressing the social determinants of health that, in a more holistic approach, are linked with social integration, other NOVA research projects have been developed focused on employment, education, language skills, public policies and law, among other areas. An example is the work developed by NOVA School of Law, namely related to European Union Asylum and Migration Law, as well as the work at the College of Social and Human Sciences (NOVA FCSH) dedicated to the issues of integration of migrant groups, including main obstacles faced and ways of accessing integration channels, such as the Portuguese language, health and the labour market.

IMPACTS TO SCIENCE AND SOCIETY

Over the past years, NOVA has been committed to research with potential for societal impact through biomedical, socio-behavioural, health economy and policy research in a multidisciplinary approach. Example of this effort is the NOVA Migration and Health group, within the NOVAHealth, that promotes collaborative research and knowledge to address the challenges of human mobility and migratory flows to health status, by combining background, expertise and vast experience of research in this field of different members from several NOVA Schools.

NOVA has contributed significantly to advance knowledge of the complex relationship between migration and health and to develop innovative evidence-based solutions that are effective, responsive and sustainable in addressing the needs of the most vulnerable groups, reducing health and social inequalities and promoting migrants' health, well-being, and social integration.

Several research projects developed at NOVA addressing migration within different disciplines have providing a privileged opportunity for diverse Master and PhD students of NOVA Schools to develop their academic work and competencies on these fields. In addition, the topic of Migration has been a focus in the curricula of NOVA Masters and Doctoral programs in the Health field, but also in many other areas linked to migration studies, such as Globalization Studies, Political Science and International Relations, Inter-ethnicity and Transnationalism, International and European Law, among others.

NOVA track-record of participatory research in a multisectoral approach has contributing to increase know-how on best approaches to reach most vulnerable groups, to collect data and to translate it into co-created tailored and context-congruent responses. The multicentric research has also providing a platform to share, collaborate, develop, mentor, and disseminate interdisciplinary knowledge and effective solutions.

At societal level, the collaborative partnerships with the communities and key stakeholders have increasing their ownership and involvement in research initiatives, contributing to enhance their advocacy, empowerment and capacity building for promoting health and achieve health gains.

With the view to further support social integration of migrant populations, the new initiative called "NOVA Refugee Clinic (NRC)" will be developed to provide a holistic service that guarantees an immediate and quick response to short-term legal or medical problems while enhancing the success of integration through linguistic, social and entrepreneurial support. The NRC – Legal Clinic, a research laboratory of the NOVA School of Law and the R&D Center on Law and Society (CEDIS), has been launched. In a near future, the NRC dedicated to health research and support will also be implemented involving several NOVA Schools.

In addition, NOVA is present at the Migrant and Ethnic Minority Health Section of EUPHA (European Public Health Association), that brings together researchers, policymakers and practitioners working in the same field for knowledge sharing and capacity building across European countries.

All the projects and initiatives intend to contribute to the achievement and are fully aligned with the Sustainable Development Goals. Overall, a main drive of research at NOVA has been to help societies to become more inclusive and equitable, and therefore potentiate the benefits of a successful migrant integration, as a way for attaining health gain for the whole population.

ONGOING RESEARCH

We herein highlight some of the ongoing projects on Migration and Health developed by, and many of them in cooperation of, several NOVA Schools. One example is the 5-year project *ELEVATE – EarLy dEtection of cerVical cAncer in hard-to-reach populations of women through portable and point-of-care HPV Testing*, developed by a consortium of partners from 8 countries in Europe and Latin America, including NOVA

National School of Public Health, and funded by the Horizon 2020. This project aims to develop an innovative solution to improve cervical cancer screening in hard-to-reach populations, including migrants, that consists of a community-based strategy and a test combining self-sampling with a new technological, low-cost, portable, rapid HPV testing device (https://elevate-hpv.com). A qualitative component has been conducted to understand who are the most-at-risk populations of women being under-screened, what are the barriers they face to screening, and what are the best strategies to reach those women. An innovative intervention will be designed and implemented to pilot a new HPV self-sampling test and device in diverse settings.

Health Literacy, Health Promotion and Social Cohesion for the Prevention of NCDs among Migrant Populations, a 3-year project, is designated one of the National Health Literacy Demonstration Projects by the WHO Regional Office for Europe and is conducted by NOVA National School of Public Health within a multisectoral consortium. It aims to optimize health literacy, health promotion and social cohesion in support of prevention of NCDs among migrant populations, thus contributing to improve health outcomes and reduce health disparities. As a community-based project, it takes a grounded approach to produce evidence on real health literacy needs and co-design, implement and evaluate health literacy-based multisectoral interventions that embody innovative, locally relevant, culturally and context congruent solutions for prevention of NCDs among migrants.

The specific area of migrant children's health has been given special attention, with research being developed by IHMT, initially with the project *Cohort Study for Immigrant and native Children in Amadora Municipality – CRIA cohort study*, awarded by FAMI. More recently, the study *Health determinants and needs of children on the move in a pandemic context: A longitudinal study for Lisbon and Tagus Valley Region* (funded by FCT) aims to analyse health trajectories of migrant children, and to provide evidence on the impact of COVID-19 on health inequalities. It intends to identify main health needs, understand barriers to health care and its determinants, identify more suitable early-childhood interventions, in order to contribute to improve existing public health strategies for a more inclusive approach.

Evaluation research has also been a focus in ongoing projects such as the project *Evaluation of the impact and implementation of Social Prescribing (SP) in primary healthcare units in Lisbon,* developed by NOVA National School of Public Health, in collaboration with primary care health services. In a mixed-methods approach, a longitudinal, prospective study is being conducted to explore the extent to which the SP intervention leads to enhance migrant patients' quality of

life, well-being, and activation, as well as to understand its impact in health systems to support scalability.

In a multidisciplinary and multicentric approach, *MINTEGRATION – Integrating Immigrants as a Tool for Broad Development: Experimental Evidence for Portugal and Cape Verde*, by NOVA School of Business & Economic (promoted by NOVA SBE), proposes to experimentally evaluate the impact of an active immigrant integration program among immigrants and their relatives in Cape Verde. The program to be evaluated will be multi-dimensional and is expected to promote better quality employment of migrants, better access and usage of health or education services, and to improve other integration indicators.

Other ongoing projects focus on the social determinants of health that strongly influence migrants' social integration and consequently their health, such as the project *PPEACE – Public Policies and Reception of Foreign Citizens* of NOVA FCSH and IPRI-NOVA, the project *READ4SUCCEED: Improving Reading Skills of Migrant, Refugee Children and Children from Low Income Neighborhoods through Animal Assisted Reading* of NOVA FCSH, CICS.NOVA and Linguistics Research Centre of NOVA University Lisbon, and the project *Mentoring Success of Immigrant College Students* of NOVA SBE.

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PANDEMICS

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PANDEMICS: A BRIEF INTRODUCTION

Classically a pandemic (from Greek πᾶν, pan, "all" and δῆμος, demos, "local people" the 'crowd') is an epidemic of an infectious disease that has spread across a large region, for instance multiple continents or worldwide, affecting a substantial number of people (1). A widespread endemic disease, with a stable number of infected people is not a pandemic.

In 412 BC, Hippocrates described what today are known as the first cases of influenza. Since then, an uncountable number of outbreaks, epidemics, and pandemics caused by different pathogens were documented. Almost 2500 years after the Hippocrates' report the extraordinary advances in understanding the etiology, transmission, prevention, and treatment of infectious diseases paved the way to a better understanding and rational implementation of effective public health measures capable of reducing its burden. However, new infectious diseases are often not easy to prevent, and the present pandemic continues to spread quickly in many parts of the world killing millions of people, especially in most vulnerable regions and population groups.

The first known plague was described by the Greek physician Galen in his treatise *Methodus Medendi* (Methods of Treatment). The disease was brought by Roman troops after a campaign in the Near East. It happened between 165 and 180 AD and was caused most probably either by smallpox or measles. It killed the Emperor Antoninus and continued to spread to Gale. It is estimated that about 10 million people were killed, with a peak of 2000 deaths a day in the city of Rome.

Since then, many other plagues were described with more or less detail in several historical sources. Among the most "famous" we can consider, in chronological order, the Plague of Athens (430 BC) that killed Pericles, and was possibly caused by typhus, the Plague of Justinian (around 542) which arose in the Byzantine Empire, spread to all the Mediterranean Basin and Near East with an estimated number of up to 100 million deaths, and was caused by the bacteria *Yersinia pestis*, the Black Death, that affected Europe and parts of Asia and Africa in the 14th century. According to different sources it killed up to 200 million people and was also caused by *Yersinia pestis*. In the middle of the 19th century, a Plague began in the province of Yunnan, China, and spread to all continents. According to the WHO, it caused the death of about 12 million people, mostly in India and China, and was active for more than 100 years affecting several countries. More recently, in the 20th century, the Influenza pandemics of 1918, also known in some countries as the "Spanish", infected 500

million people and caused between 20 and 100 million deaths, according to different sources. The WHO reported five influenza pandemics in the last 140 years with the more deadly being the one reported in 1918. This is consistent with a previous estimate by the WHO of a new influenza pandemic arising every 20-30 years.

PANDEMIC FEATURES

Historically, measures of pandemic severity were based on the case fatality rate, the proportion of deaths from a certain disease compared to the total number of people diagnosed with the disease. However, case fatality rate might not be an adequate measure of severity during a pandemic response because: 1) Deaths may lag several weeks behind cases, making the case fatality rate an underestimate; 2) The total number of cases may not be known, making the case fatality rate an overestimate; 3) A single case fatality rate for the entire population may obscure the effect on vulnerable sub-populations, such as children, the elderly, those with chronic conditions, and members of certain racial and ethnic minorities; 4) Fatalities alone may not account for the full burden of the pandemic, such as absenteeism or demand for healthcare services.

Classically, the so-called "epidemiological chain of pandemics" is characterized by the causal infectious agent, the correspondent reservoir, the exit door of the agent, the mode of transmission, the elimination doors as well as doors for entry into the host, the susceptibility of the host, and the environment. The characteristics of the infectious agent are essential for measures of controlling, and include: infectivity (the ability of organisms to penetrate and multiply in the host), pathogenicity (potential to produce symptoms of different severity), virulence (the ability to produce serious or fatal cases), infective dose (amount needed to initiate an infection), vulnerability (to the environment, chemicals, physical substances, therapeutic agents, vaccines), invasive power (the ability to spread through the host's tissues and organs), and immunogenicity (ability to induce immunity in the host).

The characterization of the host and its interaction with the infectious agent is vital in decision-making, defining the population at most risk and controlling asymptomatic, symptomatic and immunocompetent individuals. Latency period (period from entry of the pathogen until the host becomes infected) and the incubation period (from infection to symptom onset) constitute additional important factors to consider.

Advances in molecular biology speeded up the development of new diagnosis, prevention, and treatment tools, and allowed a rapid evolution of the knowledge of pathophysiology and immune response against infectious agents, decisively contributing to the definition of clinical conditions, and the adoption of more appropriate and effective therapeutic measures.

It is consensual that methodology of pandemic Response Plans includes the following phases: preparation, response (containment, extended containment, and mitigation), and recovery. They must always take into account the eventual need to return to previous phases as actually is happening in many countries today. They involve technical components like diagnosis (active and passive screening), treatment, preventive measures, research (biomedical and behavioral), social mobilization (information, education and communication) and partnerships. All of these components are naturally framed in an epidemiological surveillance system (including border control), which is crucial for monitoring the adequacy of other technical components. However, control of pandemic events very often faces chronic difficulties, namely the weakness of health systems, which includes obstacles in communication and coordination between the various levels of decision, poor laboratory capacity for diagnosis, limited number of trained personnel, inadequate case management, and logistic capacity.

Currently, humanity faces a new pandemic challenge caused by SARS-CoV-2 virus. The quick spread of the virus was enhanced by a huge and never observed in the past mobility of people due in part to globalization but also to the prevalence of local armed hostilities that generate a vast number of refugees and contribute to ease conditions for the pathogen to cross borders and continents.

THE COVID-19 PANDEMIC

Looking at the brief description of pandemics along the History there is one important fact that is common to almost all of them. They started with the contact of Humans with animals, mainly birds and mammals. Almost all of them had a zoonosis background. In the case of SARS-CoV-2, bats are the reservoirs and are also among the most disseminated mammal species in the world. However, the MERS epidemics that occurred in the Middle East in 2012-2013, also caused by a much more deadly coronavirus apparently was caused by transmission from dromedaries to humans. Animals serve as reservoirs of infectious agents that “jump” to humans and may

adapt to replicate causing new diseases for which there are no specific treatment and/or vaccine available. In the last decades it has been observed a closer contact between humans and other species due to climate changes that potentiate the quest for new animal habitats that may substitute the ones destroyed by direct or indirect human actions. The basic characteristics of outbreaks, epidemic, and pandemic events are known for centuries, and have been applied to fight them by our ancestors long before the identification of the pathogenic potential of bacteria and viruses in the XIX century.

The present pandemic, which killed already over 7 million people, is caused by a new virus belonging to a family, *Coronaviridae*, whose biology and pathology is relatively well known, and whose common ancestors have been hanging out for an estimated 10.000 years. However, human coronaviruses were only discovered in the decade of 1960 (2). They are responsible for about 15% of common colds. After infection, the symptoms are usually mild and the immunity may last for up to three years. So, what makes this new coronavirus different from the other members of the same family? A not very extensive list may consist of the following features: 1) It is more transmissible and spreads very rapidly; 2) It causes more severe symptoms; 3) The death rate is higher than that of most human coronaviruses; 4) It affects both the upper and lower respiratory tract; 5) It may be found and perhaps may also replicate in different organs of our body; 6) The origin is still unknown and controversial. There is no specific treatment although some progress has been made in patient management since the report of the first cases. Until now, the only drug that seems to have positive effect in hospitalized patients is dexamethasone, which has anti-inflammatory properties that may help attenuate the so-called cytokine storm in the affected organs. All attempts to repurpose other drugs to treat this infection and inhibit virus replication failed as recognized by the WHO. Hydroxychloroquine, remdesivir, ivermectin and many others, including the so-called therapy with antibody cocktails didn't prove to be effective in well controlled clinical trials. The potential benefit, if any, for patients was always found to be lower than the eventual harmful effects. Today more than 400 drugs are being tested to be repurposed in SARS-CoV-2 inhibition assays. One of the main advantages of repurposing a drug is saving time and money since all necessary safety trials have already been made before. Unfortunately, until now there are no clear good news to announce. More than 90% of the compounds didn't show any positive effect on tests in susceptible mice models. However, recently a couple of announcements of new specific anti-SARS-CoV-2 drugs were published in the social media. Nevertheless, it's potential future

use is still dependent on many factors one of the most important being its safety and efficacy in patients with severe symptoms. The development of a specific and effective anti-SARS-CoV-2 drug would be of extreme utility in controlling the burden of disease, especially in patients with more severe symptoms. The approaches being used are often not similar but state of the art techniques like the use of artificial mini lungs are under way. We hope that at least one of these drugs may reach the final phase of clinical trials with success becoming an important tool to save lives.

In face of this situation, the only hope resides in global and massive vaccination campaigns. Here, both the academy and the pharmaceutical industry undoubtedly were more successful. In a record time of less than one year, something that most people would never dream of before, the first four vaccines were approved and certified by the European Medicine Agency (EMA). Perhaps, when this book will be published others have also been approved. Many potential vaccines are also in the pipeline using classical and new approaches. Over the last decades, vaccines were responsible for saving millions of lives in the world, namely children. They became an indispensable, effective, and safe tool in controlling and eradicating infectious diseases as well as part of general public health strategic policies. We hope that in the context of the present pandemic they will also play a decisive role in controlling virus spread worldwide.

VACCINES AND ANTI-SARS-COV-2 COMPOUNDS

The term vaccine was coined by Edward Jenner in 1798 in the title of his *Inquiry into the Variolae vaccinae known as the Cow Pox* (3). He was the first to develop the concept of vaccine and to develop the first vaccine (against human smallpox). Since then, several types of vaccines have been successfully developed against 25 infectious diseases. In many cases, since the development of the first version, vaccines were improved due to a better knowledge of the performance of the immune system and the development of new state of the art technologies that allowed adding modifications that enhanced its safety and efficacy. In general, vaccines can be divided in the following types: inactivated (poliomielitis, influenza), attenuated (measles, mumps, rubella, yellow fever), viral vector (SARS-Cov-2 and other viruses), subunit (hepatitis B, papilloma), heterotypic (tuberculosis), toxoid (tetanus, diphteria), conjugate (haemophilus influenza type B), DNA, and RNA (SARS-CoV-2). All types have advantages and limitations depending on the characteristics of the

pathogen and of the host. Once approved for human use all can be considered safe since they comply with the most rigorous normative guidelines and standards. Development of severe side effects, as with any other pharmaceutical compound, are extremely rare events. The efficacy of vaccines is also variable. This may be due to different factors including the immune and nutritional status, age, health comorbidities, genetic background of the host and perhaps the microbioma (the set of commensally microorganisms that play a crucial but still poorly understood role in protecting our health and maintaining homeostasis). In accordance, some vaccines like against influenza have a relatively low efficacy (10-60%, depending on the year of manufacturing) mainly due to the high rate of mutation observed in virus proteins that are exposed to our immune system. This high mutation rate is a common feature observed in all RNA viruses like Influenza and HIV and is due to the lack of an appropriate repair system of mistakes that arise during virus genome replication. However, SARS-Cov-2, as well as other Coronaviruses, are an exception although they also have RNA genomes. They evolved a unique mechanism of RNA repair that reduces mutation rates when compared with other RNA viruses. As far as it has been reported by manufacturers, SARS-CoV-2 vaccines so far approved by EMA displayed efficacies of 70%-95% in phase III clinical trials which, if confirmed in phase IV follow ups, is a significant achievement.

On the opposite, DNA viruses, developed mechanisms that enable the repair of mistakes arising during replication making it easier to develop effective vaccines that can be administered over the years without modifications. Currently, there are dozens of vaccine prototypes against SARS-CoV-2 registered in the WHO database of clinical trials that were developed using different methods and approaches. A special mention perhaps deserves the quick and effective development of the new RNA vaccines. Until the beginning of the current pandemic there wasn't a single RNA vaccine approved for use in humans against an infectious agent. However, they display several advantages when compared to "traditional" vaccines. They are easier to design, cheaper and faster to produce, and to adapt to new virus variants. For example, the concept and design of the Moderna vaccine took only 2 days. However, RNA molecules are known to be unstable and prone to degradation. Accordingly, special care is needed to preserve them intact until administration. This may be achieved by preservation at very low temperatures (between -20°C and -80°C) but represents a serious drawback for immunization campaigns in low-income countries where this type of infrastructures and supply chains are still poorly developed. It is thus clear that different types of anti-SARS-CoV-2 vaccines are needed depending

on specific conditions of each country and region. Besides components of the pathogen, vaccines also contain other substances that are added to help improve preservation and stability. The so-called adjuvants are also commonly used. These are substances that are included in vaccine formulations to help boost the immune system. Not all vaccines, including RNA vaccines, contain adjuvants.

Without vaccines it would be virtually impossible to eradicate smallpox, one of the most contagious disease with high rates of mortality. Poliomyelitis is now the target of a global eradication campaign lead by the WHO. At present polio remains endemic only in small parts of Nigeria, Afghanistan, and Pakistan with small outbreaks still occurring mainly in low-income countries. We are on the verge of its eradication and saving lives of millions of children.

Undoubtedly, vaccines saved hundreds of millions of lives and represent essential tools in preventing and fighting epidemics and pandemics. However, the present pandemic is most probably very far from being controlled and its end cannot be predicted. Therefore, the only way to attempt reducing the burden of the disease is continuing monitoring the evolution of the virus and covid-19 and increase the global efforts to make vaccines available to the whole world population, without exceptions and discrimination. However, we shall not forget that there is an urgent need for effective, safe, and specific anti-viral drugs, capable to deal with the most severe cases and helping to control disease burden in case of failure of one or more vaccines due to the sudden appearance of new variants unrecognizable by antibodies that were previously generated. SARS-CoV-2, similarly to other viruses, would probably sooner or later develop resistance to these putative anti-viral drugs and this is something we can not prevent. There is a need for continuous research not only in the epidemiology, prevention, diagnosis, and treatment options but also in fundamental research that will ultimately produce the clues for a deeper understanding of host-virus interaction mechanisms that may play a decisive role in development of new therapeutic and preventive tools that will lead to more rational and effective control and treatment options of Covid-19.

HERD IMMUNITY AGAINST COVID-19

Since the beginning of the current pandemic the term “herd immunity” is perhaps one of the most mentioned in social media. Herd immunity refers to an indirect protection from infectious diseases that can happen when a certain percentage of a

population becomes immune to the disease. It only applies to transmissible diseases like SARS-CoV-2, measles, influenza, etc., but not for non-transmissible diseases like cancer, or vector-borne pathogens (like dengue for example). The term was coined by Topley and Wilson in an article published in 1923 (4). Herd immunity was officially observed and recognized only in 1930 during a measles vaccination campaign in the USA. It was reported a decline in new cases of measles in children that were not vaccinated, pointing to an indirect effect of protection promoted by those vaccinated that impaired the spread of the virus in the community. However, control of measles infections became possible only after mass campaigns of vaccination worldwide that began around 1960. The number of immune individuals in a given population that is necessary to achieve herd immunity varies and depends on different factors and assumptions. There is a theoretical basis for calculating the percentage of immune individuals in a certain population that is necessary to reach a steady state where the number of infections stabilizes. This critical value can be calculated taking into account the basic reproduction number "R_0" (the average number of new infections caused by each case) and "S" – the proportion of a population that is susceptible to a certain infection. In a steady state, when cases do not either increase or decrease, $R_0 \times S = 1$. Several factors may influence the achievement of herd immunity either naturally and/or by vaccination. Among them are the age (often older people display poor immunity after vaccination), natural susceptibility to a certain infectious agent which may also depend on several factors including the genetic background of an individual or population, the immune status as well as the mode and route of transmission of the pathogen (airborne, fecal-oral, body fluids, etc.). A typical example are Influenza vaccines which are much less effective in older people due to the decline of efficacy of the immune system. Just for purposes of illustration we can refer that the calculated percentage of immunization of a given population to achieve herd immunity may vary between 33%-44% in the case of influenza, and 92%-95% in the case of measles. For SARS-CoV-2 this percentage is believed by many experts to lay around 70%-90% but the spread of new, more contagious, variants may increase this number. The R_0 for SARS-CoV-2 without measures of prevention and contention may vary between 2.5 and 4. Will we be ever able to achieve global herd immunity against SARS-CoV-2? Opinions of experts are not unanimous, and unfortunately the answer to this question still lacks a solid background. Too many questions are open and awaiting more robust scientific data. Will new variants of the virus influence the efficacy of current and future vaccines? How long will immunity be hold? Will it be necessary to be vaccinated every year as it happens with influenza? How important is the role of asymptomatic

individuals in the spread of the disease and how do they contribute to formation of herd immunity? Are reinfections common events? Will we be able to develop a specific anti-viral compound? Can we predict the evolution of the virus and the appearance of new variants with specific characteristics? These are just a few examples of still open questions that need to and are currently being addressed by scientists.

Today, scientists are reinforced with new tools that enable a much more rapid, reliable and sensitive analyses and integration of data. One of these tools are the so-called evolutionary algorithms and artificial intelligence (AI). These approaches are being used in a large number of fields including the study of viruses. In the case of SARS-CoV-2 there are several AI tools that help dealing with the big data generated by scientists. Examples of the use of AI within the scope of the present pandemic are the prediction and tracking of the most vulnerable regions, contact tracing, early diagnosis, prediction of evolution of the genome and proteins of the virus, development of therapeutics and vaccines.

Despite the huge progress made so far, SARS-CoV-2 most probably came to stay among all of us. Unfortunately, vaccines are not being distributed equitably to all countries, there is a fierce competition between governments for the sake of propaganda and leadership, and of course between pharmaceutical companies for profit. Probably, when the population of high-income countries will be vaccinated, new variants already appeared in other parts of the world. These variants, with still unpredictable characteristics may travel globally and cause new outbreaks, epidemics and in the worst case, a new pandemic. New pandemics may arise suddenly and more frequently than before. The future of our children depends on how well we learned lessons from the past.

CONTRIBUTIONS OF IHMT-NOVA IN THE CONTEXT OF THE COVID-19 PANDEMIC

Founded in 1902 as a School of Tropical Medicine, the Institute of Hygiene and Tropical Medicine firstly focused on the presence of Portugal in Africa, and today is a privileged interlocutor at national and international levels, in areas such as public and international health, emerging, reemerging, and neglected diseases not only in the tropics but in a global and multifaceted perspective.

Today, research is performed within the frame of Global Health of Tropical Medicine (GHTM; please, see the activity report available at https://ghtm.ihmt.unl.pt)

a Centre supported by Fundação para a Ciência e Tecnologia (FCT). Besides traditional laboratory infrastructures, IHMT-NOVA also hosts a Vivarium, an Insectarium, a Biobank and a Bioinformatic hub. Since 2020, IHMT-NOVA is the guarantor of the leadership of the International Portuguese Language Science Center supported by FCT and under the auspices of UNESCO. The main general objective is strengthening of cooperation and partnerships with CPLP countries, with special emphasis on PALOPs. Among its specific objectives and tasks are the preparedness to respond to new epidemics, enhancement of primary health care and monitoring and control of vector-borne diseases. These objectives are aligned with the UN sustainable development goals, cooperation for development, and innovation.

The involvement in Covid-19 prevention and control, started in March 2020, soon after the diagnosis of the first cases in Portugal. Initially, a Biosafety Commission was launched in order to coordinate and implement regulations and preventive measures to create a safe environment for work of students and collaborators. Also in March, the COVID-360 IHMT/NOVA/CPLP Information Center platform was launched in order to gather relevant, credible, and updated scientific content on all aspects of the pandemic. At the same time, it was set up a partnership with Health Authorities (DGS) in a new TSF radio program "Covid-19: Questions with Answers", with the participation of two IHMT-NOVA specialists. This radio program has been 7 weeks in the air, on a daily basis, until the end of the first confinement period, with an impressive impact in listeners and society. Solely in 2020, the participation and intervention in national and international social media of IHMT-NOVA specialists, covering different aspects of the pandemics, exceeded 850. We believe, this contributed to a better understanding of the situation in Portugal, as well as improving the awareness and health literacy of citizens.

In April 2020, IHMT-NOVA started its involvement in Covid-19 diagnosis in a partnership with Egas Moniz Hospital and the Gulbenkian Institute of Science. A total of over 1300 samples were processed in a 3 month's period. Within the scope of this collaboration, a project aimed at studying immunological aspects of SARS-CoV-2 infection was started. Also in April, the 100th edition of the IHMT Newsletter dedicated solely to Covid-19 was published. Moreover, a new edition of the Annals of the Institute (V.19 – 2020) covering the theme "Pandemics, epidemics and humanitarian crises: protecting human resources in health, was launched (https://bit.ly/3ePrNza). At the same time, Master's and PhD students at IHMT had the opportunity to share their own experiences in pandemic times with a well-designed motivational video (https://bit.ly/3bqnrx5).

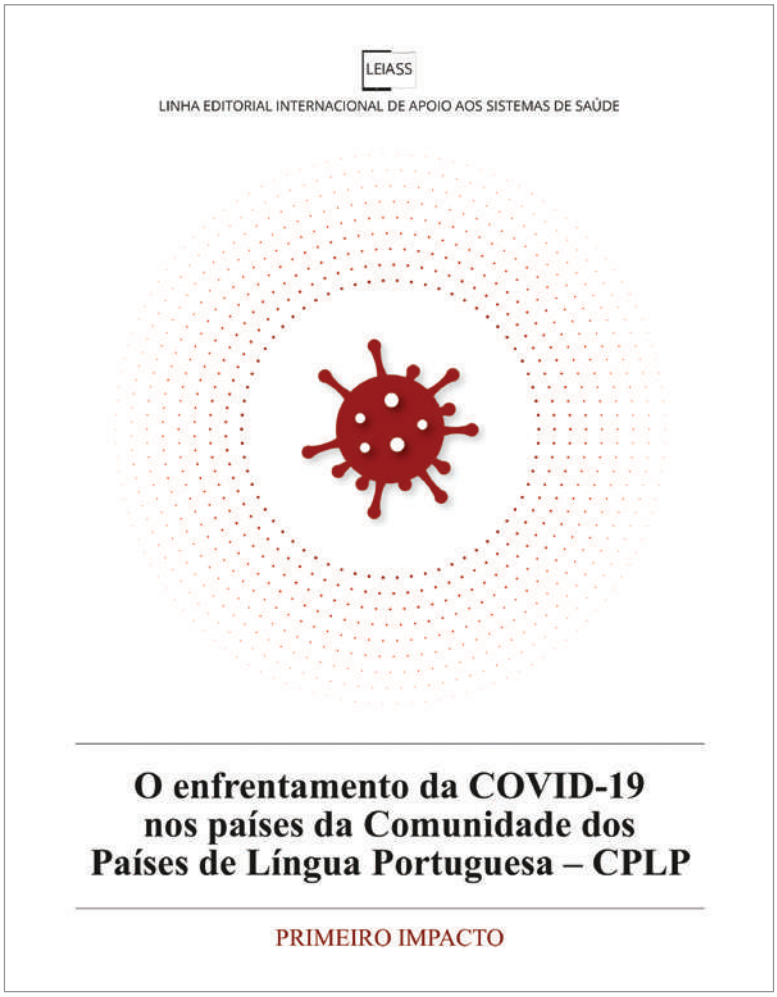
In May, IHMT-NOVA and APAH prepared a cycle of webinars on different aspects of SARS-CoV-2 and Covid-19. It involved the participation of several IHMT-NOVA and other CPLP specialists in a broad range of scientific areas. The success of these series of webinars is reflected by the participation of thousands of people from several countries, mainly Lusophone. Subsequently, IHMT-NOVA published a virtual E-book "Knowing, Organizing, Winning, Diagnosing and Investigating" containing the essential of all the webinars on Covid-19 (https://bit.ly/3pEMJN9).

Since May several important Covid-19 research projects were launched, most of them after peer evaluation in competitive calls. Among them, we would like to highlight one addressing the response to the Covid-19 pandemic in a context of social inequalities in health in native and immigrant populations. Initially performed in Amadora, it is now being implemented in other zones of the Lisbon region. As recognition of its importance and impact on society, the coordinator, M. Rosário Oliveira Martins was awarded the Human Rights Prize by the Portuguese Parliament. Currently, IHMT-NOVA researchers lead and/or participate in more than a dozen Covid-19 research projects. The large majority of them are supported by national and international agencies and institutions, like FCT (Portugal), EDCTP (EU) and Banco de Fomento Africano. All are the result of tight collaborations with different public and private partners, including the industry. Among them are CEDOC-NMS-NOVA, Gulbenkian Institute of Science, Lisbon Occidental Hospital Centre (CHLO), and several city councils in Portugal. Other projects were also implemented with partners in several countries, including Angola, Cape Verde, Guinea-Bissau, Mozambique, France, and Sweden. These projects cover different aspects of the pandemic situation, including epidemiology, immunology, public health, development of new diagnosis tools, drug discovery, and pre-clinical trials. As a result of the Covid-19 research performed by IHMT-NOVA scientists 37 manuscripts were published until December 2020 in international journals and books, including regular research articles, reviews, opinion articles, and book chapters.

As part of regular capacity building initiatives, in June we hosted a ceremony to mark the start of a project aimed at strengthening laboratory diagnosis capacity in the city of Lubango, Angola. A few weeks later, a laboratory training on Covid-19 diagnosis was performed by IHMT-NOVA and CIBIO-InBIO team for Lubango health professionals. Similar capacity building initiatives were also performed in other health institutions in Angola. Cooperation with Guinea-Bissau started later, in November 2020, but is now flowing smoothly. A first webinar on "Biosecurity in the Covid-19 Era" was launched and other initiatives are already on the ground. In the

context of research and cooperation with our partners in Lusophone countries, the new Associate Laboratory – Real, sponsored by NOVA (Translation and Innovation Towards Global Health) may become an important infrastructure to support new pandemics control activities in its different and multifaceted aspects.

As final remark, we would like to mention some ongoing initiatives aimed at preventing and controlling future pandemics, that reflect our vision of Global Health. They include different areas such as 1) Modelling global pathogen dispersion and population mobility using machine learning algorithms, 2) Drug discovery and resistance using systems biology approaches, and 3) The unique in Portugal VIASEF facility for *in vivo* arthropod experimentation.



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HEALTH PROMOTION AND HEALTH LITERACY

Isabel Loureiro
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INTRODUCTION

Health Promotion is the dimension of Public Health related to strategies, action and advocacy to improve health and wellbeing of people, under ethical values such as equity, sustainability and empowerment. Health Promotion is related to the evolution of the concept of health, the recognition of the health determinants and the value of human rights. It is deeply connected to social philosophy, from individual to groups and populations. Keith Tones (1) defined Health Promotion as the combination of Health Education and related policies to improve living conditions and facilitate healthy choices.

It has long been recognized that health follows a social gradient and that poor social and economic circumstances affect health throughout life (2). Health Promotion action includes changing health determinants through policies on social, economic and environmental conditions and improving individual and population healthy behaviors, strengthening the skills of individuals and the capacity of groups and communities to take action and gain control over the determinants of health (3)

Health Promotion transforms information into knowledge, converting its synthesis into action, including advocacy for evidence-based and ethical policies, taking into consideration values, resources, and context (4). It reflects some paradigmatic changes, such as the concept of health including the ability to adapt and to self-manage (5), an holistic and inductive approach, a systemic understanding of the health determinants and a salutogenic perspective (6) where the leveraging of all the potential resources in place is valued (7). It takes a more holistic interpretation of health, instead of a biomedical approach and a deficit model of health, with narrow focus on individual behaviour, many times called lifestyle (8) The definition of lifestyle, often taken for granted, has to be carefully examined. It is possible to think at a global level – with behaviors impacting the environment – or at the individual level, as an expression of self-identity (9) through a set of habits, but always considering habits are adopted under social, media and globalization influences.

Health Literacy called the attention to the effectiveness of Health Promotion and Health Education approaches and methods to empower people with autonomy, competence, motivation and opportunities to make their choices. The field of Health Literacy has progressed from a functional perspective to the empowerment approach, naturally reflecting Health Promotion's paradigmatic references, from the individual level to the community, organizational and political levels. Over the years it was recognized that personal skills and abilities are mediated by the context in

which health literacy is developed and applied (10). These drew greater attention to the environmental demands and situational complexities in which individuals make health decisions and to the acknowledgment of the need to address system level factors that impact health literacy. During the last decade, we witnessed investments in the development of health literate and responsive environments, recognizing that health literacy is a matter of balance between individual skills and environmental demands (11).

Health promotion is about action taken across the spectrum of health determinants, in particular towards the social, environmental and economic conditions that support health, engaging in a "healing, transformative, decolonizing and participatory ethical way" (12) to empower people for higher autonomy, social cohesion, responsiveness of organizations, political relevance and meaning of life.

STATE OF THE ART REVIEW

Health promotion captures the holistic approach of the Hippocratic theory of the four humors, searching for the equilibrium between resources and health needs and taking the human being as the center, in an integrated and interactive social, physical and biological system (13).

In 1979, Lawrence Green (14) defined Health Promotion as "any combination of health education and related organizational, political and economic interventions designed to facilitate behavioral and environmental changes that will improve health", already combining the issue of behavior change – through health education – with the required policies to make the healthy choices easier choices. This definition was adopted by the US Department of Health, Education and Welfare. With the Ottawa Charter in 1986 (15), the idea of control over health included the capacity of acting over the health determinants; behaviors being just one of their dimensions. The focus on the more disadvantaged people is aligned with the goals of Alma-Ata, the 1978 WHO declaration (16), and Health for All in 2000 (17). The five strategies designed at the Ottawa conference – build healthy public policy, create supportive environments, strengthen community action, develop personal skills, reorient health services– are aligned with the Health in All Policies document – subscribed by the EU countries in 2006 (18) -, the Adelaide Declaration(19) and with the whole-of-society and whole-of-government approaches, recognized as a requirement for accomplishing The 2030 Agenda for Sustainable Development Goals (20).

According to the Lancet paper by Davies and collaborators, in 2014 (21), the last wave in Public Health is "the cultural" one. This is related to the recognition of the importance of promoting the active participation of the population and the crucial role of social cohesion in a co-creating process of building health. Particular attention is given to democratic governance and to Health in All Policies. In 2016, at the 9th Global Conference in Health Promotion, through the Shanghai Declaration (22) on promoting health in The 2030 Agenda for Sustainable Development, three pillars were considered crucial for health: good governance; healthy cities, neighborhoods and communities; health literacy.

Uncertainty, in a complex and interdependent world in permanent evolution facing new events such pandemics or wicked problems like obesity, demands a close understanding of the situations with the required flexibility to find innovative and adequate strategies to improve health and well-being. A socio-ecological approach has been adopted as a driver for intervention in Health Promotion, together with systems and social networking theories.

Health Promotion considers the recognition of the importance of time and timing in understanding and acting on the links between the social determinants of health and the outcomes, a life course approach. This approach emphasises a temporal perspective and views health as the product of risk and protective factors that we encounter throughout life. Therefore, it looks at life experiences over different stages of life to identify the underlying biological, behavioural and psychosocial processes that operate during the life span, at specific time periods, and influence patterns of health and disease throughout life or even across generations (23). Priority for action should be given to the most vulnerable periods of life and groups, such as the investment at the beginning of life and the people living in disadvantaged socioeconomic conditions and social exclusion.

The assumption that health happens in context, also led Health Promotion to the adoption of a settings-based approach. Schools, workplaces, nursing homes, cities, neighborhoods, health services or community organizations can be especially efficient in promoting health. Working in a participatory way at the individual level or with specific population groups, such as specific age groups or any disadvantaged people, allows to address relevant issues, to co-create solutions, being responsive and attaining equity.

Thematic issues, such as tobacco cessation or breastfeeding practice, demands the development of specific competencies and, sometimes, the creation of networks that contribute to the development of knowledge in the area. One example

of this is the Baby Friendly Hospital Initiative (BFHI). In Portugal, this network went beyond hospitals, having Primary Health Care units also certified.

In fact, globally, Health Promotion and health systems are intrinsically interconnected. Health Promotion is combined with the efforts of primary health care reform that, based on the principles of social justice, are directed towards the creation of universal access. At the same time, multisectoral approach and participation in the formulation and implementation of public policies constitute an essential strategy to effectively implement Health Promotion.

Today many noncommunicable chronic diseases start much earlier in life than they did some decades ago, mainly because of high prevalence of obesity. Since life expectancy is increasing, the number of years living with a noncommunicable chronic disease, such as diabetes and hypertension, is greater than before, increasing suffering but also the burden over the health services. The investment in the ability and motivation of the patients to prevent and self-manage their health conditions is critical for the sustainability of the health system. Better results are achieved when there is productive interaction between informed and active patients with a proactive and prepared health care team (24). Here, health literacy plays a crucial role. Health literacy describes a range of skills that enable citizens to play an active part in improving their own health, engaging in community action for health and pushing governments to meet their responsibilities in addressing health and health equity.

The concept of Health Literacy has expanded in meaning to include information-seeking, decision-making, problem-solving, critical thinking and a multitude of social, personal, and cognitive skills that are imperative to a functional health-system (25). More recently, Health Literacy has been highlighting the importance of empowering individual citizens and enabling active engagement in collective Health Promotion action as well as responsiveness of the health organizations as a goal for dignity of people and adequacy of the health care delivery. Health literacy responsiveness describes the way in which services, organizations and systems make health information and resources available and accessible to people (26). For this purpose, many sectors and actors have a role to play. As the Institute of Medicine indicated (27) in 2004, and the Shanghai Declaration (22) reinforced in 2016, the education sector should integrate health education in the school curriculum at all school levels, a cultural environment of competences and values – such solidarity – should be promoted, and the competences of health professionals in communication – such as the use of plain language, strategies to enable change

and participation in the development of new models of health care delivery in their organizations – should be assured. Knowledge translation from basic and clinical research to health care delivery and empowerment of patients as well as communication across health-related stakeholders, settings, and sectors demands competent knowledge brokers (28).

The training of health professionals in pedagogical and communication techniques (such as motivational interviews, use of plain language and teach back techniques), the integration of several ongoing processes at different settings (such as school curriculum or health promotion at workplaces), community networking (such as immigrant services or support groups for breastfeeding), the development of a culture of responsiveness to the needs of all people and mass media commitment are all critical factors for improving the levels of health literacy of the populations, including the most deprived individuals.

Being Health Promotion the active dimension of Public Health to change health determinants as to improve health and wellbeing of people, implementation of changes is, naturally, critical for its success. Implementation science has been developed also because of the acknowledgment that there are many guidelines based on scientific evidence but nevertheless, not translated into new practices and thus not benefiting people.

According to the National Institutes of Health in the US (29,30), implementation science is the study of methods to promote the integration of results of research in policy, health care delivery and community interventions. For its success the understanding of health professionals and other stakeholders' behaviors is required. This creates conditions and strategies to overcome barriers in transforming evidence-based practices into routine practice, and testing new approaches to improve impact in different contexts. According to the National Implementation Research Network Active Implementation Framework, this also requires leadership in each context to guide the process in its different components such as training, coaching and organizational changes that facilitate supporting innovation. This means to produce the desired changes in the real world (30)

Such as in other fields of Health Promotion, implementation science uses quantitative, qualitative and mixed designs, action-research and participatory methods taking into account multiple levels, including patient, provider, clinic, facility, organization, the broader community and policy environment. It also requires a solid theoretical framework, trans-disciplinary research teams and analysis of its sustainability (31,32).

The limitations of traditional models of research and a concern for social inequality regarding health and new global health challenges have raised the need for more comprehensive perspectives concerning research and intervention (33).

Regarding research on complex health issues, it is essential to develop studies that contribute to identify and understand how the various determinants (genetic, individual, community, related to services, culture, economy, society and politics) influence health. In this sense, different sciences such as biology, epidemiology, sociology, psychology, environmental social and human sciences, economy, demography, medicine, political science, pedagogical and communication sciences, have helped to understand processes related to the health of populations, converted into an integrated knowledge.

RELEVANT CONTRIBUTION FROM NOVA

The involvement of NOVA in the development of the scientific area of Health Promotion has been ongoing at an international level, particularly in the European Region.

The field of Health Promotion was recognized in 1951, with the creation of the International Union for Health Promotion & Education (IUHPE), but it has caught the interest of the higher education mainly in the 9th decade of the XX century. Because of the specificity of the knowledge, values and strategies that guide good practice in Health Promotion, an EU project was initiated, in 1996, with the goal of preparing a school curriculum at master level. The NOVA National School of Public Health (NOVA NSPH) integrated this and the following projects. NOVA NSPH was a member of the European movement for developing the scientific field of Health Promotion, integrating the main European projects such as the European Master in Health Promotion (EUMAHP), the Public Health Training in the Context of an Enlarging Europe (PHETICE), European Health Promotion Indicators Development (EUPHID), and the Project for Developing Competencies and Professional Standards for Health Promotion Capacity Building in Europe (Comp-HP), which originated the release of the CompHP Core Competencies and Professional Standards for Health Promotion (34) (Barry et al., 2012) and later the IUHPE Core Competencies and Professional Standards for Health Promotion in 2016. It also has collaborated with several universities at the national and international levels, including the summer courses of the European Training Consortium in Public Health and Health Promotion (ETC-PHHP).

At the national level, the NOVA NSPH has developed a diverse training offer in the area of Health Promotion. The Master's in Public Health, created at the NSPH in 2002, had the branch of the specialty in Health Promotion, until 2017, which was always very sought. After realizing how much this field was growing and calling the interest of a broad public, it was decided to create the Master's in Health Promotion, following the competencies framework proposed by the IUHPE. This master course is certified by the IUHPE and by the Agency for Public Health Education Accreditation (APHEA). This program is intended for graduates in a range of backgrounds in health fields and intends to train a skilled workforce of health promoters with the capacity for intervention for change at different levels (individual, organizational, social, political), leading to a greater freedom of informed choice, a capacity for social transformation, and a better quality of life for all.

The PhD program at the NOVA NSPH, created in 2010, includes the specialty of Health Promotion. As part of this specialization, students received specialized training in the domains of core competencies in Health Promotion and also contributed to the development of knowledge in the field. Today there are already 14 PhD in Public Health, specialized in Health Promotion. The theses were mainly within the scope of health literacy, mental health, child obesity, healthy aging, and implementation science. At the moment there are 25 PhD thesis in Health Promotion under course.

Within the scope of the Medical Internship of Public Health, which confers the degree of Public Health Specialist to the doctor, the NOVA NSPH offers the Specialization Course in Public Health, that responds to the training needs of the internship. The field of Health Promotion has been a part of the curriculum at the NSPH, even when it was not yet included in the official curriculum designed by the National Association of Medical Doctors (Ordem dos Médicos). Specifically, the training in participatory planning has been an investment in a more engaged approach between Public Health professionals and the community and political stakeholders.

Beyond the degree academic courses, the NOVA-NSPH offers short-term university extension courses that deepen or update specific areas. In the field of Health Promotion, the School was a pioneer in launching some of them. In 1996, the first WHO training trainers course on Breastfeeding Counselling in Portugal took place. The pediatricians and nurses who attended it are the current national leaders of the breastfeeding support movement (members of the BFHI). For the last 4 years the course on "Acquisition of healthy habits since childhood", inspired by the participatory methodology of the HENRY – Health, Nutrition for the Really Young – program

in the United Kingdom, has been offered at the school, providing training for the use of a family-centred approach and development of partnerships with families.

In an effort to guide the training in the field and try to integrate the concept of Health Promotion in other sectors such as education, in 2008 and 2009, several articles on strategies, complexity of the learning process, competencies and results in Health Promotion were published in Revista Portuguesa de Pedagogia (35,36).

Indeed, Public Health and Health Promotion have evolved towards the achievement of the aspiration of increasing the years of a healthy life, reducing health inequalities and facing the multiple health problems that affect the population today (37). This evolution has stimulated the reflection on the challenges of education, research and practical implementation of this science. It is intended to produce an integrated view of the specificities of Health Promotion teaching and its role in the progression of the student's education in Public Health. The teaching proposal developed through the years aims to contribute to the formation of a critical mass of informed and skilled professionals to work in policy formulation and strategies implementation that contribute to the improvement of populations' health. In an educational policy perspective, providing knowledge, skills and values for professionals to translate theory and research into effective policies and practices is essential to strengthen the global Health Promotion (37).

Regarding research, the contributions from the NOVA NSPH illustrate the central tenets and approaches of health promotion.

Under the scope of the Portuguese collaboration with the Harvard Medical School, the NSPH developed the Project "Early beginnings: tackling childhood obesity" (38), which aimed to produce written and audiovisual contents for parents and other caregivers on promoting healthy habits from pregnancy to 5 years of age (www.papabem.pt). The project approach, focusing on the early years, parenting and health literacy, was developed in collaboration with the University of Leeds and the UK program Health Exercise and Nutrition for the Really Young (HENRY) and with the Harvard School of Public Health. Over the last couple of years, the project's approach was incorporated into a community project (Eat Well Communities), which takes a settings approach for the prevention of childhood obesity. Taking childcare as a strategic setting and using a participatory approach, the project has been supporting change in the educational environment, families and children.

The valorisation of the community level and social mobilisation for Health Promotion has been one of the strong dimensions for research. In 2009, within the collaboration with the project "New York City Food & Fitness Partnership"

from Columbia University (USA), the ecological perspective of food safety and empowerment of communities at urban settings was used in several projects in Portugal on food safety and local governance. Some examples are in the training for the Portuguese Healthy Cities Network, the BIP-ZIP project with the Municipality of Lisbon at the most vulnerable places in the city, and the national Healthy Neighborhoods Program. A reference document in territory planning and local health strategies was produced for the National Health Plan 2011-2020 (39).

Health literacy has been a major focus over the years. Since 2018, the NOVA NSPH has worked with the Faculty of Health, Arts and Design of the Swinburne University of Technology, in Australia, in the validation of health literacy evaluation tools and implementation of participatory approaches for the promotion of health literacy and health literacy responsiveness. This collaboration was later framed in a protocol with WHO for dissemination of knowledge in the field of health literacy (e.g., International Conference on Health Literacy for the Prevention and Control of Noncommunicable Diseases, 2019, Rectorate of the NOVA University of Lisbon; Health Literacy Capacity Building Workshop, 2019, NOVA NSPH) and for the development of two National Health Literacy Demonstration Projects, in the areas of diabetes and migrants' health. The NSPH has also contributed to reference documents in this area, such as the WHO Global Coordination Mechanisms document "Health literacy development for the prevention and control of noncommunicable diseases".

In the search for innovative approaches that reach a more in-depth knowledge on the health, in the last decades there has been an emergence of the paradigm of community-based participatory research (40,41). This approach comprises the equitable involvement of several partners, namely community members, governmental institutions, non-governmental organizations and researchers in the research process, with each partner assuming a central role in the understanding of the phenomenon under study and its socio-cultural dynamics (40). The NOVA NSPH has also contributed to the development of community-based participatory research and local governance. Some examples are the Participatory planning in mental health promotion, at Amora (41), and the action-research project developed at Fernão Ferro Health Family Unit with the outcome of new protocols for the maternal consultation and parenthood capacity-building (42,43). Another example is a participatory HIV research project, conducted by NOVA, with sex workers and men who have sex with men to understand epidemiological HIV dynamics and associated sociobehavioural factors among these vulnerable groups. This participatory

research produced a dynamic and interactive process of knowledge coproduction and translation into effective community-oriented health actions and policies. The participatory research reproduced an innovative alliance for HIV prevention and sexual health promotion responsive to local needs and priorities (44).

IMPACTS TO SCIENCE AND SOCIETY

The research developed over the years contributed to strengthening the application of Health Promotion principles in research and practice, through the adoption of a socioecological model and life-course lens and the integration of participation and capacity building in research. Participatory research in itself may be an important vehicle to equity and empowerment and a powerful mechanism for increasing implementation of evidence-based and locally relevant interventions.

The role of NOVA NSPH in society in the field of Health Promotion has taken several forms. Besides its participation in strategic committees at the national level (such as Baby Friendly Hospital Initiative, Nutrition during the 1000 first days of life, expert group for the National Health Plan, National Health Council), NOVA NSPH has been playing national roles such as the coordination of the Health Promotion & Education at the ministry of education (45) and the coordination of the Department of Health Promotion and Prevention of Noncommunicable Diseases at the National Institute of Health (46). The involvement in some reference groups such as the Gulbenkian project on "The future for health" indicates the external recognition of the importance of the field of Health Promotion.

The involvement of the NOVA NSPH in healthy policies at different sectors – health, education, social welfare, economy, environment – and at different levels – international, national and local – has been oriented to respond to the health needs through the distribution of resources and the creation of physical and social environments that promote health and the well-being, ensuring that each citizen has an equal opportunity to make healthy choices and the right to live in a friendly, safe and supportive environment.

The evolution of Health Promotion led to consolidating a set of principles, such as those concerned with socio-ecological and salutogenic perspectives, a holistic, multi-sector approach, a concern for sustainable development, a commitment to social justice and equity, a participatory approach to individual and community capacity-building and respect and sensitivity regarding cultural

diversity. Indeed, Health Promotion, as a dynamic discipline, has evolved to answer health issues in the current globalized world, assuming a preponderant role in a contemporary society (47). Nevertheless, it is critical to continue to reflect and develop its theoretical, research and action fields (47,48).

ONGOING RESEARCH

Investing in the understanding of the best ways to reach the most vulnerable populations as to empower them, improve their health literacy and identify policies that leverage their potential and assure a life with dignity are some of the challenges of research in Health Promotion, contributing to its relevance. NOVA NSPH keeps running National Health Literacy Projects with WHO, developing kowledge about how to improve health literacy through a cocreative process – one project with immigrants and the other with people with type 2 diabetes or at risk of developing it.

NOVA NSPH is involved in evaluating the impact of national policies at the systemic crisis of Covid-19, such as the Healthy Neighborhoods Program, a bottom-up approach that co-creates solutions to improve quality of life by local communities. Also, the most critical and sensitive periods of lifecycle, such as the beginning of life, is an area where the commitment of NOVA NSPH with public organizations is ongoing. An example of implementation evaluation is the "Papa Bem" project.

The digitalization of the world and the use of new technologies will have an impact on strategies for participatory planning and research and on methodologies for evaluation in complex contexts. New approaches will emerge as well as new opportunities to increase more evidence-based practice with more "practice-based evidence"(49).

Arts in health can realize the full potential of people contributing to their enjoyment, fulfillment and creative lives. Cultural education facilitates the capacity for integration and can be an opportunity for giving people voice. Health Promotion seems to be an appropriate field for developing this area and there is already some ongoing research, such as some PhD thesis. The European Union intends to invest in culture as a strategy for promoting mental health, now aggravated by the present pandemic situation. The Portuguese Presidency of the European Union Council has invited the NOVA School of Public Health to contribute to this goal.

NOVA plays a crucial societal role in Health Promotion, meeting its local and global challenges, to improve health, equity and capacity for well-being.

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CHAPTER VI

THE OTHER FACE OF MEDICINE

A

VALUE IN HEALTH

Ana Londral
José Fragata

1. INTRODUCTION

Healthcare sector has been facing an unprecedent pandemic crisis, but the situation beforehand was not immaculate, being characterized by uneven access issues, extreme variability of outcomes, quality and safety issues and an ever-expanding spiral of costs. Furthermore, this increase in costs was not related with the pressure on demand or the toll of technology complexity, neither was it associated with the expected improvement in access and quality, as well as generated satisfaction for patients[1].

Several attempts to mend the healthcare system have focused on the pressure for volume production and improving efficiency, as well as quality promotion – mostly without significant success. It has than became clear that "the fix" to the healthcare system would come from a totally different view, the one introduced back into 2006 by Michael Porter and Elizabeth Teiseberg[2] from Harvard – the Value Approach, that the authors claimed would fix the health system[3]. Not by adding in more technology, not by putting pressure on production, but by focusing on the value created for patients first and players following. Value being represented by the equation where outcomes, also as perceived by patients, figure on the numerator and the total cost needed to achieve those (long standing) outcomes, will figure on the dominator; in a word, how much good care (perceived by the patient) can we buy by euro spent. The *International Consortium for Health Outcomes Measurement* (ICHOM) consortium was created to implement this concept in practice globally.

NOVA University launched the first ICHOM conference in Portugal in 2017, then we created a Value for Health research group and engaged into a multitude of actions, namely the Cascais Summit, in 2019, where public awareness to the topic was created and players were asked to sign a commitment agreement regarding this new Value Vision to promote the needed Healthcare change.

That was when the Ministry of Science and Technology launched the call for Collaborative Laboratories and we decided to submit a proposal, that would become successful and originated the Value for Health CoLAB. We now have the proper tools – research and technical – to go ahead and promote the needed change in our health system, by using the Value Vision.

Value for Health CoLAB was certified as a collaborative laboratory by Fundação para a Ciência e Tecnologia, in November 2018, and started to implement its research and innovation agenda in May 2019, with the public funding for scientific employment.

NOVA University of Lisbon led the vision of Value for Health CoLAB (www.vohcolab.org) and involved Fraunhofer Portugal, CUF group and Vodafone. The vision for this CoLAB was that, by investing in monitoring methods that follow the patient longitudinally, either in the hospital settings and at home or residential care, we will be able to create methodologies for objectively measuring health outcomes and costs towards new sustainable interventions in healthcare, that deliver high value to the patients.

2. STATE OF THE ART

Studies at the global level show that at least 20% of health expenditure has no beneficial impact or added value for patients, in other words, it is wasteful[1,4].

A big change was proposed by Porter and Teisberg [1], the early thinkers of the concept of Value-based Healthcare (VBHC): moving from a focus on activity to focus on outcomes. The whole paradigm change reinforces a patient-centered approach to Healthcare, that replace the institutionally focused payment systems, based on fee-for-service, which promote fragmentation in the delivery of care, by integrated care reimbursement models, based on value-based reimbursement[5]. Thereby, it drives the integration of health and care, in the way that value to the patient should consider outcomes measured by all providers in a full cycle of care.

Porter's value-based healthcare was a kick-off to bring the concept of Value in Healthcare to the discussion, among worldwide leaders[6]. The concept is evolving and is being discussed within various communities. In Europe, Porter's model has been discussed as insufficient for the scope of nations that are committed to universal health coverage.

2.1. Value(s)-based Healthcare

The original thinking of VBHC needs adjustments for when considering universal systems, where there is a legal commitment to cover the healthcare needs of all population, with budgets that are finite. Sir Muir Gray suggests proposes the Triple Value healthcare model, implemented in the NHS England RightCare programme, to face the challenges of sustainability, equity and innovation in universal health systems[7]. This model addresses value in the three following levels: (i) *Personal value* (at the level of Patient), i.e., ensuring that each patient's values are used as a basis for decision-making; (ii) *Technical value* (at the level of Intervention), i.e.,

ensuring that resources are used optimally for a given condition; and (iii) *Allocative value* (at the level of Population), i.e., ensuring that the resources are allocated in an optimal and equative way to serve populations.

In this triple value model, the clinician is not only responsible for maximizing the outcomes for a specific patient with the least use of resources, but also for preventing inequity related to age or other social factors.

The Expert Panel on effective ways of investing in Health (EXPH) takes a further step and adds a fourth value to cover a solidarity-based healthcare system: *Societal value*, i.e., ensuring that resources that are allocated promote social cohesion, based on "participation, solidarity, mutual respect, and recognition of diversity" [8].

The concept of Value(s)-based Healthcare is suggested by EXPH as a wider perspective of value; in particular, as a framework for European welfare states with a common goal for health systems to be more effective, accessible and resilient. VOH. CoLAB is adopting this value(s) vision, by exploring the personal, technical, allocative and social dimensions of Value in Healthcare, in its research and innovation agenda.

2.2. The role of digital transformation in Healthcare

Outcomes' measurement is the starting point for assessing and increasing value in healthcare[9]. In the actual context, citizens are actively empowered by technology to report health outcomes in daily context[10-12]. Remote monitoring, self-management care, process optimization in healthcare delivery, prediction analysis for prevention of complications and data interoperability are some of the domains where digital health plays a major role in innovation towards VBHC[13].

On another perspective, there is a growing need to validate digital tools for healthcare through value assessment. After the COVID-19 outbreak, and in the recent context of the German *Digital Healthcare Act*, a breakthrough in the use of digital health tools is now happening, worldwide[14]. In consequence, VBHC methodologies will be important to evaluate the digital services that bring value to the patients and orient the decision-makers in technology adoption and scaling.

3. RELEVANT CONTRIBUTIONS FROM NOVA

Contributions from knowledge domains in Medical, Economics, Social and Engineering sciences were gathered to build Value for Health CoLAB (VOH. CoLAB). This interdisciplinary context pushes the core team to integrate different

scientific areas in resolving the complex problem of understanding and measuring value in Health.

The research objectives started in building a framework of methodologies and tools to collect outcomes and costs in real contexts of healthcare. Three knowledge units were created, each led by a PhD researcher: Data science in Healthcare and software development, Value assessment, and Health and Digital Literacy.

3.1. Telemonitoring health outcomes in cardiac surgery and home hospitalization

Patients' follow-up during the postoperative period after cardiac surgery is critical, with hospital readmission rates of 15 to 20% during the first month and 30% in the first year[15-16]. With the primary objective of reducing the risk of readmission, we started working with the Department of Cardiothoracic Surgery of a public hospital in Lisbon, Hospital de Santa Marta – Centro Hospitalar Universitário Lisboa, together with researchers from NOVA Medical School and Fraunhofer-AICOS, to implement a digital telemonitoring system. Vodafone Portugal supported the project with 4G connection. The digital system was developed and tested in a pilot study with 35 patients, after being approved by the Ethical Committee of the hospital. In this pilot study, the selected patients were instructed before hospital discharge, to daily report outcomes during the first month after surgery. Patients were engaged to collect patient-reported outcome measures (PROMs) from a mobile app and an IoT kit. A literacy session was prepared and delivered to each patient that participated in this pilot study before hospital discharge. The clinical team was empowered to daily monitor the patients in a digital platform, where PROMs could be visualized and managed. We developed a digital platform to collect PROMs from multiple sources – chatbot, IoT devices, text messages – and to facilitate clinical teams in the communication and management of those outcomes. Figure 1 depicts the involvement of patients and nurses, as well as the telemonitoring system that was developed under this study. The VBHC approach of our research was based on pathway design with business process modeling, participatory research to involve patients and clinical teams, statistical analysis of outcomes data and time-driven activity-based costing (TDABC)[17] to calculate the costs.

The pilot study demonstrated the feasibility of the telemonitoring system to collect outcomes. Results demonstrate a decrease in the average of critical incidents related to patients' readmissions when comparing patients that were telemonitored with a paired control group. High satisfaction was measured with the Net Promoter Scale, with 84% of the patients classified as promoters. Costs related

to the telemonitoring service are being incorporated in the digital platform to be automatically measured. The research team was awarded with a research grant to proceed the work in two topics: (i) to develop machine learning models to support risk prediction of patients based on long-term outcomes; and (ii) to design a long-term telemonitoring program for cardiac surgery and scale-up the study to assess the value of this telemonitoring system in a clinical study with 150 patients. The project *An intelligent system to improve patients' safety and remote surveillance in follow-up for cardiothoracic surgery* (www.beatnik.vohcolab.org) is funded by FCT, under the call Data Science and Artificial Intelligence in Public Administration 2020.

Figure 1 – From left to right: Participatory research with patients and nurses' involvement; digital platform developed by VOH.CoLAB to manage patient-reported outcomes; IoT kit that is used by the patients after cardiac surgery, developed by Fraunhofer-AICOS.

In the same topic, VOH.CoLAB joined two Portuguese startup companies and a private hospital from CUF, to develop a digital system to collect clinical and patient-reported outcomes in the context of home hospitalization. This project was awarded with the highest rank, in a call from ACTIVAGE consortium (www.activage-project.eu), an innovation action funded by H2020 and led by Medtronic Iberia. The challenge was to validate, in a Portuguese context, an IoT ecosystem that was developed by the European consortium. Using participatory research methodologies, we worked with the clinical team in the hospital, for an agreement of the requirements, designing the process and defining the outcomes that are relevant for the context of home hospitalization[18].

3.2. Cost analysis with Time-Driven Activity Based Costing: the case of Knee Arthroplasty

The growing ageing population and increase in obesity, are the main cause of high prevalence of hip and knee osteoarthritis, one of leading causes of global disability. According to OECD data[19], from 2000 to 2015, Portugal performed an average of 47 total knee replacement surgeries per 100 000 population, in public hospitals in the mainland. In the same period, the Portuguese rate of knee replacement has

increased 9.11% per year. Joint replacement is a surgical intervention that improves the quality of life of patients, by reducing pain and improving joint function[20].

The CUF group, the largest private healthcare provider in Portugal, proposed a study to analyze costs in the Knee Surgery pathway for patients diagnosed with knee osteoarthritis (KO). Two hospitals in the region of Lisbon were selected to participate: CUF Descobertas and CUF Santarém. These hospitals follow different care delivery processes, one of them with a specific perioperative program, but collect outcomes based on the same standard set of ICHOM for Hip and Knee Osteoarthritis. Cost analysis is the missing module to assess value in this pathway.

VOH.CoLAB developed a framework to calculate costs based on the TDABC methodology[17]. The methodology consisted of mapping the in- and out-patient pathways to identify resources, obtain time estimates for each process and resource, estimate the cost of supplying patient care resources, and calculate the total cost of patient care based on the calculation of the capacity cost rate (Figure 2). The framework included the development of a digital cost simulator that implements the method and the data structure that is need for cost analysis in Knee Surgery pathway. The costs of an average patient were calculated in the two different contexts of the two hospitals. Results demonstrated that TDABC methodology allows the detailed characterization of costs along the care process of each patient, enabling comparison between different care delivery chains. Although, such methodology is complex to implement, as data collection is patient-driven and not system-driven. The cost simulator that was developed is a proof-of-concept and revealed the need of future improvements in the existing information system (IS) infrastructure. Data collection needs interoperability from different data sources (clinical, financial, and logistics) along the patient pathway. Future research should focus on developing effective and efficient interfaces to allow importing process, activity, resources, and time information from the existing IS, in order to automatically calculate the total costs of care per patient.

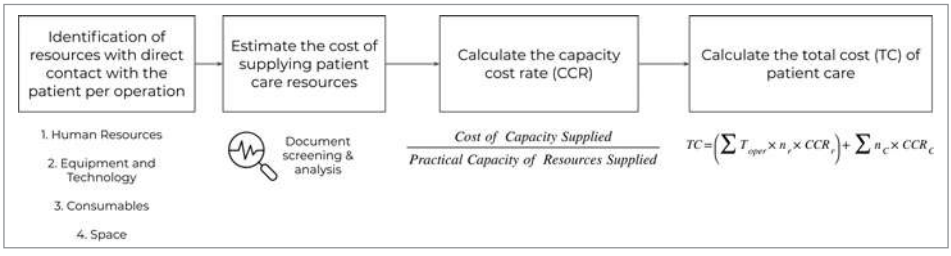

Figure 2 – The methodology followed in the cost analysis for Knee Surgery.

The evaluation of Value in Knee Surgery interventions at CUF will be possible as a next step, with the integration of data from this study, for cost analysis, with health outcomes' measures, from ICHOM standard set.

3.3. Designing patient pathways: the case of Amyotrophic Lateral Sclerosis (ALS)

Patient pathway design is the first step of the process of outcomes and costs assessment. By designing pathways, it is possible to characterize the patient journey and define stages or time stamps where the outcomes should be measured in order to assess value in the healthcare process. VOH.CoLAB started a research work in collaboration with the Portuguese Association of Amyotrophic Lateral Sclerosis (ALS) and clinical experts in the disease. The aim was to support the association in clearly characterizing the pathway of ALS patients, namely the patient journey, the pain points, and the costs, in multiple domains such as clinical, social, economic, and personal.

ALS is a neurodegenerative disease that has no cure and causes low health outcomes. Patients experiment a fast process of function loss related to walking, using upper limbs to daily activities, speaking, eating and, ultimately, breathing[21]. The lack of awareness and coordination of ALS care pathway prevents patients from early and efficient support, contributing for a significant delay in diagnosis and treatment or interventions that can increase the quality of life, as also the social and regional asymmetries in care.

We used a mixed-method approach, with interviews to patients, caregivers, social workers, and clinical experts, to assess the needs in multiple domains for the different stages of the disease, the pain points, the social and economic impact. Clinical data analysis from a set of patients was used to extract the average times from diagnostic to the different stages. After the qualitative analysis of the interviews, we designed a patient pathway. This was validated using the Delphi method[22] with a panel of experts in ALS healthcare. The resulting ALS pathway enlightens the decision-makers on the main pain points and unmet needs in ALS.

As future work, we will use the ALS patient pathway to define a set of health outcomes and socio-economic indicators, aiming at implementing a national study to monitor value in healthcare of ALS patients.

3.4. Intelligent pathways and outcomes collection in Ageing

In the context of the societal challenge of Ageing, intelligent and well-designed digital services are important tools to fulfill the third goal for the

sustainable development defined by United Nations: "Good Health and wellbeing" for 2030. Telecare digital solutions can support early risk detection of frailty, as also personalized intervention and interaction with senior citizens who live at home, in order to provide effective delivery of preventive healthcare.

The SNS24 is a telephone and online service of the Portuguese National Health Service. As the Contact Centre of the National Health Service, it is an integral part of the SPMS-Shared Services of the Ministry of Health, E.P.E. In 2018, SNS24 started a pilot program called Senior Proximity, in collaboration with primary care centers in two different regional areas, to target and support elderly population with frailty. The main objectives of such program were to use a telecare service to follow a group of elderly citizens with frailty to: prevent health occurrences; early detect needs; promote integrated care in health, social and safety dimensions; and to contribute for a healthy and active ageing.

From 2020, VOH.CoLAB is working with the team of SPMS that implemented the pilot program, in a research project led by NOVA University of Lisbon, namely the Medical School and School of Science and Technology, to support the upscale of the senior telecare program to a national level. The project *Intelligent frailty pathways for the portuguese elderly population and impact analysis of the telecare service SNS24 Proximidade Sénior* (www.frailcareai.vohcolab.org) is funded by FCT, under the call Data Science and Artificial Intelligence in Public Administration 2019. In this project, AI experts are joining clinical experts in Ageing and telecare service professionals to develop intelligent tools that aim at improving the efficiency of intervention pathways, as well the cost-effectiveness of the national public health telecare service for senior citizens with frailty. Data from the pathway of elderly population in NHS will allow the development of risk prediction models for frailty, based on machine learning techniques. Also, it will be developed an intelligent expert clinical decision support system for automatic guidance of telecare intervention of this population.

In the same context of healthcare for Ageing population, VOH.CoLAB is leading the innovation project EasyHealth4Covid (easyhealth4covid.vohcolab.org) with three Portuguese companies that are developing a telecare system for the elderly. This project is funded by Portugal 2020. The telecare system includes a videoconsultation application and telemonitoring system to monitor physiological signals that are relevant for such population: electrocardiography, blood pressure, temperature, oximetry, and glucose. VOH.CoLAB is working with User-Centered methodologies to develop simple digital interfaces to collect outcomes that matter to the elderly population and to their caregivers. We are implementing the system

in a Residence, to validate technology, measure user satisfaction and evaluate the value of such a digital health service.

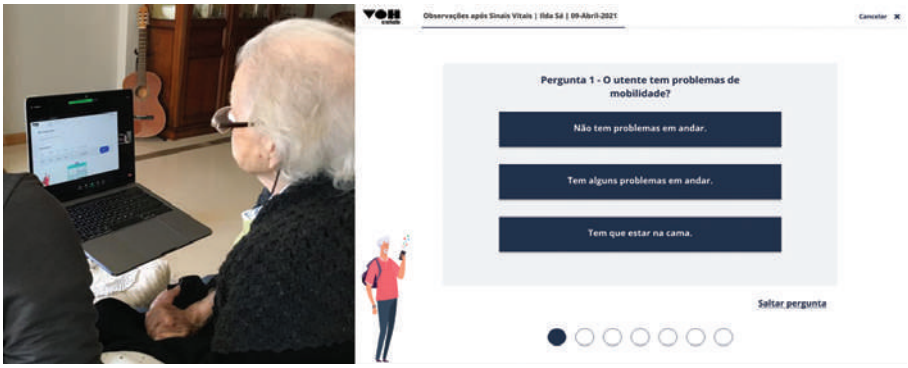

Figure 3 – A photo of one session with a volunteer participant while assessing user experience during the development of our digital application to empower elderly to report outcomes.

3.5. Patient literacy and engagement in post-Covid rehabilitation: a digital communication module

In the context of COVID-19 pandemic, the long-term symptoms of the disease are still unknown, but a significant number of patients present motor and cognitive disabilities after acute symptoms, resulting in the need of patient-centered rehabilitation programs[23]. Com@Rehab project was proposed by an interdisciplinary collaborative research work integrating three organic units of NOVA University – School of Science and Technology, School of Social and Human Sciences, and Medical School – in collaboration with the VOH.CoLAB, the Hei_Lab research center and the Center for Medicine and Rehabilitation of Alcoitão. This project was awarded with the Santander/NOVA Collaborative Research Award 2020. The goal is to develop new care pathways for Neurorehabilitation to support the rehabilitation of post-COVID patients in hospital or home environment. Com@Rehab integrates an awarded system of interactive rehabilitation technology VR4NeuroPain® that combines virtual reality and a glove with biosensors[24].

The goal of this project is to develop a Digital Communication Module for the virtual reality system, that is focused on the development of communicative skills of the various agents involved in the rehabilitation process, while empowering them with technology literacy. This project explores collaborative partnerships in the scientific areas of Linguistics, Technology, and Medicine, towards the development of guided patient-centered health interventions using virtual reality and sensor technologies.

4. IMPACTS TO SCIENCE AND SOCIETY

The research and innovation agenda of VOH.CoLAB is strongly oriented to develop scientific knowledge that sustains the paradigm change in Healthcare, the focus on value to each patient to build sustainable Healthcare pathways.

Patient pathways that have been explicitly addressed in the activities of VOH.CoLAB were: cardiac and knee surgery, ageing, emergency services, home hospitalization, neurodegenerative diseases and neurorehabilitation. The research areas that are being developed by the research team are: telemonitoring, digital service design, data science and artificial intelligence for healthcare data, patients' literacy, patients' pathways design, cost analysis in healthcare, patient-reported outcomes, and value networks in healthcare.

By closely communicating with patients' associations, clinical teams, and companies, VOH.CoLAB is leveraging the social relevance of the academic research activities, acting as a hub between NOVA University of Lisbon and the Healthcare sector. Its proximity to market and society has attracted young university students and researchers to develop research or work in traineeships, integrated in the activities of the core team of the VOH.CoLAB.

The active participation in international networks or research & innovation programs, such as *AHA Lisbon – European Innovation Partnership on Active and Healthy Aging* (EIP-AHA), *European Innovation and Tecnhnology* (EIT) Health and ICHOM are a demonstration of the impact of our research activities. Future international collaborations will be fostered to implement VBHC in international initiatives. We are developing tools and methodologies that will contribute to build innovative, efficient, and sustainable health systems that are needed for the post-pandemic context.

5. ONGOING RESEARCH

The implementation of VBHC philosophy and method poses a huge challenge. It introduces a methodology that truly changes the way we view the delivery of Healthcare today, based on continuous measurement and improvement initiatives. VBHC concept is truly disruptive and needs to be put into practice, while simultaneously scientifically validated. NOVA University is actively working with society, developing interdisciplinary training initiatives and research

methodologies that will empower stakeholders to assess and improve value in Healthcare, as also to support systematic and sustainable changes, at a national and global level.

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B

HEALTH ECONOMICS IN PORTUGAL AND THE ROLE OF UNIVERSIDADE NOVA DE LISBOA

Pedro Pita Barros

1. THE INITIAL YEARS

Health economics was introduced in Portugal in the eighties of the twentieth century by António Correia de Campos, professor of the National School of Public Health (then part of the Ministry of Health, currently integrated as an organic unit at the Universidade Nova de Lisboa). The lines of research, and intervention, of health economics in this first wave focused mainly on two aspects: (i) equity and its measurement in health systems; and, (ii) economic evaluation (designation covering cost-benefit analysis and variants applied to the health sector). In addition to the National School of Public Health (ENSP), there was also relevant scientific activity at the Instituto Superior de Economia e Gestão (ISEG), then at the Technical University of Lisbon, with Carlos Gouveia Pinto. The nineties of the twentieth century sees the expansion of the group of people in Portugal who dedicate themselves to the themes of health economics, with a growing number of groups of researchers in other schools of economics and management: Faculty of Economics of the University of Coimbra, Faculty of Economics of the Universidade Nova de Lisboa – today, Nova School of Business and Economics, Faculty of Economic and Business Sciences of the Catholic University of Portugal – today, Católica Lisbon School of Business and Economics, in a first phase, and then the School of Economics and Management of the University of Minho, the Faculty of Economics of the University of Porto, the Faculty of Economics and Management of the Portuguese Catholic University (located in Porto), the Higher School of Management, Hospitality and Tourism of the University of Algarve, among others. Over the years, new health economics research centers have been established in the various schools of economics and management of higher education and polytechnic education in Portugal, although in several cases they are concentrated on a very small number of permanent researchers. Alongside these different groups, there is also a growing community of Portuguese researchers and health economics experts working in international institutions and universities in other countries, maintaining close collaboration with research centers in Portugal.

Scientific research in health economics interacts closely with the management of health units and with health services research (which has an evolving definition, and can be seen as how access to health care is processed, what are the costs of providing health care, and what outcomes for patients emerge). That is, at the crossroads of economy and management applied to the health sector. The understanding of what is health economics in Portugal cannot be disconnected

from this proximity to health management. A sign of this proximity is the Portuguese Association of Health Economics, created in 1986, under the leadership of António Correia de Campos, attracting the collaboration and participation of many hospital administrators, as well as other health professionals in its events.

2. SCIENTIFIC PRODUCTION IN HEALTH ECONOMICS

The scientific publication in the field of health economics was analysed in Mateus and Moura (2014), which directly collected information from associates of the Portuguese Association of Health Economics, from bibliographic research at PUBMED and ORCID (open and voluntary registration of researchers) and from personal web pages. The studies obtained were classified by the authors according to the proposal of Alan Williams (Culyer and Newhouse, 2000), which considers eight major areas: A- what is health and what its value is; B- what influences health; C- demand for health care; D- supply of health care; E- analysis of market equilibrium; F- economic evaluation; G- planning, budgeting, regulation and monitoring mechanisms; and, H- overall evaluation of the health system. Currently, since this classification is still a valid one, it would be natural to make a separation, within the demand for health care, between C1- protection against uncertainty – public and private health insurance; and, C2- demand for health care in case of need. The conceptual reason for this subdivision is in the role that private health insurance, public health insurance systems (such as the National Health Service), and various protection mechanisms, have in the relationship between demand and supply of health care.

In the analysis of Mateus and Moura (2014), they considered Portuguese authors, an option that currently would leave out researchers of other nationalities that do research in health economics based in Portuguese institutions. They included articles published in scientific journals as well as master's and doctoral theses, covering the period from 1979 to 2014.

The growth of scientific production in health economics, measured in this way, grew in a sustained and significant way throughout this period, and especially from the beginning of the 21st century.

In the information collected by Mateus e Moura (2014), the three institutions with the highest number of scientific publications are the National School of Public Health (Universidade Nova de Lisboa), the Faculty of Economics of the University of Coimbra

and the Nova School of Business and Economics (Universidade Nova de Lisboa), giving a prominent place to the Universidade Nova de Lisboa in the national panorama. The same implication results from two other exercises of searching for sources of information on scientific production. Consulting the portal "Investigação em Economia in Portugal: Pessoas e Instituições" (http://cefup-nipe-rank.eeg.uminho.pt), in the classification of the Journal of Economic Literature I- Health, Education and Welfare, it is possible to consult the articles that may be considered belonging to the area of health economics, from 1970 to 2020. The researchers included are based on national institutions, regardless of their nationality, and the information base covers only articles published in scientific journals. It excludes, therefore, books and master's and doctoral theses, considered in Mateus and Moura (2014). Again, the Universidade Nova de Lisboa emerges as one of the three main institutions, along with the University of Lisbon and the University of Minho (all with 18 entries of scientific articles, followed by the University of Porto, with 16). The University of Coimbra has 5 scientific articles.

A third exercise consists of taking the research in health economics as disseminated in the national conferences of the Portuguese Association of Health Economics, considering the last three conferences (2015, 2017 and 2019).This covers the period after the review of scientific production of Mateus and Moura (2014). In this exercise, only the oral communications presented were included, as a way of having a first view of the most recent panorama of the main universities and schools involved. Due to the presence of co-authorship, it is necessary to follow some rules. In particular, the following criteria were followed: (i) an article co-authored by researchers from the same institution counts only once; (ii) an article co-authored by two researchers from different institutions counts once in each institution; (iii) a co-authored article with three or more researchers from different institutions is considered to be only from the institution of the person presenting the work in the conference, with the exception of the situation in which only one researcher is in a higher education institution, in which case the communication is considered in the production of that institution (being verified whether it corresponds to a master's or doctoral thesis); (iv) two categories are considered for other cases: other institutions (which cover small numbers of higher education institutions or non-academic institutions) and international (researchers from institutions outside Portugal, academic or not, and researchers may be Portuguese nationals or not). This methodology for dealing with communications in which the papers have multiple authors with various affiliations (including cases of the same author having multiple affiliations)

seeks to reflect the diversity and intensity of participation by institution. There is of course the possibility to discuss the criteria used for classification (for example, the introduction of a discount for the number of authors, or different ways of dealing with communications in which the basic article has many co-authors).

The following tables contain the results of this exercise, by university in Table 1 and with breakdown of the academic units of main universities, in Table 2.

	2015	2017	2019	2017-2019
Universidade Nova de Lisboa	35%	18%	31%	28%
Institutions outside Portugal	16%	15%	15%	15%
Universidade de Lisboa	9%	16%	13%	13%
Universidade de Coimbra	13%	11%	11%	12%
Universidade do Porto	8%	14%	8%	10%
Others	7%	9%	13%	10%
Universidade do Algarve	3%	6%	5%	5%
Universidade Católica Portuguesa	7%	6%	1%	5%
Universidade do Minho	3%	4%	3%	3%

Table 1 – The conferences of the Portuguese Association of Health Economics, by university of origin
Source: own elaboration

	2015	2017	2019
Nova SBE	22,7%	11,4%	19,5%
Institutions outside Portugal	16,0%	15,2%	14,9%
Others	6,7%	8,9%	12,6%
FE – Coimbra	13,3%	11,4%	11,5%
Nova ENSP	9,3%	6,3%	10,3%
UL – IST	6,7%	10,1%	8,0%
FE – Porto	4,0%	8,9%	5,7%
U Algarve	2,7%	6,3%	4,6%
FM – UL	0,0%	5,1%	4,6%
EEG-Minho	2,7%	3,8%	3,4%
UCP – Lisboa	5,3%	6,3%	1,1%
Nova NMS	2,7%	0,0%	1,1%

	2015	2017	2019
FEng – Porto	0,0%	2,5%	1,1%
FM – UPorto	2,7%	2,5%	1,1%
ISEG – UL	2,7%	1,3%	0,0%
ICBAS – UPorto	1,3%	0,0%	0,0%
UCP-ICS	1,3%	0,0%	0,0%

Table 2 – The conferences of the Portuguese Association of Health Economics, by entity of origin
Source: own elaboration

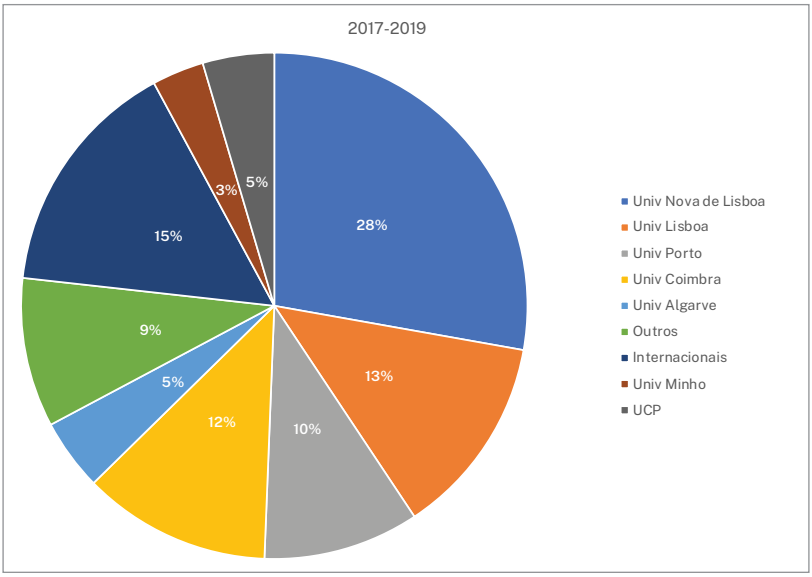

Figure 1 – presents in a graphical way the values of Table 1.
The conferences of the Portuguese Association of Health Economics, by entity of origin
Source: own elaboration

These three sources of information give a diverse view of what has been the evolution of health economics in Portugal, in its scientific component, and the role of Universidade Nova de Lisboa, from introduction of field in Portugal to current participation in scientific research. Although Universidade Nova de Lisboa appears as main institution in the growth of the field in any of these approaches, it still has an underestimation of its contribution. The sources consulted do not include a presumably important volume of articles in scientific journals in nearby fields,

such as public health or political science, which could eventually also be published in journals such as those included in the economics research ranking.

The disaggregation by academic units of the same university is useful because it reveals a trend of the last decade in health economics in Portugal – the emergence of groups of researchers who dedicate themselves to health economics themes in academic units that do not have, as their main scientific activity, research in economics or management. Two groups of institutions emerge. On the one hand, medical schools, where the introduction of principles of economic evaluation in the research carried out in their traditional fields translates into a growing interest in knowing better what is done in this specific area. It is also probably the result of economic evaluation studies for interventions (medicines, but not exclusively) implying a deep interaction between the two scientific areas. Thus, communications have emerged at the conferences of the Portuguese Association of Health Economics by researchers from medical schools, many of them associated with economic evaluation studies, some focusing on macroeconomic aspects of the health sector. On the other hand, engineering schools, through researchers who mostly develop and apply operational research methods to health sector problems. They are schools that have created a growing presence, with several nuclei of the same institution. It is, moreover, this participation of operational research groups that underlies the top position of the University of Lisbon in the image resulting from the consultation of the ranking of research in economics.

These different sources also highlight the situation of the University of Minho, where a researcher of foreign nationality publishes regularly in international journals of health economics, but is largely absent from the national (Portuguese) scientific community (which is present in the national conferences of the Portuguese Association of Health Economics). On the other hand, the University of Coimbra with a very active group of researchers with relevant contributions in the area of measurement of health outcomes, which do not generally lead to publications in scientific journals of economics or health economics, comes with a great underestimation of its significance in the national-based scientific production in the "Ranking of Research in Economics".

In general, each group working in health economics in each academic institution evolves towards a specialization in particular issues, while still maintaining, broadly speaking, equity as the most transversal theme to different groups in schools of economics, and the efficiency of health care providers as the theme most shared by groups using operational research methods. That is, a significant part of

the scientific production in health economics published in scientific journals of the field has no connection with the community that produces works that are included in the general topic of health economics but published in journals of other areas.

The growth of scientific production in the field of health economics has been, therefore, largely the result of a widening of frontiers of the field, in which researchers from nearby areas when dealing with health economics issues with methodologies in these areas bring contributions to the scientific growth of the area of health economics.

It is also interesting to note the presence, at the conferences of the Portuguese Association of Health Economics, of communications from researchers or professionals of public entities and private companies, revealing the applied nature of the research that is produced. Applied research covers both data processing, case study and economic theory applied to health economics problems.

Thus, in a personal interpretation, the growth of research in health economics has been present in what in economic jargon are called extensive margin and intensive margin. Institutions that traditionally have researchers involved in health economics have strengthened their activity (increased scientific production in health economics through expansion of the intensive margin). Researchers from institutions with little tradition in health economics increase their presence (growth in scientific production by expansion of the extensive margin).

3. HEALTH ECONOMICS AT UNIVERSIDADE NOVA DE LISBOA

As mentioned above, the Universidade Nova de Lisboa has been a leader university in health economics in Portugal. There is also room for this participation of the Universidade Nova de Lisboa to be strengthened through the extensive margin, bringing in contributions to health economics from other academic units than the ones that have been contributing so far.

The participation of researchers from Nova Medical School in activities that lead to scientific production in the field of health economics is still at an early stage. In the applications of operational research, on the other hand, there is, for the time being, no participation in discussions and analyses at the level of what is observed from the Instituto Superior Técnico of the University of Lisbon. There is, therefore, a possibility of extensive margin to be explored, which could strengthen the role of the Universidade Nova de Lisboa in the field of health economics in Portugal. Of

course, in other Portuguese universities there is also room for the growth of these intensive and extensive margins.

The historical tradition based on the National School of Public Health, in the first decade of health economics in Portugal, extended, within Universidade Nova de Lisboa, to other academic units. There are now researchers in health economics at the Nova School of Business and Economics, the Nova Medical School and the Institute of Hygiene and Tropical Medicine, suggesting the ability of Universidade Nova de Lisboa to continue its role in the growth of health economics in Portugal.

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C

HEALTH SYSTEMS, THE SARS-COV-2 PANDEMIC AND THE "EUROPEAN HEALTH UNION"

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INTRODUCTION

The traditional understanding of health systems as a key element of national sovereignty was already obsolete, when considered from the perspective of the European health policies and European health law. The COVID-19 pandemic reinforced the belief that health is a global public good and needs to be delivered as such. The vulnerability of healthcare systems affects the health of its citizens, endangers the global community, negatively influences economic growth, hinders the trust of citizens in formal powers and threatens social cohesion, even in the European Union Member States. The United Nation's Sustainable Development Goals (SDGs), adopted in September 2015, include an ambitious, yet crucial, purpose to promote universal health coverage, in order to achieve SDG 3 "*Ensure healthy lives and promote wellbeing for all in all ages*" (1). The performance of our healthcare systems is, nowadays, intrinsically connected with external and multisectoral determinants, regulatory frameworks, research and data, with natural prominence of the European Union (EU) influence and action.

On the other hand, the COVID-19 pandemic has shed a spotlight on critical weaknesses of Member States' health systems and on the need to provide access to equitable, efficient and quality healthcare across the EU. Hence, the idea of *a European Union of Health* is unavoidably based on the principles of cooperation and solidarity, but also needs strong political commitment to much needed reforms.

Notwithstanding a desirable convergence in terms of access to and coverage of health care between States, especially given the founding principles of the European Union (2), the heterogenous political organization and philosophy, have dictated a divergence between and within Member States. Conflicting with the principles of autonomy and sovereignty, the development of a single approach to guarantee the right to health of all EU citizens, universal coverage and equity has proven to be a hard bone to chew. However, the SARS-CoV-2 pandemic has demonstrated that this might be a bone that European Union is interested to chew.

FROM NATIONAL HEALTHCARE SYSTEMS TO GLOBAL HEALTH

In the field of health, the evolution of the role played by the EU has been gradual, but consistent with the path taken from economic union to political union. Starting from a motivation anchored in the goal of economic growth and the free

movement of persons and goods, only later welcomed social concerns, in particular those related to public health, until the principle of solidarity appeared, in recent days, repeatedly invoked to justify a future European Health Union and to further commit the EU to a leading role in global health.

In the wake of the COVID-19 crisis, this branch of European social policy has gained an unavoidable leading role vis-à-vis the typical objectives associated with the execution of the internal market, with some arguing the need to change the legal basis applicable to public health (3).

It is important to go back to 1997, to identify a similar political path. In the aftermath of the "Bovine Spongiform Encephalopathy (BSE) Crisis", with the EU facing strong criticism for a late and insufficient response to this cross-border threat, the need to strengthen the powers of the European bodies in public health issues was required. This process culminated with the approval, in the Treaty of Amsterdam (4), of the principle of horizontal protection, among other legislative reinforcement measures. As it seems to be the case with the current Covid-19 crisis, the BSE public health emergency had in its origin a clear linkage between human and animal health.

The codification of the also called *health in all policies* approach, in Article 9, and Article 168(1) of the Treaty on the Functioning of the European Union (TFEU) (5) and in Article 35 of the European Union Charter of Fundamental Rights (6), formally recognized the multisectoral and transdisciplinary nature of public health, and intended health impacts to be considered in all relevant policies.

The acknowledgment of the interactions between human health, animal health and the environment is not new but has been revitalised. The *One Health* concept foresees a transdisciplinary approach for the design of programmes, policies, legislation, and research, in which multiple sectors collaborate to achieve an improved public health. The control of zoonoses and the combat against antimicrobial resistance are two of the main immediate targets, but it is expected that this collaborative method could help solve some of many other emerging global problems. Of utmost important to this strategy is ensuring that the measures are implemented across national and even European frontiers. Another challenge is to make sure that the efforts and processes adopted go beyond a mere response to the COVID-19 pandemic.

For the time being, the European Commission's proposal for a European Health Union, (7) building on the need for a stronger EU health framework presented at the end of 2020, continues to have Article 168(6) of the TFEU as its enabling basis, focusing on strengthening coordination and cooperation efforts, particularly in relation to cross-border threats.

A BRIEF OVERVIEW OF THE EUROPEAN ENABLING LEGAL BASIS IN THE FIELD OF PUBLIC HEALTH

The affirmation of European law regarding the national health system has benefited from the gradual extension of the powers enshrined in the EU's founding treaties concerning public health, as well as from the strengthening of the principle of horizontal protection which requires health to be considered in the remaining Community areas. Both circumstances should be understood in the broader context of the acceptance of the social protection objectives, in particular health protection, which has been spearheaded by the adoption of the EU Charter of Fundamental Rights. The European Court of Justice's (ECJ) performance is, in turn, a clear element in consolidating the Europeanisation of public health law and policies, an achievement definitively reinforced after the process of approval of the Directive on the application of patients' rights in cross-border healthcare (8).

The founding treaties of the European Union were silent on the protection of health or public health. This did not, however, prevent Community bodies from intervening in matters relating directly or indirectly to health, such as the quality of medications or the safety of foodstuffs, even if this intervention was legitimised, in the context of trade policy, by the powers exercised in connection with the execution of the internal market.

The Maastricht Treaty (1992) (9) recognises for the first time public health as a branch of European policy, giving it an autonomous title. Under Article 129 of the Treaty, the European Community (EC) takes on an explicit and specific competence, although focused on measures to encourage, support, and coordinate the national activities. The Treaty of Amsterdam (1997) extends the EC's legislative powers to include the adoption of measures to ensure the quality and safety of organs and substances of human origin, blood, and blood derivatives, as well as veterinary and phytosanitary measures. It enshrines the protection of health as a goal of the Community's action, going past the previous limitation to mere prevention. Finally, the strengthening of the principle of horizontal protection, henceforth reaffirmed in the precept devoted to public health (see Article 152 TEC in the version resulting from the Treaty of Amsterdam), requires the Community bodies to protect health in all policies and measures, thereby making health protection a fundamental task of the Community. The Treaty of Nice made no significant changes in this area, especially when compared with the Treaty of Lisbon, which approves the TFEU.

The TFEU strengthens incentives for cooperation in combating common safety threats, particularly in cross-border matters (including surveillance, alerting in the event of such threats and combating them), increases the complementarity of health services in cross-border regions and to further extends the legislative powers in the area of public health. In the latter area, measures establishing high quality and safety standards for medications and devices for medical use (see Article 168(4)(c) of the TFEU (5)) will be included in the set of possible legislative measures.

The principle of horizontal protection favours the adoption of health-related measures in different fields of action, such as: agricultural policy, consumer protection, environmental protection, free movement of workers, right of establishment, provision of services, innovation and development. As we argued, the emphasis is placed on the competence to approximate provisions having, in most cases, a direct bearing on the establishment or functioning of the internal market (Article 114 of the TFEU(5)). In any event, it is important to ensure that social policies are not merely market-making instruments.

Alongside public health and the above-mentioned fields of action, where there is a health impact, fiscal government policies have had direct and significant consequences in the national health systems, making it impossible to exclude them from a broader sense of a European health policy (10).

SCOPE AND INSTRUMENTS OF PERFORMANCE IN PUBLIC HEALTH MATTERS

The EU's competence in public health matters is now based on a combination of Articles 4(2)(k), 6(a) and 168 of the TFEU (5). This is a competence focused on public health[1] and, in this field, particularly attentive to common safety problems.

[1] The concept of public health remains dependent on different doctrinal conceptions and therefore cannot be subsumed into an unequivocal and universal definition. Moreover, this is a reality that reveals multiple possible facets and perspectives, which present it at times as a science, as a practice, as a discipline, as a State power and even as a social problem. In very broad terms and aware of the simplicity and neutrality it entails, we have chosen to invoke the characterisation of Gostin, *Public Health Law – Power, Duty, Restraint* (2000), which brings public health back to the efforts of a society to ensure that its population is healthy, with emphasis on the preventive and community aspects of health.

The action of the Union "*shall be directed towards improving public health, preventing human illness and diseases and obviating sources of danger to physical and mental health*" (see Article 168(1) of the TFEU (5)). The fight against major scourges, as well as health information and education and the surveillance of serious health threats, are the highest goals of European policy, since they are expressly set out in the TFEU. The list seems to be merely illustrative (11).

A critical element in the shape of the European public health formal competences is the recognition that Member States have sole responsibility for the organisation and provision of health services and medical care and that it is incumbent on them to define their health policies (Article 168(7) of the TFEU (5)).

It is possible to identify four lines of action in the European intervention, achievable through different possible implementation instruments: support and coordination, programming of European action, cooperation with third-party countries and legislative intervention. Each task may have more than one implementing instrument (*e.g.*, programme measures both support the implementation of support and coordination measures and serve the definition of the broad lines of the European policy).

Incentive and coordination measures have been essential in contributing to the improvement of national policy, as is the case in other areas of social protection. From the range of possible coordination measures, the emphasis is on the collection and processing of data, standardisation and monitoring of indicators, preparation of studies and reports, risk analysis and preparation of action programmes.

Another of the EU's instruments for action is the definition of the broad lines of health policy, through which areas of strategic development are prescribed and financial investment is planned.

The programme measures that define and characterise the strategy of action can be of a general or sectoral nature. In the European case, there was a gradual replacement of piecemeal and sectoral plans by programmes of a strategic and more general nature. We must argue that this transformation heralded the emergence of a European health policy

The eight thematic action programmes implemented since 1986 were followed by three multiannual action programmes (12).

In May 2020, the legislative proposal for the Union's fourth action programme in the field of health was presented as a (first) response to the pandemic crisis. The EU4Health (13), into force since 26th March 2021, aims to improve the Union's preparedness and response to cross-border threats and to fulfil a European Union

of Health, by investing in the availability of medicines and innovation, improve cancer care, and boost digital health while strengthening the Member States' health systems.

TIMES OF CRISIS: THE SARS-COV-2 PANDEMIC

The efforts undertaken in the past decades to create a common health ground were put to the test with the pandemic and despite the three main areas involved (public health, regulation, and fiscal governance), it were mainly the first two, public health and regulation, that were in the spotlight.

Actually, the efforts undertaken to develop and regulate EU internal market, namely in eliminating protectionism, for instance, have proven to be paramount for procurement of SARS-CoV-2 vaccines and related negotiation process (14), which allowed for a more equitable and fair access to vaccines for smaller, and poorer countries. The European Commission entered into Advanced Purchase Agreements with individual vaccine producers on behalf of Member States, financing a part of the upfront costs faced by vaccines producers from the Emergency Support Instrument as a down-payment on the vaccines purchased by Member States.

However, and probably because it happened much earlier than vaccines procurement, the Member States competed against each other, while buying Personal Protective Equipment (PPE) and other medical equipment essential for providing care for COVID-19 patients in March/ April 2020. Soon it was evident that surpassing other Member States would later jeopardize not only bilateral relations but the EU fundamental principles and foundations. In March 2020, a strategic reserve of essential medical equipment within RescEU (16) was created with the European Commission financing most of the stockpiling costs and managing distribution (14) and procurement being made by Germany, Romania, Denmark, Greece, Hungary and Sweden for all Member States (17).

The recognition of professional qualifications (18), was decisive during pandemic times, with no need for special regimens for EU health professionals acting in different countries. This allowed less affected Member States to offer assistance (i.e., medical teams) to most affected EU countries to provide support mainly to COVID-19 patients, without additional bureaucracy (i.e., recognition of legal right to practice). It was the case in Italy, Belgium and Portugal (17).

If in terms of regulation, the success of a common health ground was unquestionable, the same was not to say about public health. For long, Member States had recognized the need to have common policies and procedures in terms of health emergencies (19). The fortunate lack of a common, large public health threat failed to test the policies developed so far as well as the articulation between EU institutions such the ECDC and the member States.

The frailty of the route to a European Health Union in terms of health policy has been documented and founds its bases in the limited EU actions (10). This became largely evident in the first months of the SARS-CoV-2 pandemic, with institutions like the World Health Organization taking a leader role, and ECDC a secondary one.

In fact, the way EU Member States approached the pandemic in early 2020 showed inadequate coordination, with countries closing schools and ordering lockdowns with repercussions in EU circulation without notice. The same happened when countries relaxed measures in Summer 2020, with different criteria and rules to citizens' circulation: some countries demanded quarantine for travelers from some destinations, some demanded negative COVID-19 tests to enter the country, and some did not have any of those measures in place. Furthermore, even if the joint acquisition of COVID-19 vaccines was seen as a relative success, the process of vaccine rollout revealed many fragilities. Firstly with constant delays by pharmaceutical companies in delivering the contracted amounts of vaccines. Secondly, as a consequence, Member States unilaterally approving vaccines which were not yet approved by EMA. And lastly, with countries suspending vaccination with AstraZeneca vaccine, against the recommendation of EMA, thus threatening the confidence of citizens on this vaccine and delaying the vaccination process.

As the pandemic grew stronger it became evident the need to have not only stronger institutions but a more centralized decisional process, first within countries, and then between Member States (14) and, as so, in the EU. The adoption of measures to tackle the pandemic and guidelines for managing the several aspects of the pandemic (from contingency plans to contact tracing) started to be discussed and recommended at the EU level (14) aiming at a uniformized uptake at national and subnational levels, with growing relevance and recognition of ECDC and EMA and structures such as the Health Security Committee (20).

The reinforcement of EMA's mandate to play a key role in accelerating the approval of medicines and monitor medicine shortages in EU is useless if Member States do not agree on respecting and strengthening this mandate. The same can

be said for the ECDC and the need for a stronger, more comprehensive EU framework for preparedness, surveillance, risk assessment and actions to improve early warning and crisis response.

The DG SANTE – DG for Health and Food Safety, responsible for EU policy on food safety and health and for monitoring the implementation of related laws, and deriving from the lessons learnt from COVID-19 proposed in November 2020, a new health security framework, drawing upon the 2013 Directive on Cross Border threats to health (21). This framework recognizes that "public health measures need to be consistent, coherent and coordinated to maximise their effect and minimise the damage" and further proposes strengthening the mandate of the ECDC and extending the mandate of the EMA (6).

The new framework along with its legislative initiatives might represent the foundations for a more solid health policy to be ratified and shared between Member States.

CONCLUSION

Although the mechanisms of EU coordination during the pandemic were put into force, with frequent informal ministerial meetings, European Councils, coordinated response to the economic crisis and coordinated mechanisms of vaccine rollout, the pandemic has unveiled the need for stronger mandates by European institutions, such as the European Centre for Disease Prevention and Control (ECDC) and the European Medicines Agency (EMA).

This struggle to control the pandemic crisis and the multiple setbacks associated with the European response urged a reinforcement of the EU's position, warranting the defence of a European Health Union, formally assumed by the European Commission in November 2020. This defining step in the evolution of the European intervention, which recognises health as a key requirement to the preservation of society and of our way of life, seeks to reinforce coordination between Member States and the potential sharing of efforts, and even management, at the EU's level. In fact, despite being centred on the defence of public health and on combating cross-border threats, the current legal and institutional framework has proven to be of little effectiveness and inadequate in meeting the ambitions of the representatives of the European institutions and of the European citizens. Among other legislative reforms, a new Regulation on serious cross-border threats

to health, reinforced mandates for the European Centre for Disease Control and Prevention and the European Medicines Agency and a future Health Emergency Response Authority (HERA), are some of the proposals for strengthening European action in the field of heath.

According to the TFEU, Member States remain legally responsible for the definition of their health policy and for the organisation and delivery of health services and medical care, including the management and the allocation of the resources assigned to them.

Despite the subsidiarity principle, increasing preparedness and resilience of healthcare systems are explicit goals of the most recent European health-related proposals, alongside with promoting efficacy and equity. All these goals are to be considered within a broader health strategy, based on solidarity and on the premise that a better health is a driving force for sustainable development. This comprehensive approach should, in the future, not be limited to preventing and controlling existing cross broader threats.

Even though this is the desirable path, it is probably the right time to question if Member States still have exclusive powers over their health systems, and whether any possible transmission of powers was formally legitimised, namely by an appropriate legal base.

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D

HEALTH LAW AND ETHICS, BIOLAW AND BIOETHICS

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I. INTRODUCTION

The subject of the present book chapter has a significantly wide scope, scientific interest and social relevance. Conscious of these characteristics, NOVA University of Lisbon has played a pioneering role in bringing the subjects of Health Law, Biolaw, Bioethics and Human Rights in health to the center of academic discourse in Portugal. Through the dedicated teaching and inclusion of autonomous disciplines in several of NOVA's Courses in the Health area, students of different scientific backgrounds became acquainted with the importance of these (at the time) innovative subjects. Furthermore, solid, collaborative and multidisciplinary research on these issues enlarged the span of scientific knowledge, while strengthening the ties within the academic community and widening the University outreach. Accordingly, a robust ethical compromise developed within the walls of the University, which later transpired into contacts with other institutions and society members, leading to the establishment of competent bodies to conduct comprehensive ethical reflections, debates and reviews of health research.

NOVA University gathers strength from its diversity. The academic endeavor undertaken throughout the years on the subjects of Health Law and Ethics, Biolaw and Bioethics is no exception to this fundamental characteristic of our University. Particularly, the efforts of scholars, researchers and students at the School of Law, NOVA Medical School and the National School of Public Health have developed different and complementary views on these subjects, simultaneously building bridges within the University and with the outside community, while contributing to expand and enrich scientific knowledge on different dimensions of these wide-ranging topics.

This Chapter, co-authored by members of different NOVA Schools, tries to capture this diverse and collaborative cultural trademark. Despite the lack of comprehensiveness attributable to the space limitations inherent to a text of this nature, we have tried here to: i) review the state of the art in the field (by clarifying the scope of connected but not superimposable concepts, discussing the international framework, which provides a legal structure in the field in Portugal, and highlighting important ethical, legal and social issues of specific and related topics); ii) present specific contributions to the field from NOVA University (including teaching, research and ethical review of health research), and iii) synthesize and underline the broad impacts to science and society of work done on these fundamental disciplines.

We hope the following text does justice to the efforts of those at NOVA University that throughout the years have expressed a longstanding passion and interest for these fascinating subjects.

II. STATE OF THE ART REVIEW

A) Health Law, Biolaw, Bioethics and Human Rights – related and complementary but not identical fields

Health Law identifies with the more traditional part of the juridical aspects linked to health and life sciences, composed of the juridical tools (legislation, doctrine, and jurisprudence) that apply mainly to the health care act and settings. The term "Health Law" is sometimes used as a synonym of the expressions "Medical Law", "Health Care Law" or "Biomedical Law". Nevertheless, these last terms always evoke a narrower scope and tend to be more focused on the legal problems of the medical profession, health care, and biomedical developments. The study of Health Law normally covers issues such as access to care, health systems organization, patients' rights, health professionals' rights and duties, strict liability, healthcare contracts between institutions and professionals, medical data protection and confidentiality, informed consent and professional secrecy (1). As a complement to a definition of Health Law, it is necessary to mention the more recent term Biolaw, as the legal field that covers the social consequences that arise from biotechnological developments. Recent developments in Medicine and Biology have created an even greater challenge for the Law, defying some of its traditional fundamental concepts. The concept of "**Biolaw**" is usually defined in connection with the field of Bioethics as: "*The taking of agreed upon principles and practices of bioethics into law with the sanctions that law engenders. Biolaw includes legislation on bioethical issues, interpretation of such legislation and case law made by judges"*(2). The transformation of Bioethics in Biolaw is, however, not an easy task. Biolaw must first of all be adaptable to the ever-changing developments of scientific knowledge and, secondly, it must reflect a consensus between society and the scientific community in order to promote adherence and applicability of its norms.

The term "**Bioethics**" was first used to describe a kind of ethics that would include not only our obligations to other human beings but to the biosphere as a whole(3)(4). Later in 1988, Van Rensselaer Potter defined Bioethics on the cover of another book as "*Biology combined with diverse humanistic knowledge forging a*

science that sets a system of medical and environmental priorities for accept- able survival" (5). The concept evolved and in 1995 the Encyclopedia of Bioethics defined bioethics as the "*systematic study of the moral dimensions — including moral vision, decisions, conduct, and policies — of the life sciences and health care, employing a variety of ethical methodologies in an interdisciplinary setting"* (6)Nevertheless, it is almost impossible to define the exact content of Bioethics as its boundaries widen every day. In this sense, the UNESCO "Universal Declaration of Bioethics and Human Rights" (2005) showed how Bioethics again enlarged its scope almost returning to its primitive "ecological" dimension, an assertion that is supported by different articles of the declaration (*e.g.* 16 and 17). Bioethics is, as mentioned, intrinsically linked to Health Law and as such, evolution in this field influences the shape of the legal framework in all the overlapping areas of these two connecting fields.

In parallel, although **Human Rights** have always been historically linked to medicine and health (the 1947 Nuremberg trials and Code), the importance of Human Rights to coexist with Health Law and Bioethics is more recent (7). In accordance, Human Rights influenced the movements that lead to establish Patients' Rights as a fundamental piece of contemporary Health Law and are also the cornerstone of the 1997 Council of Europe Convention for the protection of Human Rights in Biomedicine (Oviedo Convention) and the 2005 UNESCO Universal Declaration on Bioethics and Human Rights. These principles are generally reflected in European Health Law and demonstrate how the spirit of Human Rights is present there and is crucial in an area where the concepts of humanity and human dignity are always at stake.

B) International Biolaw in Portugal

Portugal has a very high level of protection in what concerns human rights and biomedicine, as the main legal texts that have been adopted in this field by the United Nations, Council of Europe and the European Union apply directly in its territory.

The universal bioethical norms and principles promoted by the most important United Nations Agency in bioethics, UNESCO, are mainly proclaimed in three Universal Declarations, adopted by acclamation by UNESCO's General Conference: the Universal Declaration on the Human Genome and Human Rights (November 11, 1997), the International Declaration on Human Genetic Data (October 16, 2003), and the Universal Declaration on Bioethics and Human Rights (October 19, 2005). These Declarations, as reflected in their title, anchor the fundamental principles in

bioethics and aim to promote the full respect for human dignity and human rights in what concerns the recent applications of science and technology, especially of Life Sciences and Medicine. They address to States that have to take all legislative, administrative or other measures, required to give effect to the principles that are set out in these texts, having also in mind international human rights law(8).

This interrelation between Bioethics and human rights is recognized by the Portuguese State that not only considers the UNESCO'S Declarations as an integral part of Portuguese Law (article 8 of the Constitution of the Portuguese Republic), but also has signed and ratified the most important international treaties adopted at a regional level in the field of Biolaw, which recall in their texts the essential principles of bioethics established in the referred Universal Declarations.

The most important Biolaw's Covenant in Europe is Council of Europe' Convention for the protection of Human Rights and Dignity of the Human Being with regard to the Application of Biology and Medicine: Convention on Human Rights and Biomedicine, open for signature in Oviedo, on April 4, 1997. Portugal was one of the first States to sign it, without any reservations or declarations, thus recognizing the main importance of the first legally-binding international text designed to protect human dignity and fundamental human rights and freedoms against the misuses of the accelerating biomedical developments. Portugal has also already signed and ratified all the Protocols that develop in specific fields the principles contained in this Convention: the Additional Protocol to the Convention on the Prohibition of Cloning Human Beings (open for signature on January 12, 1998); the Additional Protocol to the Convention concerning Transplantation of Organs and Tissues of Human Origin (open for signature on January 24, 2002); the Additional Protocol to the Convention concerning Biomedical Research (open for signature on January 25, 2005), and the Additional Protocol concerning Genetic Testing for Health Purposes (open for signature on November 27, 2008).

These international treaties as a whole provide a common framework in Europe for the protection of human dignity and human rights with regard to the new biomedical developments that have been signed and ratified at a national level by the Portuguese State. Also included in this common framework are other Council of Europe's treaties also signed and ratified by Portugal, namely the Council of Europe's Covenant against Trafficking in Human Organs (open for signature on March 25, 2015) and the Council of Europe's Convention on the counterfeiting of medical products and similar crimes involving threats to public health (open for signature on October 28, 2011).

Although the control of the manner in which Portuguese internal law ensures the effective implementation of the provisions of these Conventions is mainly done by reports on their application at a national level, Portugal still has the duty to provide for appropriate sanctions that will be applied in the event of an infringement of those provisions. The Portuguese State has already provided for those sanctions (as well as corresponding guarantees), which constitutes a wider protection with regard to the applications of Biomedicine than the protection provided by the aforementioned European Treaties. For example, the national law provides a higher level of protection than international law regarding the conditions applicable to protect persons who are not able to consent to research, when the research does not have the potential to produce results of direct benefit to the health of the concerned person.

National Courts, especially the High Court of Justice, often refer to provisions of the Convention of Human Rights and Biomedicine and of its Additional Protocols when deciding national cases on unlawful infringements of the principles set forth both in national law and in these European treaties.

This internormativity between national and international legal sources also occurs with European Union's provisions that have the same main objective: to protect the human being against the possible misuse of the new applications of Life Sciences and Medicine(8). The Charter of Fundamental Rights of the European Union, solemnly proclaimed by the European Parliament, the Council and the Commission, on December 7, 2000, centers the Union on the common values of the peoples of Europe, also preserved by the Council of Europe Treaties on Biomedicine, *i.e.* the inviolability of human dignity, freedom, equality and solidarity. Article 3 ("Right to the integrity of the person") of the Charter reaffirms part of the principles already included in the Convention on Human Rights and Biomedicine in the context of medicine and biology.

Several Regulations and Directives on the field of Biolaw are also very important to ensure a high level of health protection in the territories of the European Union member states, namely the General Data Protection Regulation (Regulation (EU) 2016/679 of the European Parliament and of the Council of the 27th April 2016, on the protection of natural persons with regard to the processing of personal data and on the free movement of such data; Directive 2002/98/EC of the European Parliament and of the Council of 27 January 2003 setting standards of quality and safety for the collection, testing, processing, storage and distribution of human blood and blood components amending Directive 2001/83/EC, and Directive 2011/24/EU of the European Parliament and of the Council of the 9th March 2011 on the

application of patients' rights in cross-border healthcare. The Portuguese State has already brought into force the laws, regulations and administrative provisions required to comply with these and other Directives which have provisions that aim to ensure a high level of human health protection, as determined in Article 168(1) of the Treaty on the Functioning of the European Union.

The jurisprudence of the Court of Justice of the European Union is also very important for national courts, especially when applying internal dispositions that implement Union Law. The preliminary rulings given by the Court of Justice concerning the interpretation of acts of the European institutions are also essential when a Biolaw question is raised before a Portuguese court and this court considers that a decision is necessary to reach a judgment.

Portugal accords a very high level of protection to human rights and dignity, protecting present and future generations from threats that may result from the improper use of biomedical developments (9). It is considered one of the countries in the world that most respects the main Biolaw principle of the primacy of the human being over the sole interest of science or society.

This normative compass, supported by a core of sound ethical principles, is fundamental to face the challenging ethical, legal and social issues arising from different scientific and technological developments in the healthcare and health research areas.

C) Ethical, Legal and Social Issues (ELSI) of specific science and technology developments in the health area

i. Biobanks

Biobanks, which in general terms can be described as organized collections of biological material (organs, tissues, biofluids and genetic materials) and/or associated information, can vary significantly in terms of nature, size, aim (population-based biobanks for biomarker identification, disease-oriented biobanks for epidemiological studies and disease-oriented general biobanks, for example), duration, ownership (public, private, or partnerships of these, held by hospitals, research institutes, pharmaceutical companies and patient organizations) or governance model (10).

In particular, biomedical research biobanks (BRB) have gained impressive momentum in the last decades, particularly because these research infrastructures potentiate the collective capacity to understand human biology and medicine while contributing to fight disease and improve quality of life. These infrastructures also

provide a near-perfect environment for collaboration between scientists, clinicians, ethicists, legal scholars and practitioners, health managers and other professionals. Growing numbers of BRB have been created throughout Europe in the last decades (11,12). In parallel, as a result of relevant developmental efforts, Portuguese BRB have also been created and implemented. Subsequently, a national biobank consortium (Biobanco.pt), which includes a biobank from NOVA University, has been set up to inventory national infrastructures, catalogue samples, harmonise procedures, establish common ethical standards and guidelines and promote national and international research[1].

Despite these largely positive impacts, BRB face significant challenges. In order to maximize their benefit for health research and public health, biobanks need to be ethically robust, legally compliant (with national and international legal frameworks) and gather significant public support(13). Therefore, ethical, legal and social issues (ELSI) of BRB deserve particular attention (14). Relevant ELSI of BRB include (but are not exclusive to): i) the protection of the rights to autonomy, confidentiality and privacy of participants while also respecting the public interest of scientific research; ii) selecting the need, method, scope, level of detail, and periodicity of informed consent, grounds for sample withdrawal; iii) security measures that should be adopted; iv) balancing non-commercial use of human biological material for scientific research purposes and the development of commercial products directly arising from stored and shared samples, waiving and retentions of property rights; v) promoting public trust and inclusion; vi) incentives for donor participation; vii) ownership, governance, business and management issues; viii) access to research results, at individual, institutional and global levels; ix) ensuring maximum quality of sample preservation and management, while facilitating sample access and sharing (10,14–17).

Notably, not all these issues have received similar scientific, political and legal attention. Nonetheless, biobank regulation has been a sound and popular topic of international and national biolaw in the last decades. For example, European Research Project "EUROGENBANK" examining human and non-human biobanking at a European-wide scale, included work developed in the National School of Public Health of NOVA University, which started in 1998 and culminated with the publication of important recommendations for biobank regulation(18). In particular, Portugal has specific legislation on biobanks (Law 12/2005, of January 26th).

[1] www.biobanco.pt, last access April 14th, 2021

Despite criticism from some sectors(19), lack of legislative update and case-law on the matter, the national legal framework has permitted the structured creation and implementation of biobanks in the country, as well as their current harmonization toward a national hub (Biobanco.pt), as mentioned above.

ii. Genetics, genomics and precision medicine

Since the completion of the Human Genome Project (HGP) in 2003, technological progress has resulted in the ability to sequence human genes and genomes for very competitive prices (20). More recently, the arrival of the $1,000 dollar genome has been announced, which promises to take us one step further to an announced revolution in healthcare – the era of precision medicine where treatment and prevention strategies are precisely tailored and provided to the right individual at the right time (21).

In parallel, the advancement in biomedicine has given rise to multiple "OMICS" that study the totality of different molecules in the organism – transcriptomics (RNA), proteomics (proteins), metabolomics (metabolites), microbiomics (microbial genomes), and others (20). The knowledge generated by these disciplines is being translated into different health tests for screening, diagnosis and prediction of disease risk, promising to empower doctors, patients and consumers alike. Furthermore, combining the analysis of these individual data with environmental exposures (exposomics) and social contexts has the potential for long-arching public health impact as health interventions can become more accurate, timely, fair and cost-effective (22,23).

In order to shape and accompany the fast pace of progress in this promising area of biomedicine, balanced and precise inputs from ethics and the law are necessary but mainly still lacking. Hence, studies on the ethical, legal and social implications (ELSI) of precision medicine and public health in general, and of OMICS health tests in particular, are key to increase public involvement and trust and lay the groundwork for future regulation. The ELSI of genetics have been extensively studied in the last decades (13,17). However, in order to fully understand the potential of genetics, genomics and precision medicine, while guaranteeing that citizen's rights are properly balanced with public interest, significant more work needs to be done in the future. Specifically, "classical" and emergent ELSI must be analysed, assessed, debated and transformed in ethical guidelines and biolaw. Relevant ELSI, include (and again, are not exclusive to): Informed consent-related issues (e.g. respecting autonomy, communication of risks/benefits); methods and

duties-related with return of test results and incidental findings; assessment of test analytical validity, clinical validity and utility; laboratory certification and quality control measures (including oversight bodies); advertisement practices and marketing regulation; privacy, confidentiality and data protection-related issues (data access, database security, professional secrecy, information sharing with third parties); biological material protection, sample destruction, ownership of research materials, ownership of research materials and industrial property rules (*see biobanks above*); expertise of professionals involved (including academic curricula update); test accessibility and affordability; insurance and health plan coverage; potential for discrimination of the tests results; health and genetic literacy levels, public debate and public awareness.

In addition, gene therapy and genome-editing technologies (CRISPR/Cas9, for example) have become recently available, which raises particular ELSI as well. Issues related with overall acceptability of genetic interventions (*which conditions? which life-stage? somatic-only or germ-line too?*) (24), equal and fair access to extremely expensive healthcare interventions and the potential for genetic discrimination, just to name a few. In order to provide fair and balanced ethical guidelines and/or legal rules that respond to these issues, disease mechanisms, individual circumstances of patients, potential benefits and expected harms of technologies must be taken into account, which demands higher efforts to bridge the long-withstanding chasm between international and national law on one side and science on the other. In conclusion, hype must be distinguished from hope, science must be promoted, strengthened and directed toward the common good, while respecting and fulfilling the human rights to health and to enjoy the benefits of science.

iii. Digital Health

The digital revolution, which has steadily reshaped different dimensions of modern life, has recently started to transform healthcare by proposing innovative solutions to both classic and emergent medical problems. Prominent examples of such proposals include the advent of telemedicine and electronic health records, the conception of wearable, implantable, injectable and even ingestible digital medical devices, the development of health mobile apps as well as the creation and application of artificial intelligence algorithms to multiple health settings (25). Correspondingly, the collection, storage, analysis, sharing, understanding and integration of large quantities of health data is becoming increasingly possible, opening the door to more timely and accurate predictions of health outcomes.

As is often the case with technological advancement, progress in digital health raises compelling ELSI, which require dedicated attention and scrutiny by clinicians, researchers, healthcare providers, managers, and other relevant health stakeholders alike. The most relevant ELSI of digital health include privacy, confidentiality and health data protection issues; the assessment and validation of digital health innovations and obstacles to a fair distribution of their risks, costs and benefits; increasing transparency in AI applications[2]; avoiding, detecting and correcting algorithm bias in healthcare settings; protecting and securing increasingly interconnected medical devices; promoting health and digital literacy toward professional and patient empowerment; and, more broadly, the risk of dehumanisation of care, the moral status and ethical judgment of machines and, ultimately, the future contours of human nature and social interaction in medicine and healthcare.

In summary, digital health has laid down the promise to revolutionize healthcare by transforming the way health and disease are perceived, managed and dealt with in the future. Proportionally, the resulting ethical, legal and social challenges are broad and multifactorial. Contributing factors include deeper social inequality, a lack of common platforms and cross-disciplinary languages to deal with increasing technical complexity, and, ultimately, the contrast between the vertiginous pace of scientific progress and the timed responses provided by law and ethics. Overall, as the ELSI of digital health accumulate, grow in intricacy and elicit wider social ramifications, their critical debate assumes paramount importance (26,27).

III. RELEVANT CONTRIBUTIONS FROM NOVA UNIVERSITY OF LISBON

A) Teaching

NOVA University has a long-standing tradition in teaching Health Law and Ethics in Portugal. For example, it was the first Portuguese higher education institution to integrate a Health Law course (15–hour course taught at the National School of Public Health (ENSP-NOVA) as part of the Health Administration Specialization

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[2] See *e.g.*: European framework on ethical aspects of artificial intelligence, robotics and related technologies

https://www.europarl.europa.eu/RegData/etudes/STUD/2020/654179/EPRS_STU(2020)654179_EN.pdf

EU guidelines on ethics in artificial intelligence: Context and implementation https://www.europarl.europa.eu/RegData/etudes/BRIE/2019/640163/EPRS_BRI(2019)640163_EN.pdf

Course). Later, the course "Health Law and Bioethics" was introduced in NOVA School of Law in 1997, as a course that aimed to provide a broad and transdisciplinary overview of health law in both international and national Law, as well as of the emerging Bioethics issues in Medical and Life Sciences. It also included the main ethical theories to provide students the required knowledge to identify and solve practical dilemmas in healthcare. It was first taught by Professor Paula Lobato de Faria (until 2002) and afterwards, by Professor Helena Pereira de Melo (since 2006 until the present date) (28).

Nova School of Law was the first Portuguese Law Faculty to offer in its Law degree a specific Health Law and Bioethics course where the bioethical issues and human rights problems of the recent Biomedical progress were addressed. The course has been updated and is now taught at a master level, in the Master in Law – specialization in Law and Technology, under the curriculum name "Life Sciences Law". Nova School of Law is also a founding member of ABIO – Association for the Biolaw Study, founded on 2016, within the consortium University's Tagus Tank (Tagus Academic Network for Knowledge), with the purpose of studying the legal issues that arise from the application of Biomedical and Life Sciences' progress. Professor Helena Pereira de Melo has been part of ABIO's board of direction since its foundation.

In parallel, there is also a long-established tradition and experience in teaching Health Law, Deontology and Bioethics courses at NOVA Medical School. For example, the course of Medical Deontology (for Medical students, taught since the School's origins until 1998); Deontology, Bioethics and Medical Law (Medical Degree, from 1998 until 2014); Ethics, Deontology and Medical Law (as part of the *Medicine and Society* course, from 2013 to the present date); Ethics and Biomedicine and Ethics and Health Care (since 2013/2014 to the present date); Bioethics (MSc-level 2002-2005); and Introduction to Medical Ethics / Research Ethics (PhD-level 2009-2020). Currently Bioethics and Research Ethics courses are taught in several MSc and PhD courses at the NOVA Medical School.

Identically, Health Law and Ethics-related disciplines are taught widely at NOVA University of Lisbon within ENSP-NOVA, in Portuguese and English, mostly to PhD, MSc and other post-graduate students (from very different scientific and professional backgrounds, including medicine, nursing, physiotherapy, nutrition, management, economics, law, engineering, sociology, among others). These curricular units include: Biomedical Law (until 2013); Health Law; Research Ethics; Bioethics and Health Management; Personalised Medicine and Digital Health; Health Law and Ethics; and Public Health Law and Ethics.

Notably, Bioethics and Health Research Ethics are also part of the Research Ethics Course of NOVA Doctoral School[3], which was the first course taught in this flagship project, in 2013.

We apply a multidisciplinary perspective (combining the life sciences, health and biomedicine, with law and ethics in health and research) and value the collaboration within NOVA University *(e.g.*"*Research Ethics in Public Health*" – *International Global Public Health Doctoral Programme, NOVA National School of Public Health/ NOVA Institute of Hygiene and Tropical Medicine/ NOVA Medical School, and the University of Porto (Institute of Public Health/Faculty of Medicine);* "*Innovation, Change Management and the New Healthcare Client*" *and* "*Data Privacy, Security and Ethics*" – *NOVAIMS Post-Graduate Courses; and* "*Biobanks and Biological Samples Management*" – *NOVA Medical School/University of Aveiro),* and in collaboration with other national and international academic institutions *(e.g. Erasmus Mundus Joint Doctoral Program on Dynamics of Health and Welfare – Evora University/ L'École des Hautes Études en Sciences sociales EHESS (Siège) and Linköping University; PhD in Health Sciences – Faculty of Medicine, University of Coimbra, PhD in Sport Sciences and Physical Activity in Health – University Institute of Maia – ISMAI; PhD Programme Molecular Medicine Institute (iMM)/Faculty of Medicine, University of Lisbon/Instituut Pasteur; Msc Courses – Erasmus University Rotterdam; Law School, University of Salerno)*. In particular, ENSP-NOVA established a long-lasting collaboration with the Center for Health Law, Ethics & Human Rights of the Boston University School of Public Health to advance the academic collaboration on the issues of Health Law, Bioethics and Human Rights, which resulted in the organisation of four International Seminars, the set-up of a Public Health Regulation Analysis Center (LEXBIOLAB project) and the publication of several books on related topics (*see below*).

B) Research

Past and ongoing research at NOVA University of Lisbon in the Health Law, Ethics and Bioethics areas has been developed as a combination of two main characteristics: trans-disciplinarity and internationalization. By bringing together different disciplines (life and health sciences, biomedicine, law and ethics) and

[3] For more detail, see: https://www.unl.pt/en/study/doctoral-school/nova-doctoral-school and https://www.unl.pt/en/courses/study/escola-doutoral/research-ethics-course-1, last access April 14th, 2021

different international points of view, research in these areas has contributed to build a strong, diverse, tolerant, inclusive and forward thinking University.

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Importantly, research in the aforementioned areas at NOVA University of Lisbon is and has been the result of work developed at different **multidisciplinary research centers**, namely the Comprehensive Health Research Centre (CHRC)[4], the Public Health Research Centre[5], the Interdisciplinary Social Sciences Centre (CICS.NOVA)[6] and the CEDIS – Law & Society Research Centre.

As aforementioned, past and ongoing research includes different **international collaborators**, e.g.: Boston University School of Public Health, Erasmus

[4] For more details see: https://novaresearch.unl.pt/en/organisations/comprehensive-health-research-centre-chrc-p%C3%B3lo-nms and https://novaresearch.unl.pt/en/organisations/comprehensive-health-research-centre-chrc-p%C3%B3lo-ensp, last access April 14th, 2021

[5] http://www.cisp.ensp.unl.pt/#, last access April 14th, 2021

[6] https://www.cics.nova.fcsh.unl.pt/?lg=uk, last access April 14th, 2021

University Rotterdam; Salerno University; Fluminense Federal University (Niterói), University of Cantabria,

Finally, research done at NOVA University of Lisbon in the area of Health Law and Ethics and Bioethics has resulted in multiple PhD and MSc thesis on different areas, including biobanks ELSI; privacy, confidentiality and health data protection; patient safety; advanced directives and living will; cross-border healthcare; mental health and human rights; direct-to-consumer genetic tests; digital health ELSI; adoption of AI in healthcare.

C) Ethical Review of Healthcare Research

Ethics in health and health research (in theory and in practice) is ingrained in NOVA University's philosophy and action as demonstrated by the aforementioned expertise in teaching and research activities. For this reason, the University has set up different competent bodies to address the ethical challenges facing the academic community in general and health research issues in particular. In addition to the Ethics Council (a University-wide ethical body, with broader competences[7]), ethical review boards tasked with reviewing and evaluating health research activities from an ethics standpoint exist in different NOVA Schools[8]. Particularly, the recently-created Ethics Committee of the National School of Public Health, the accomplished Ethics Committee of the Institute of Hygiene and Tropical Medicine and the Ethics Committee of the NOVA Medical School, which deserves a more detailed analysis, due to its relevance and long-standing tradition.

The particular case of the Ethics Committee of the Faculdade de Ciências Médicas / NOVA Medical School (CEFCM) – 15 years in defense of ethical standards

The Institutional Review Board of the Faculdade de Ciências Médicas / NOVA Medical School of the NOVA University of Lisbon (CEFCM – Comissão de Ética da Faculdade de Ciências Médicas) was created in early 2006, by proposal of the late Professor José António Esperança Pina, former Dean of the School and Rector of

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[7] For more information, please see: https://www.unl.pt/en/nova/ethics-council, last access April 14th, 2021

[8] *e.g.:* http://www.nms.unl.pt/main/index.php?option=com_content&view=article&id=1884&Itemid=752&lang=pt

https://www.ensp.unl.pt/escola/comissao-de-etica/ and https://www.ihmt.unl.pt/organizacao/conselho-de-etica/

the University, to face the needs for independent review and monitoring of biomedical research involving human subjects and non-human animals undertaken by its individual researchers or research groups.

The CEFCM was thus one the first academic Institutional Review Boards to be created in the NOVA University of Lisbon and in Higher Education Institutions in Portugal. For this reason, the CEFCM soon accepted to review research projects from other NOVA University Schools, who did not hold an Institutional Review Board (IRB) of their own, namely the FCT-UNL (Faculdade de Ciências e Tecnologia), the ITQB-UNL (Instituto de Tecnologia Química e Biológica) and the ENSP-UNL (Escola Nacional de Saúde Pública).

The membership of the CEFCM has, since its onset, fostered equality and diversity, assuring, amongst its members, the essential expertise in different basic and clinical biomedical specialties and in non-human animal research and welfare, as well as including a non-affiliated lay member representing the society. All members act entirely *pro bono*.

Most of the projects submitted to the CEFCM are reviewed using a 'primary reviewer' procedure: one of the members is chosen as rapporteur to review the project considering his/her expertise. Each project is then presented by the primary reviewer at the formal CEFCM meeting, and subsequently discussed by all the members present, which receive the full set of each project's documents for consultation prior to the meeting. At the end of the discussion, a decision is taken to either send the PI a letter of approval, a letter of opinion requesting further clarification(s) or information, a request for resubmission or a letter of disapproval.

On very rare occasions, when a research project does not involve more than a minimal risk and the reviewal process is urgent, an 'expedited review' is considered, the reviewing process being carried out by the Chairman of the Review Board, alone or assisted by one or more members. A decision on the project is taken out of a formal meeting of the CEFCM. If a disapproval is considered, then the decision will have to be taken in a formal meeting of the IRB.

With the development of numerous MSc and PhD programs in the Faculdade de Ciências Médicas / NOVA Medical School, the CEFCM has extensively broaden its scope of action reviewing and monitoring research projects aimed at obtaining an academic degree.

Our institution, and rightly so, requires the approval by CEFCM of all master's or doctoral research projects as an essential requirement for the defense of the respective theses. In particular, the CEFCM has faced the challenge of reviewing

research projects (namely those aimed at obtaining an academic degree), which are submitted after initiation or after completion. The CEFCM decided that these projects, although not eligible for formal approval, are worthy of an ethical compliance assessment regarding study design and conduction. This innovative procedure allows master's and doctoral students to defend their theses and ensure the publication of their results, while the CEFCM safeguards the ethical framework of the process.

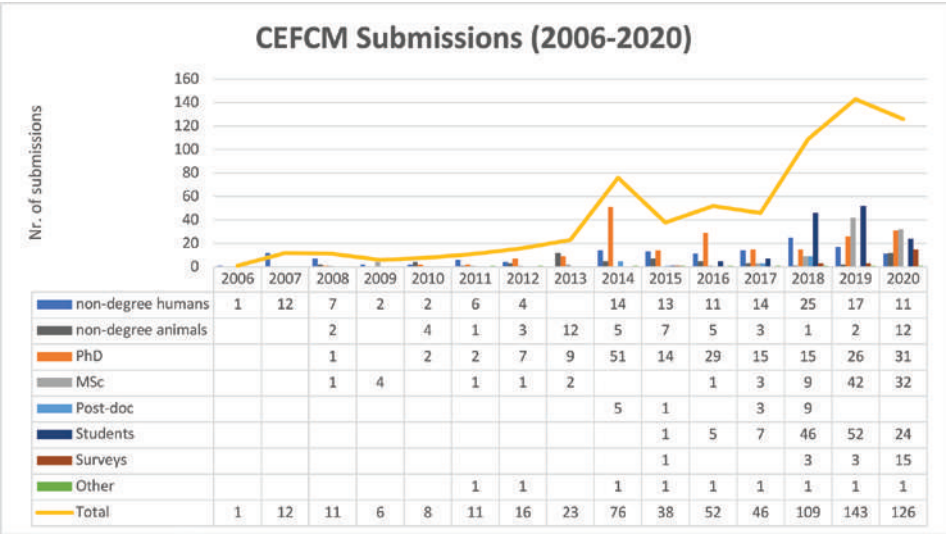
In more recent years, students have become more involved in research, in most of the cases within the assessment process of a given course's curricular unit. Along with these projects, many surveys with the participation of students and/or teaching and non-teaching staff from the medical school have been ethically reviewed by the CEFCM.

The review of research projects with non-human animals has undergone important changes imposed by the publication of Decree-Law 113/2013 of 7 August which aims to protect animals used for scientific purposes. Thus, an institutional flowchart has been created for the approval of projects with non-human animals that requires, in addition to the authorization of the competent authority, the obligation of the opinion of the vivarium and the Animal Welfare Body (ORBEA) of the NOVA Medical School, before formal review and eventual final approval by the CEFCM.

Notably, one of the challenges faced by Ethics Review Committees in Portugal is mutual recognition of decisions, especially in multicenter research projects. It is a long time wish of investigators involved in multicenter research projects to ideally submit the project to a single ethics committee, while the others tacitly validate its decision. Although this objective has not yet been achieved, as of today, several initiatives have been undertaken, to encourage cooperation between Portuguese Ethics Research Committees, which review clinical research projects. One of these meritorious initiatives was the call made in June of 2012 by the National Council of Ethics for the Life Sciences (CNECV) for a joint meeting of all Portuguese Ethics Research Committees, including those already existing in Higher Education Institutions. This meeting resulted in the creation of an informal working group which led to the establishment of the 'Redética Association' in March 2017, which aimed to promote cooperation between the Portuguese Institutional Ethics Committees and stimulate the study and discussion of topics of Ethics and Bioethics. CEFCM was actively involved in the work of this group, namely the one that led to the proposal for a revision of Decree-Law 97/95, of 10 May, which regulated the Ethics Committees for Health in the country. This revision contributed decisively to the publication of the Decree-Law 80/2018 of 10 October, which replaced the previous

legal framework encompassing the Academic Institutional Review Boards existing in Higher Education Institutions. Legal recognition of the Academic Institutional Review Boards was then finally achieved.

The following Figure summarizes the activity of the CEFCM[9] during its first 15 years of existence.



	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020
non-degree humans	1	12	7	2	2	6	4		14	13	11	14	25	17	11
non-degree animals			2		4	1	3	12	5	7	5	3	1	2	12
PhD			1		2	2	7	9	51	14	29	15	15	26	31
MSc			1	4		1	1	2			1	3	9	42	32
Post-doc									5	1		3	9		
Students										1	5	7	46	52	24
Surveys										1			3	3	15
Other						1	1		1	1	1	1	1	1	1
Total	1	12	11	6	8	11	16	23	76	38	52	46	109	143	126

IV. IMPACTS TO SCIENCE AND SOCIETY AND CONCLUDING REMARKS

The right to the enjoyment of the highest attainable standard of physical and mental health is a human right facing significant challenges. In order to fulfill this right, awareness, support and investment in health promotion, healthcare access, quality, safety and innovation are paramount.

While trying to deal with the most intricate issues that confront our societies, the best practices often result from the tension between different and sometimes conflicting perspectives. Bridges can be built, alliances can be forged, inclusion can be promoted, collaboration and integration can be fostered.

[9] The Article 26 of the Statutes of the Faculdade de Ciências Médicas / NOVA Medical School (Universidade Nova de Lisboa – Reitoria – Despacho 8032/2018 de 2018-08-17) pertains to the Institutional Review Board (CEFCM).

Universities play a fundamental role in this endeavor by promoting the respect for persons and the environment, while fostering meaningful and sustainable societal outreach. Accordingly, teaching and researching activities conducted with full intellectual freedom can be directed towards real societal needs and aspirations. This is particularly relevant in the case of academic work in the Health area and NOVA University is no exception.

In addition to teaching and research activities, different Health Law, Deontology and Ethics, Biolaw and Bioethics projects and initiatives conducted at NOVA University epitomize this spirit, be it through the monitoring of citizen's knowledge about their health-related rights, the analysis of public health regulations and recommendations for their correction and update, the information about the role of law and ethics and their applications in healthcare (in historical, present and future terms) to lay audiences, or the support of political decision making in the health sector. All these activities are, in fact, closely aligned with the mission of NOVA University:

> "*The NOVA University of Lisbon, as a public higher education institution, has the mission of serving society at local, regional and global levels, by advancing and disseminating knowledge and understanding between cultures, societies and people, through education and training, research of excellence and the provision of services sustained in a strong sense of community and with the following components: (...)*
>
> ***Teaching with an international profile (...) focused on its students and endowing them with rigorous knowledge, creativity, a critical spirit and a sense of citizenship and justice that allows them professional success and leadership*** [...]
>
> ***Collaborative, responsible and internationally relevant research, favoring interdisciplinary areas and including research aimed at solving problems that affect the society*** [...]
>
> ***Service provision that promotes solidarity and sustainable development, in terms of health, economic, technological, cultural and social, based in the Lisbon region and committed at national and international level, paying particular attention to the Portuguese-speaking countries*** [...]
>
> ***A broad base of interinstitutional participation aimed at the integration of different scientific cultures, with a view to creating innovative synergies in all areas of its activity.***"
>
> art. 2nd, al. a) to d) of the Statutes of the NOVA University of Lisbon, (DR 2nd Series, Nº. 26 of 06-02-2020)

Law and ethics are fundamental normative orders in the Health area and their essential roles have become more evident in recent decades. For example, legislation (and respective regulation) in the Health area is a fundamental element for the formulation of policies, the implementation of intervention plans or the construction and execution of educational programs, with a view to influence individual behaviors and societies while seeking to promote lifestyles more consistent with health and well-being. To different degrees depending on the particular setting and challenge, Health Law and Ethics continue to be powerful instruments for creating, debating, clarifying and balancing rights and duties in healthcare, establishing principles and models of healthy behavior, resolving conflicts of interest between different stakeholders in the same health issue and establishing a standard of equity and justice with regard to the necessary balance between individual interests and rights and public and collective health and, not least, imposing limits and ensuring an equitable distribution of resources (and costs). Hopefully, the work done so far at NOVA University in this area inspires present and future generations of scholars, researchers, students and citizens in general to continue the progress achieved so far. Their work will be welcome to assist in the global task of fulfilling the fundamental human rights to health and to enjoy the benefits of scientific progress and its applications in a more sustainable, just and fair world.

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CLINICAL INVESTIGATION, INNOVATION AND ETHICS

Maria Alexandra Ribeiro

1. INTRODUCTION

Clinical investigation or clinical research can be defined in several different ways, but it refers to research with human beings or their data. According to the National Institutes for Health (NIH)[1], clinical research means any research carried out on humans either through direct interaction or through the collection and analysis of blood, tissues, or other samples focused on improving knowledge of diseases, developing diagnostic methods and new treatments or medical devices to ensure better patient care [1]. Biomedical research (or medical research) albeit related to health and prevention and treatment of diseases is much wider including the understanding of the underlying life process which affects disease and human well-being.

Clinical research includes several types of research such as clinical trials, behavioural, genetic or epidemiological studies among others, with clinical trials being the key instruments for advancing medical knowledge and for improving healthcare. Modern views on clinical and biomedical research entail joint efforts throughout international cooperation and innovation in order to create value and deliver schemes or products to improve people's health.

Tough regulations, ethical guidelines and laws are in place in order to protect the rights and the safety of research subjects but the current ethical framework of contemporary clinical research is facing new demands. The "digital age" has now a central role in clinical research. Electronics, computer sciences, the internet and other modern technologies are reshaping clinical research. Collection, analysis and wide sharing of personal data and electronic health data have become the "gold mine" of clinical research not only for clinical trials for new medicines but also, regarding research with genetic databases, "big" data in general and artificial intelligence systems or "machine learning". Clinical research with databases has huge potential of benefits for improving health and healthcare but it comes along with new ethical and regulatory challenges, and (un)predictable threats.

Not intending to make an exhaustive discussion or literature's review regarding clinical research, ethics and innovation, I will try to address from where the ethical framework on human research emerged and questioning its applicability to contemporary research on human's data focusing on the former examples of clinical research under the "digital age".

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[1] NIH was the first government's institution in United States responsible for biomedical and public health research continuing to be a reference in this area.

2. CLINICAL RESEARCH AND ETHICS

Human volunteers are key actors in clinical research, so protecting their life, health, dignity and welfare is an undeniable requirement. From the Kantian idea of respect for persons by treating them always as an end and never as a mean to achieve other purposes, do not comply with the ethical requirements of clinical research would be unethical and a violation of this basic principle, despite the recognized need for developing medical knowledge and new therapeutics.

History showed, however, that this has not always happened. The ethical framework in place today were primarily a response to past abuses, the most notorious in Germany in the Nazi war camps or other unethical studies that took place in the United States such as the Tuskegee or Syphilis studies, among others, as denounced by Henry Beecher in 1966 [2]. Notwithstanding, the existence of previous documents establishing ethical considerations on human experimentation requiring explicit consent from the participants, the recent medical research history was darkened by those clear violations and disrespect for human dignity. In fact, many research ethical guidelines were developed in the 20th century in response to such a period. Some of the best known are the Nuremberg code (1947), "Universal Declaration of Human Rights" (1948), Helsinki Declaration (first published in 1964) and the Belmont Report (1979). Several other ethical guidelines are in place today. The "International Ethical Guidelines for Health-related Research Involving Humans" from The Council for International Organizations of Medical Sciences CIOMS (2002; last version from 2016), the UNESCO "Declaration on the Human Genome and Human Rights" (1997) and the "Oviedo Convention"[2] (1979) and it's additional protocols "Prohibition of Cloning Human Beings" (2001) and "Biomedical Research" (2005), are just a few examples.

Having in mind the ultimate goal of clinical research in developing generalizable knowledge improving human health or increases understanding of human biology, researchers have the responsibility not only to adopt the highest ethical standards towards participants' protection, complying with the ethical

[2] Convention for the protection of Human Rights and Dignity of the Human Being with regard to the Application of Biology and Medicine: Convention on Human Rights and Biomedicine. This is the only international legally binding instrument on the protection of human rights in the biomedical field.

guidelines but also to guarantee the quality and the integrity of the data collected and reported. At the point, in which medical decision depends upon clinical research results, research integrity is of utmost importance. Not having a positive definition of research integrity nor a well-grounded and standardize orientation, "scientific integrity" definition remains wide open but understood as a part of the ethical framework of clinical research [3]. Several statements or declarations on research integrity have been proposed in the last decades [4,5,6,7,8,9], all of them establishing values and principles for responsible research, reinforcing the codes of conduct of researchers and institutions and the compliance with the practices accepted in the scientific community for carrying out scientific research.

The "Singapore Statement on Research Integrity" [4] from 2010, considering that the value and benefits of research are vitally dependent on the integrity of research, establishes four principles – honesty, accountability, professionalism, and stewardship-, and fourteen responsibilities for the ethical conduct of research, among others, towards data sharing, compliance with regulations and social responsibilities. Additionally to the "Singapore Statement", recognizing research typically as a cross-boundary collaboration, it was proposed in 2013, further responsibilities at the individual and institutional levels regarding collaborative research, creating the "Montreal Statement on Research Integrity in Cross-Boundary Research Collaborations" [5]. Responsible Conduct of Research had already been stated by the Office of Research Integrity (ORI) from the United States in 2007, in the document "Introduction to the Responsible Conduct of Research"[6]. The European Commission recognises "The European Code of Conduct for Research Integrity" from All European Academies (ALLEA) [7] as the reference document for research integrity for all EU-funded research projects and a model for organisations and researchers across Europe. In Portugal, the Foundation for Science and Technology published in 2015 its code of conduct [8] for providing a model for responsible research for researchers, institutions, universities and funding agencies. A more recent document on research integrity is the "Declaration on research integrity in responsible research and innovation" from the UNESCO Chairs in Bioethics' at the University of Barcelona and the Portuguese Catholic University [9].

Promoting reliable science, responsible research and respecting ethical principles must be a priority for all of those involved in research and particularly in clinical research. Scientific integrity, clinical research and ethics are a three-way relationship.

3. ETHICAL PRINCIPLES: FROM THE BELMONT REPORT TO CONTEMPORARY ETHICAL VIEWS

Understanding this three-way relationship – scientific integrity, clinical research and ethics- is time to focus on the ethical principles guiding clinical research, like those established in the "Belmont Report" and other ethical guidelines and discuss their adequacy regarding the challenges of the modern technological enterprise of clinical research.

In 1974, the United States created the "National Commission for the Protection of Human Subjects of Biomedical and Behavioural Research" to identify the basic ethical principles that should underlie the conduct of biomedical and behavioural research involving human subjects. This Commission was in charge of developing guidelines, which should be followed to ensure that such research was conducted following those principles. In 1976, the "Belmont Report" was published, identifying three basic Ethical Principles for the Protection of Human Subjects of Research: Respect for persons, Beneficence and Justice. In practice, these principles apply respectively to the requirement of obtaining informed consent from the participants, assessing risks and benefits of the research and finally proper and careful selection of research participants.

From the "Belmont Report", respect for persons encloses two moral requirements or ethical principles: the requirement to acknowledge their autonomy and the requirement to further protect those with diminished autonomy. The principle of beneficence requires doing no harm and maximizing possible benefits and minimizing possible harms, and the principle of justice establishes the moral requirements to implement fair procedures and outcomes in the selection of research subjects.

Despite the multiplicity of ethical guidelines on clinical research, a very coherent and systematically framework was proposed by Emmanuel and colleagues in 2000 [10] in which authors established seven principles for conducting ethical research with humans: social and clinical value; scientific validity; fair subject selection; favourable risk-benefit ratio; independent review; informed consent; and respect for potential and enrolled participants. These seven requirements create guidance for evaluating the ethics of clinical research studies from which the sequence of principles is necessary.

The classic ethical framework from "Belmont Report" and from those others principles emerged, thought from research settings in which clinical researcher or physician interacts directly with research subjects or patients, is far distant from

the today's clinical research landscape, and no longer provide sufficient guidance [11,12]. Present research using new technologies such as those applied to clinical trials, human genetic databases, "big" data research or artificial intelligence systems pose new ethical (and regulatory or legal) questioning, since the research conducted with large and diverse sets of online data require new practices for ethical and responsible research [11,12].

Albeit Belmont's principles remain valuable, research ethical governance tends to emphasize the primacy of informed consent [12,13,14] and the protection of the vulnerable [13]. Likewise, risk-benefit assessment deserves a new approach. Despite central, it puts great emphasis on informed consent as a condition for acceptable research risk, though consent, while important, does not always represent nor a sufficient nor a necessary condition for acceptable risk in research [14]. Independently of obtaining informed consent, it seems that some levels of risk could not be acceptable at all, or at least only in some particular research circumstances. Contrarily, considering the low level of risks, informed consent might give no additional protection to research participants, while hampering research [12,14]. Thus, the scientific or social value of the research is likely fundamental for ensuring the acceptability of exposing participants to net research risks [15]. Some current research policies towards studies designed for guiding medical practice, being focused on the protection of participants and their beneficence or preventing useful health-related data sharing might hamper some studies that could provide immediate benefit or evidence that could improve the medical treatment of patients, becoming ethically flawed [12,13,15].

Understanding that current ethical principles and regulations cannot cover every possible situation, particularly considering the advances in science and technology, there is no doubt that the contemporary clinical research innovativeness, evolving medical technology, genetic research, therapeutic interventions, and innovations challenge society to maintain the highest moral and ethical principles [16]. For this, rethinking clinical research ethics [12,13,14] or rethinking the ethical framework with new ethical guidelines [11,13,16,17,18] is a central issue towards clinical research innovation.

Another issue about rethinking the ethical framework for clinical research is the finding that research with humans outside clinical or biomedical research has increased a lot recently. Normally, these types of research even though involving humans are less regulated or not regulated at all. Nevertheless, some strategies or aims of these studies pose some risks to the participants and other poses large

risks. Albeit these studies should follow the ethical guidelines regarding respect for participants and their rights, usually they do not need ethical approval relying on the moral' standards of the researcher. Assuming no particular reason for leaving non-biomedical research projects outside the ethical review, The European Network of Research Ethics Committee (EUREC) published in March 2021 a position paper on this issue [19], recommending policymakers and research institutions to give more attention to this issue welcoming members from non-medical research ethics committees for support them in carrying out their tasks. Aligned with the recommendations for ethical review outside biomedical research, good clinical practices should also apply. Despite the guideline ICH-GCP(R2) [20] is normally strictly used for clinical trials, the guideline itself considers that the principles established may also be considered for other clinical investigations that may have an impact on the safety and well-being of human subjects. This was further developed by myself under the EUREC collaboration with the "SIENNA Project" establishing how this guideline could support research with humans outside the particular scope of clinical trials [21].

Concluding, it would be expected that research ethics governance involving human beings or their data, might progress through a condition where every type of clinical research undergoes ethical revision proportional to the nature of the study not hampering research nor innovation or unreasonably make this research impossible mission. This means a "tailored" ethical approach without losing two major goals: protect research subjects and their rights, and promote responsible ethical research governance.

4. RESPONSIBLE RESEARCH, INNOVATION, AND IMPACT ON SCIENCE AND SOCIETY

In this section, I will focus on present opportunities and challenges regarding clinical trials with medicines, and examples on clinical research innovation, human genetic databases, "big" data and artificial intelligence and their major ethical issues, and possible impacts on science and society.

Clinical trials

Clinical trials have been considered as the paramount of clinical research, through which new medicines or medical devices are studied to measure their safety and/or effectivity or adaptations of existing therapies for different patient

populations, different doses or other new circumstances, contributing to innovation and improving clinical practice.

Clinical trials are becoming increasingly complex, undergoing substantial evolution and innovation, with novel trial designs, complex protocols using the internet and mobile devices for patient's outcomes report and analysis. Collection, analysis and widespread sharing of electronic health and web-based services can be used for secondary purposes speeding up clinical trials for new medicines [22], being already in place guidelines for the industry [23].

The European Regulation (EU) 536/2014 of 16th April on clinical trials on medicinal products for human use, expected to enter in force in December 2021, will bring new opportunities for innovation and ethical challenges as I had already discussed elsewhere [24]. Compared with present legislation governing clinical trials with medicines, the Regulation brings two major changes with ethical impact. The first one is the establishment of a new category of clinical trials, based on risk, the "Low-intervention clinical trials" in which the investigational medicinal products are used following the marketing authorisation or its use is evidence-based and the trial poses no additional risk or burden to subjects compared to normal clinical practice. This new legal provision contributes to innovation, improving and simplifying procedures, mainly for non-commercial trials responding directly to the real needs of patients and physicians. The other change brought by the Regulation is the possible exclusion of the ethics committees from assessing the scientific aspects of the study, foreseeing a less effective protection of research's subjects [25,26,27]. This could hinder the goal of responsible research and encloses a setback on the well-established ethical framework for clinical trials, with no expected increased value for innovation.

Another innovativeness in clinical trials enterprise is their conduction outside the site facilities, the so-called "Decentralized Clinical Trials". In general, these clinical trials make use of the new technologies such as digital health and telemedicine, mobile technologies, internet websites or other technologies for remote collection of data or monitoring of patients in real-world settings, allowing for potential improvement of clinical trials, more participant-centred on their needs, and a more inclusive clinical research trade [28,29]. Also, the involvement of social media in the conduction of clinical trials such as for the recruitment of research's participants or dissemination of the results [30], for those no legal, ethical or good clinical practices provisions and consensus are in place, is a matter of concern. As reported in a non-interventional clinical trial, the decentralized setting showed to be operationally feasible and well accepted by patients with faster recruitment and

improved access to patients in the decentralized arm [31]. Nevertheless, no matter these decentralized clinical trials seems to be attractive and feasible, the use of these technologies is challenging the classic way of assessing and protecting the rights and well-being of the research's subjects. Issues like cybersecurity and data privacy protection laws will come across with ethical issues and new challenges, and Europe is starting with the first steps on decentralized clinical trials. On the 16th of March 2021, the Swedish Medical Products Agency announced the implementation of their pilot project "Patient-centric, decentralized and virtual clinical trials" for conducting clinical trials decentralized and virtually in Sweden [32].

Further, exemplifying how technology had been incorporated in clinical trials is the increased tendency for using electronic consent (e-consent). Informed consent documents have become very long, extremely complex and thus less informative losing the ethical value for guiding research with humans as the expression of subject's autonomy and voluntariness, becoming mostly a legal contract. Being a new and improved tool for helping subjects to understand the study, e-consent requires a responsible ethical approach when obtaining consent from the participants and for this, the innovativeness of e-consent is not exempt from ethical questioning.

Finally, the "digital age" is quite well patent in the "Cancer Core Europe" (https://www.cancercoreeurope.eu/), a research infrastructure that aims at improving cancer health in Europe through the establishment of a high-level shared platform, bringing together several research projects from preclinical to clinical research and clinical trials. This infrastructure allows collaborative efforts in cancer research bringing discoveries to benefit patients and deliver more personalized medicine [33,34]. This Platform supports several task forces from clinical trials, data sharing, genomics, ethics and legal activities among others and is connected to the consortium "Cancer Prevention Europe" [35], able to support and drive innovation linking healthcare systems together, and ensuring alignment of policies towards prevention and translational research.

Genetic databases

Human genetic research, crucial for the diagnostic and the treatment of genetic diseases, includes several areas such as genetic testing, gene therapy, genetic databanks or genomics. The new ethical considerations on genetic research are to find out whether the classic ethical framework, constitutes a proper approach or if new or additional principles are required, mainly when considering genetic research involving biobanks or databases. There is no consensual agreement

regarding this issue as discussed by Bernice [36]. Anyhow, the author proposed that the classical ethical framework is sufficient to guide human genetic databases' research, considering that there is no need for a new ethical framework and it should not be replaced, refuting new ethical guidance based on community values such as solidarity, altruism and familiar mutuality, because those lack sufficient justification. According to the author, the most controversial issues are related to consent, confidentiality and access to the genetic databank, feedback to study participants and benefit-sharing, which require further discussion or resolution for concrete and practical guidance to adapt the global framework to the needs of local research and healthcare institutions. Nevertheless, genetic research from databases or biobanks poses different and undetermined dimensions of risk related to privacy of the information, which depends on particular circumstances such as being a rare disease in a small community, on mechanisms to secure confidentiality or on possible discrimination [36].

Further, genetic research gains additional complexity regarding the rapidly evolving genomic testing and additional controversial questionings regarding consent and data management, and its novel ethical and confidentiality constraints which require new approaches for the process of informed consent and sharing genomic and health-related data [15,37] or genetic data. Despite common agreement on the requirement for getting some form of consent for allowing research involving these databases, the form of consent goes through the more conservative approach requiring new consent to each new project to those more focused on "broad consent" taking into account the not yet defined and future projects. Between these two extremes, several intermediate proposals are in place as discussed by Bernice [38] and others.

The controversial issue on returning of information from genetic databases' research to study participants is related to the right to know or not to know which is a complex issue taking into consideration that genetic information and the results of genetic research do not only concern the subjects but also all the genetically related members of their family. Finally, benefit-sharing involves controversial understandings and practical difficulties. Despite repeatedly used in guidelines and regulations for genetics' research, this was tentatively first defined by Schroeder in 2006 [39]:

> "benefit-sharing is considered as the action of giving a portion of advantages/profits derived from the use of human genetic resources to the resource providers to achieve justice in exchange, with a particular emphasis on

> the clear provision of benefits to those who may lack reasonable access to resulting healthcare products and services without providing unethical inducements".

When talking about research on genetic databases and benefit-sharing we are no longer discussing the narrowed research inside an institution, a region or a country. Now we are talking about sharing and (secure) authorize access to banks of genetic data and other data relevant for health around the world [37,40,41]. In fact, "The European Declaration of Cooperation Towards access to at least 1 million sequenced genomes in the European Union by 2022" [40], signed by Portugal, for delivering cross-border access to their genomic information will improve understanding and prevention of disease, allowing for more personalised treatments and targeted drug prescription, in particular for rare diseases, cancer and brain-related diseases. The same goal has been established by the "Global Alliance for Genomics and Health (GA4GH)" in 2013 (https://www.ga4gh.org/), for responsible and effective sharing of genomic and clinical data in a way that is as simple as using the "worldwide web". The ethical discussion on consent, confidentiality and data management is now under cross border considerations and scrutiny; the ethics of genetic or genomic data sharing no longer relies (exclusively) on local regulations.

With these lines, I point out some ethical concerns regarding research with genetic databases. But, emerging technologies had open unlimited opportunities for genetic research, for intervening in the genome and changing genetic and epigenetic features in a tailored manner, raising more profound ethical discussion on what might be good or (un)acceptable [42,43]. Although impossible to discuss here it could not be disregarded when talking about clinical research, ethics and innovation.

Big data

From the European Commission recent policy definition:

> "Big data refers to large amounts of data produced very quickly by a high number of diverse sources. Data can either be created by people or generated by machines (…) [and] it covers many sectors, from healthcare to transport and energy." [44]

In the context of clinical research, big data comes from health records or other biomedical sources such as clinical trials, genetic databases, through methods such

as DNA sequencing or other research with humans, mobile apps or online sharing platforms. The use of data from health records and their retrospective analysis for research healthcare purposes is not new. What is new is the possibilities for wide sharing, large-scale use and mining of personal data for clinical or biomedical research [41,45,46,47,48,49,50] and public health [41,51].

The use of electronic health data and "big" data in research have social, ethical and regulatory challenges or even some obstacles [12,46,47,48,49,50,51,52]. Despite the undeniable value and benefits of this large–scale use of data in clinical or biomedical research, there are some challenges mainly due to privacy issues. Balancing the need to make available these data sources to achieve their full potential for research while respecting privacy as the basic human right of limited access by others to aspects of their person is a challenging matter. In fact, behind the ethical considerations of using personal data there are also legal issues regarding data ownership, privacy and consent to be addressed to find the right balance between public demands for autonomy and privacy, and find feasible ways for researchers to get access and use data for research [38,48,52].

Informed consent and risk-based assessment as the classical ethical approaches regarding clinical research with personal (health) data are generally inadequate regarding "big" data research [47,52,53]. Solutions such as de-identification of data or use of anonymized health records might difficult or hinder "big" data research [12,50], while keeping the risk of re-identification [49,50], especially regarding data on human DNA. The general public concern about privacy breaches is real. In fact, the classic ethical and regulatory approach seems to not properly address "big" data research opportunities and challenges, requiring a new ethical framework as proposed by several authors [49,51,52] having into consideration not only current and already known difficulties but having in mind future research possibilities [47,50]. Principles such as data minimization and purpose specification, to ensure a minimum amount of information to be collected from human subjects or health registries and data use restricted to those authorized by the subjects [47,52] must be endorsed. Moreover, minimal administrative requirements with no access to electronic health registries by unauthorized users together with new computing safeguards like research access only to aggregate data and not to individually identifiable data [50] will determine better security. These provisions would allow for simplified research processes [50,52] while keeping the ethical safeguards driving for better public awareness of the enormous value of "big" data research. The increasing involvement of patients in their care decisions together with their openness for

sharing their data for research [50] would fully explore the potential of "big" data clinical research, for better healthcare knowledge and personalized medicine.

Privacy and security, trust and accountability are key elements for ethical "big" data use and research complying with individual protection and rights and social benefits.

Artificial Intelligence (AI)

As proposed within the European Commission's Communication on AI[3]:

> "Artificial intelligence (AI) refers to systems that display intelligent behaviour by analysing their environment and taking actions – with some degree of autonomy – to achieve specific goals. AI-based systems can be purely software-based, acting in the virtual world (...) or AI can be embedded in hardware devices (...)." [54]

The big potential and promises for AI systems are that they can analyse data automatically to extract patterns and relevant knowledge, and thus "make decisions". Using "big" data, AI poses growing ethical considerations regarding privacy and security of information, data management, access, and usability [46]. But more disturbing is that AI algorithms will make important decisions in the field of medicine proposing a solution in such an efficient and quick manner that no human being would ever be able to do. AI in healthcare promises to be faster, more accurate, personalized and more cost-effective.

Who will be responsible when the answers will be given automatically without human's involvement? How much can we trust in AI systems or machines? These are just some questioning when we are facing AI as a reality in clinical research and clinical practice.

In fact, several applications in medicine such as the use of AI systems for screening, prognostic, decision support or even treatment recommendation are in place in several areas from ophthalmology to oncology or mental illness.

Medical ethical considerations on AI had been reported [55,56,57] and AI systems and "machine learning" (ML) are becoming increasingly more relevant in

[3] Communication from the Commission to the European Parliament, the European Council, the Council, the European Economic and Social Committee and the Committee of the Regions on Artificial Intelligence for Europe, Brussels, 25.4.2018 COM(2018) 237 final.

clinical research and medical practice [18,57,58,59], in a way that the integration of AI in healthcare is already being made through clinical trials. Outside clinical trials, McCradden and colleagues [58] proposed a three-stage process for the evaluation and validation of ML models for clinical care in order to be subject to ethics review and oversight. Through this model, authors aim to enhance the scientific reproducibility of healthcare AI research and ML tools to maintain a high standard of empirical validation and ethical review to protect the rights and interests of patients. Clinical trials using AI have been initiated only in the past two years [18] but at the end of March of 2021 the clinical trials registry (clinicaltrials.gov) showed 341 clinical trials (from which 139 were interventional studies) when searching for "artificial intelligence" as the "condition"; some of them already completed. Despite this, guidelines for protocols and for reporting clinical trials for interventions involving AI are quite recent [60,61] and will need continual adaptation since AI is a rapidly emerging area.

New ethical guiding principles are essential for performing and reporting clinical trials of medical AI in a transparent way and without causing (any) harm [18] and for guarantying that AI systems or devices are effective in the research study, but most of all that they are safe, effective, equitable, and reliable in clinical practice, thus respecting medical ethics [56,62].

Besides ethical questioning of AI applications such as responsibility and transparency, several ethical principles regarding AI have been published and AI initiatives announced in the last years [63], including the document on "Trustworthy of AI system from the High-Level Expert Group" on AI from European Commission [64], mentioning that even with good intentions, AI systems can cause unintentional harm. In this document, lawful, ethical and robustness are the three components to be met throughout the AI system's entire life cycle, establishing that the trust of AI relies on the compliance with applicable laws and regulations and the adherence to ethical principles and values and that AI systems need be robust, both from a technical and social perspective. The four ethical principles considered to be the foundation of trustworthiness of AI are respect for human autonomy, prevention of harm, fairness and explicability. Another pragmatic approach to AI's trustworthy considers other principles such as transparency regarding the operations visible to the user; credibility, meaning acceptable outcomes; auditability for efficiency to be easily measured; reliability in controlling AI systems performance as intended; and recoverability, which mean that manual control can be assumed if required [55].

Whatsoever a more specific ethical framework, we can assume general principles based on trust, transparency and responsibility for doing no harm governing AI regarding clinical research. More insightful ethical, social or philosophical reflections are in place for today and the future about AI possibilities [59,63,65,66], nonetheless impossible to further discuss.

Arriving here it is clear that new technologies changed the face of clinical research. Bringing new opportunities and appealing new methods or approaches, emerging technologies also raises ethical, social, legal and political concerns regarding "an open horizon" for clinical research.

Responsible research and innovation mean to contribute to developing new therapeutic alternatives and greater access to innovative medicines fulfilling the most modern vision of personalized medicine and the therapeutic approach, for better care of patients in general, and each patient in particular. The expected benefits from clinical research innovation nor the trust of the society in the science and scientists could be threatening for a loose research ethical compliance. Facing the new challenges, health research regulation should move from strict and prescriptive rules towards more flexible principle-based regimes also ethically sound, involving researchers, regulators and the public to managing risks (and benefits) and defining regulatory objectives [12].

Bringing together ethics, innovation and responsible research, facing the challenges and taking the opportunities, clinical research will positively affect science and society.

5. CONCLUSION

Clinical research, innovation and ethics go together. There is no innovation for clinical practice without clinical research, as there is no clinical research without persons or their data. Protecting participants and respecting their rights means complying with ethical requirements, good clinical practices and research integrity. The ethical framework for clinical research built towards a confined research in which researcher and subjects interact directly or researcher uses a small amount of data, has changed tremendously with the emerging technologies, which bring new opportunities and benefits but undoubtedly social and ethical questioning.

Innovative clinical research highly supported on "digital age" technologies – from clinical trials to genetic databases, "big" data research or artificial intelligence

– address common ethical concerns regarding not only the use of personal and sensitive data but also, most of all the large data availability and data sharing. Between restrictions and expectations, the most demanding issue regarding the contemporary enterprise of clinical research is keeping high ethical standards and the paramount consideration of protecting the rights and welfare of research subjects not hampering scientific and clinical research and therefore innovation.

Considering the growing trend towards the globalization of clinical research, an internationally recognized common governance towards the ethical and scientific integrity of global clinical research will better protect research subjects wherever they are while maintaining respect for human rights and global sharing benefits and risks. Meanwhile, as technology evolved faster than the regulations and the ethical challenges and dilemmas accompanied clinical research, precautious and responsible conduct of each researcher becomes leading guidance for guaranteeing respect for human dignity and keep our intrinsical nature of human beings.

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F

THE OTHER FACE OF MEDICINE: EHEALTH IN THE EUROPEAN DIMENSION

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The World Health Organization (WHO) defines eHealth as: "the cost-effective and secure use of information and communication technologies in support of the health and health-related fields including healthcare, health surveillance and health education, knowledge and research" [1]. From the European Commission (EC) point of view, eHealth can be defined as "tools and services that use information and communication technologies (ICTs) to improve prevention, diagnosis, treatment, monitoring and management of health-related issues and to monitor and manage lifestyle-habits that impact health" [2]. Its scope is extremely wide and encompasses a wide variety of sub-domains of digital health such as Electronic Health Records (EHR), Electronic Medical Records (EMR), Telehealth and Telemedicine, Health IT systems, Consumer health IT data, Virtual healthcare, Mobile Health (mHealth), Big data systems and Artificial Intelligence used in digital health.

Nowadays, countries around the world are shifting their health systems developing the co-creating concept with both citizens (including patients) and health workers, taking advantage of the immense opportunities posed by data and digital technologies centered on the people rather on the systems [3]. This is providing the visionary shift from the well-established system-centric implementations to the prescient human-centric approach.

With a considerable slowdown in population growth, an acceleration in population ageing and an increase on citizens mobility in their professional, leisure and on holidays, Europe constitutes a multidimensional challenge for eHealth that has only became more evident with the outbreak of COVID-19 pandemic [4]. The recent outbreak of COVID-19 exposed some difficulties of the European health services, especially Intensive Care Units.

The EU 75.000 intensive care beds serve a population of 441 Million citizens, but its highly heterogeneous distribution between countries (e.g. Germany 29.2 and Portugal 4.2 beds per 100 000 inhabitants) and the lack of staff and/or support of the necessary means for swift cooperation and knowledge sharing creates serious constraints in several EU countries. In fact, Europe faces an urgent, unmet need in increased ICU capacities highly trained medical specialists and specialized nurses due to the demographic challenge of an ageing population and emerging global health crises, reinforced by globalization and travel patterns. This need becomes even more obvious when all nursing measures require the presence of at least two members of staff next to the patients which becomes particulary dangerous when caring for infectious patients.

Also, and although it is still early to have a complete evaluation, it is also likely that European ageing population was a factor on the high prevalence of COVID-19 crisis. The rapid increases in the elderly population are predicted for the coming decades, due to the ageing of post-war baby boomers. Eurostat projects that the ratio of people aged 65 and over relative to those of working age (15-64 years), namely the old-age dependency ratio, is projected to increase from 28.8% to 51.6% between 2015 and 2060 in the EU-28's populations [5]. In parallel, due to the slightly declining proportion of children, the total age dependency ratio, is projected to rise from 53.2% in 2016 to 79.7% in 2080 [6]. The progressive decline in physical and cognitive skills prevents elderly people from living independently and from performing basic instrumental activities of daily living. These trends are putting significant pressure on age-related public expenditure in the EU, which is estimated that, by 2060, will reach 12.9% of gross domestic product DP) for pensions, 8.3% of GDP for health care and up to 3.4% of GDP for long-term care [7].

Indeed, more and more frequently Europeans are living in one European country, working in another European country and spending their leisure time and holiday in various (European) countries. While citizens are mobile within Europe, their paper and electronic health records are stored stationary at non interoperable general practitioners and in hospitals even in their home countries. This situation results in a lack of appropriate access to medical background information which can be life-threatening in case of acute illness.

In a nutshell: EU is facing a serious shortage of health workers while its healthcare needs are constantly raising. This situation can only be tackled with an appropriate health strategy that recognizes digitalization as part of the solution. The potential of eHealth allows to empower citizens and healthcare providers with tools to manage and to collaborate via shared decision-making that result in better patient outcomes and experience.

Citizens become central for the medical decision as active participants in the data collection process, observing how their health fluctuates over time, and understanding how certain actions and behaviors may influence their health outcomes. Also, medical doctors are enabled to intervene sooner and make changes to a citizen's treatment protocol or instruct the citizen to visit the medical doctor and/or the hospital. In critical situations such as COVID-19 pandemic, eHealth is fundamental to promote knowledge sharing, and contribute for public health preparedness and response.

In its agenda for the creation of the European Union Digital Single Market (EU-DSM), the EC identifies Health as one of the main sectors benefiting from the digital transition, given the potential benefits that digital services have to offer citizens and workers in this area. Numbers show that digital health could save €99 billion in healthcare costs to the EU GDP and enable 11.2 million people with chronic conditions and 6.9 million people at risk of developing chronic conditions to extend their professional lives and improve productivity. [9] This would add €93 billion to the EU GDP in addition.

To foster eHealth adoption and development, the Commission's Communication on the Transformation of Digital Health and Care [8] identifies 3 pillars around activities should be based:

1. Secure data access and sharing: To facilitate greater access to cross-border healthcare, the Commission is building the eHealth Digital Service Infrastructure to allow e-prescriptions and patient summaries to be exchanged between healthcare providers. The first cross-border exchanges started in 2019, with the goal of having all the other EU countries on board by 2025. In the longer term, the Commission is working towards establishing a European electronic health record exchange format that is accessible to all EU citizens.
2. Connecting and sharing health data for research, faster diagnosis and improved health: The second pillar of the 2018 Communication intends to tap into the huge potential of health data to support medical research with the aim of improving prevention, diagnosis, treatments, drugs and medical devices.
3. Strengthening citizen empowerment and individual care through digital services: Digital services can empower citizens, making it easier for them to take a greater role in the management of their own health from following prevention guidelines and being motivated to lead healthier lifestyles, to managing chronic conditions and providing feedback to healthcare providers. Health systems will also benefit from innovative care models that use telehealth and mHealth to address the rising demand for healthcare, helping to shift progressively towards integrated and personalized care systems.

Following this strategy, EU has been implementing a set of supporting mechanisms to research and development on eHealth in the recent years. Much work is being developed to use technology for preventing, slowing the development of, or dealing effectively with effects of the health impairments that can have a significant

improvement on quality of life and bring significant savings in the cost of healthcare services in modern society. Smart technology solutions enabling the monitoring of the elderly and immediate intervention upon the above conditions can improve prevention and induce more effectively manage the health conditions, leading to more independent and healthy living of the elderly. Figure 1 depicts an example of this technology, deploying shoes equipped with sensors to monitor quantitative gait parameters such as: stride length, velocity and heel-strike ankle. In eHealth scenarios data can be streamed to a tablet directly to provide real-time analysis for the diagnostic workup or for telehealth treatment in the patient's activities of daily living.

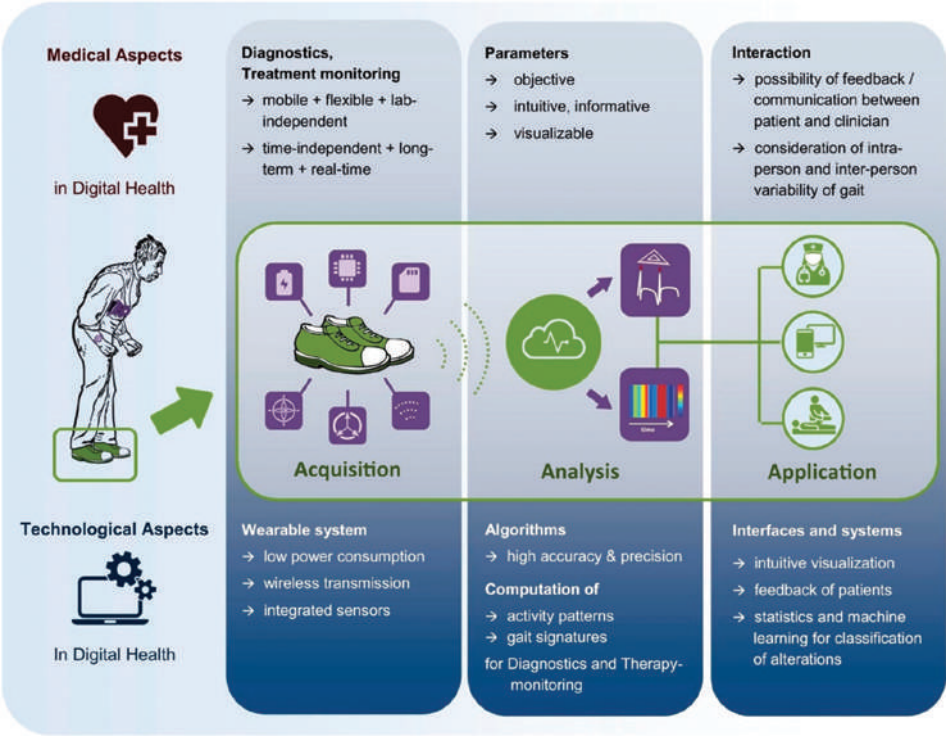

Figure 1 – IoT data collection using wearables [10]

Over recent years, there has been an increase in interest in eHealth monitoring systems situated at homes, leading to the creation of Health Smart Homes. Such technologies can facilitate the monitoring of patients' activities and enable healthcare services at home. They improve the quality of elder population well-being in a non-obtrusive way, allowing greater independence, maintaining good health,

preventing social isolation for individuals and delay their placement in institutions such as nursing homes and hospitals.

Their development was enabled by major advances in wireless technology and computing power, leading to an increasing number of connected medical devices that can generate, collect, analyse and transmit data. The data, along with the devices themselves, are creating the Internet of Medical Things (IoMT) – a connected infrastructure of medical devices, software applications and health systems and services. The IoMT is rapidly transforming the healthcare delivery. More specifically, connectivity between sensors and devices is enabling health care organizations to streamline their clinical operations and workflow management, and improve older adults care, even from remote locations, such as their home. Also, and while there has been a dramatic growth in the volume of "best practices" in healthcare, they are essentially "parked" at the threshold of the individual (local) clinical practice setting, inhibiting the implementation of effective health maintenance, personalised medicine and value/evidenced-based healthcare.

EHRs are characterized by limited interoperability coupled with limited ability to store, manage, utilize and integrate genomic, environmental, phenotypic, and citizen-generated health data. For the benefits of eHealth, personalised medicine and evidence-based practice to be realized, data must be untethered from its source, unlocking the potentially huge research, clinical and financial potential of an asset that is barely useable in its raw form at most healthcare institutions throughout EU. Based on the wide reach of mobile networks and services, smart devices and wearables, EU citizens can be empowered with the capacity managing, collecting, accessing and sharing own health and healthcare data at the centre of her/his active and healthy life in a modern digital society.

Thus, the development of solutions that provide an easy-to-use, secure, constantly accessible and portable health data and services prototype within the EU and beyond, advancing citizen health and wellbeing, nurturing digital health innovation by enhancing interoperability and bridging the gap between political intent and capability for action by the citizen, are key. These solutions need to allow citizens to managing own and own-generated health data in the EU and beyond, with data in actionable formats providing for smart processing and analysis. Also, they need to take into consideration aspects such as interoperability of assets and tools, as well as secure, seamless communication of health-related data. The General Data Protection Regulation1 (GDPR) and the technology convergence of digital services are key factors that need to be taken into consideration while developing and

implement these kind of services. 'Wellbeing services' and 'personalised (precision) medicine' are increasingly important and enabled by the power of omics approaches and personalization unleashing the potential of eHealth.

The eHealth Digital Service Infrastructure (eHDSI) is an example of a major initiative to establish a European Electronic Health Record (EHR) exchange format that is accessible by all EU citizens. Piloted since 2012 and launched in 2019, this infrastructure allows electronic prescriptions and patient summaries to be exchanged between healthcare providers across national borders [11]. Another example of the developments being made in this direction, is the Smart4Health project (Figure 2) [12] that is developing, testing and validating a platform for the citizen-centred health record with integrated abilities for aggregation of data, for data sharing and for data provision/donorship to the scientific community.

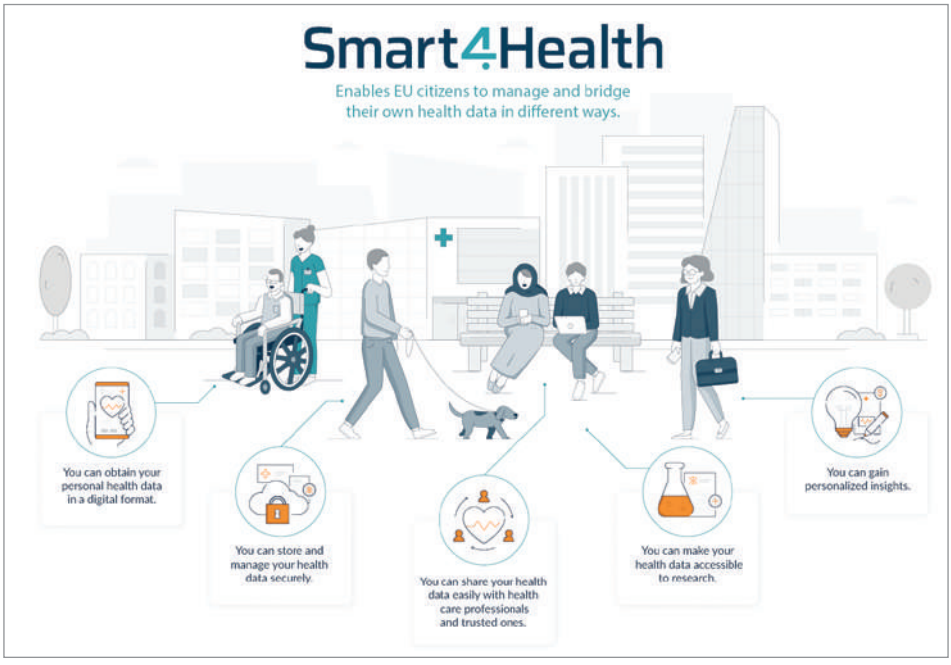

Figure 2 – Smart4Health General concept [11]

The vision is that interoperable EHR enables to unleash the potential to facilitate cross-border treatment of patients in Europe, to reduce adverse treatments (e.g. of medication allergies or unnecessary diagnostics like X-Rays and treatments) and provide healthcare workers with the right information at the right time to make

the right decisions together. Also, the aimed level of interoperability and information empowers patients to engage in the prevention and treatment of diseases.

EU support to eHealth was substantially reinforced during the outbreak of COVID-19, with EC recognizing the need for medical technologies, digital tools and artificial intelligence analytics to improve surveillance and care. On the patient side, these technologies and tools are being particularly relevant for the development of fast, cost-effective and easily deployable sampling, screening, diagnostic and prognostic systems, using AI to enable rapid diagnose of patients at critical stage, while taking the appropriate first steps for treatment without further delay.

The usage of low-cost sensors, smart wearable devices and robotics and/or AI in combination with telemedicine and continuous remote monitoring of patient parameters allows to rapidly grow systems scalability and geographical coverage for several thousands of patients. In fact, telemedicine has proven to enhance care in infectious disease management as well as improves the treatment and process quality as well as the efficiency of the supply of relevant patient groups comprehensively and measurably. The application of telemedicine together with the data-driven approaches using AI-enhanced clinical decision support models is being implemented in EU project ICU4COVID [12], aiming to promote improved sharing of recommended models for better treatment and subsequently rehabilitation. Also, ICU4COVID is working towards the protection of healthcare practitioners and general public by using robots and telemonitoring to significantly reduce exposure of the healthcare workforce, other patients, and the healthcare environment at the site, moving the concept from telemedicine to the holistic telehealth. As mentioned, the workforce enhanced collaboration is fundamental in acute crisis situation. The availability and support provided in routine exchange during the daily rounds are key to create confidence and trust among the staff, when facing overwhelmingly critical situations. With ICU4COVID [13] a network of European Digital Health Hubs supported by telemedicine infrastructure and services in establishment in ICUs in Europe.

These examples, coordinated at European level by the Innovation Hub for Digital Health and Wellbeing at UNINOVA[1], are international research, development and innovation flagships that make plain the digital health competences available in Portugal.

Hence, a main challenge for eHealth is not on the development of new technologies. Instead, it looks forward to working on the definition of a common ground

[1] UNINOVA – Instituto de Desenvolvimento de Novas Tecnologias (www.uninova.pt)

for interoperable health data that encompasses semantic interoperability and FAIR[2] principles [14], standards adoption and privacy protection as well as human and institutional capacity. This cannot be achieved without the support of regulators and policy makers working to make genuine digital transformation in health. Regarding data, a clear policy on access, treatment and ownership should be put in place and such support will be provided by certification authorities. When it comes to interoperability and protocols, shared rules and common standards should also be set up, regulators and the private sector playing a key role here, e.g., HL7 and Fast Healthcare Interoperability Resources (FHIR) standards. Procurers, (such as European/national/regional authorities, agencies, hospitals amongst others) will be included in the dialogue, stimulating the demand concretization and supporting the adoption of the services to enable the developments of solutions and bring them to the market.

The Digital Transformation of Portugal and Europe of the Health sector requires a necessary effort that should not be limited to Research and Development. Portugal and the European Union must significantly empower and advance its digital capabilities. This includes digital technology, as well as digital skills required for the Portuguese health sector workers and stakeholders. To achieve it, Portugal must develop key digital infrastructures and strengthen its industrial base, grow its flexibility in terms of resilience in services, technology, and infrastructure for digital. Its implementation will require substantial public and private investment and joint efforts that no single Member State can guarantee alone by itself.

Certification of AI/ML/algorithms in connection with GDPR, but also medical device regulation (MDR) is critical. Documentation for especially class IIa and IIb certification is quite difficult as they are now, and should be addressed well supported by testing facilities. Moreover, it will be able to support planning the validation protocol and other specifications in advance and enable rapid and seamless clinical validation of AI and ML applications in multiple sites. Regulatory sandboxes will play an important role in AI and MDR compliance. It will empower the validation and certification of AI solutions, economic assessments and links to existing mechanisms in Member States such as those that have been set up under the medical devices' regulation and current and future frameworks for Health Technology Assessments. This will ensure that innovations and new solutions are adapted to the needs in the clinical settings, that the solutions take the local/regional background conditions

[2] FAIR – Findable, Accessible, Interoperable, Reusable – https://www.go-fair.org/fair-principles/

into account when analyzing data, and that the user groups (citizens, patients, professionals) are able to participate in the development of new supportive digital tools, through participatory design and development processes that put the user at the center of development.

DTH (Digital Twins in Healthcare) has significantly advanced, mostly empowered from advances in science and technology. Their benefits in view of services will enable a sound digital transformation in the Health sector in Portugal for better prevention approaches, faster and more accurate diagnoses, personalized treatments, and care, delivering a mature framework to structure cooperation and leverage on synergies between academia, private sector, regulators and end-users. This requires the mapping and link of actors and initiatives, developing a blueprint of an inclusive ecosystem to share knowledge, and facilitate understanding between developers, users, and decision-makers throughout the relevant health related sectors. Hence, the resulting 'roadmap' on DTH would take into consideration in a co-creation approach the different stakeholder groups, identifying the needs of end-users, determining the necessary enabling infrastructure and considering a framework for the deployment of digital companions, taking both the technical and societal dimensions.

To achieve the other face of medicine through eHealth, a dedicated education program should be put in practice aimed to work with highly qualified interdisciplinary experts in the health sector, at the interface between IT, computer science and medicine and other related health areas, like health care staff and citizen wellbeing. This program will empower interdisciplinary skills in research or business with an application focused on the health sector in multiple ways, including the areas of health economy and healthcare provision. It will provide skills to analyze, design and implement complex and secure IT systems and infrastructures in the healthcare field, and deal with ethical and legal issues of relevance in the conception of health systems. Entrepreneurship topics and the innovation method of Design Thinking are an integral part of the learning curriculum.

Concepts and Methods, Technologies and Tools and Specialization for eHealth:

- Health Systems and Sciences for Digital Health: Comprehensive overview of selected areas of digital health in a national, European and international context. Processes, actors and care goals of digital health are examined in detail: current standards of health care, research and patient-centered treatment. Integration and monitoring of digital solutions are of concern.

- Software Architectures for Digital Health: Gain knowledge of complex digital systems, networked software infrastructures and interoperable digital health applications. Concrete concepts and procedures of the software development process for applications from the life sciences are concerned.
- Digital Health Business and Process Transformation: Modelling, analysis and evaluation of existing and new digital health processes using common IT systems, infrastructures and applications. Strategies and concepts for solving problems of health care actors and the management of health data are communicated.
- Ethics, Law and Compliance for Digital Health: Ethical and legal framework conditions to be observed in the design and implementation of software systems and in the handling of data from the health sector. Good clinical practices, handling of study data, legal requirements (e.g. eHealth legislation) and recommendations (e.g. National and European) are discussed. Get insights on the assessment of the risks associated with the use of health data, to ethically and legally assess the conflict situations that arise in business and society and to prevent them by acting in a legally compliant manner.
- Scalable Computing and Algorithms for Digital Health: Technical concepts and innovations and their translations into daily routines in areas such as telemedicine, wearables, big data technologies and cloud computing.
- Digitalization of Clinical and Research Processes: Digitization of clinical and research processes, taking into account data quality, reproducibility and stability.
- Acquisition, Processing and Analysis of Health Data: Process chain of data collection, processing and analysis. These include big data from heterogeneous data sources, data with high acquisition frequency and fast processing times.
- Health Data Security: Safety-relevant aspects in the area of the use of data in the field of digital health.
- Principles of IT Systems: Fundamental knowledge of complex IT systems, operating systems, network (basic concepts, Internet and WWW), which are required within the scope of Digital Health.
- Fundamentals of Programming: Fundamental knowledge of the programming languages, development tools, strategies and selected aspects of programming complex software systems required for Digital Health.

- Introduction to Principles in Medicine: Fundamental principles and concepts of selected areas of medicine as well as the teaching of competences for the documentation of medical concerns in the field of digital health.
- Fundamentals of Healthcare Systems: Basics for working in European and international health systems with their concrete requirements and special characteristics are imparted.
- Digital Future in Health: Get updated on how digital is supporting local delivery of the health services and what a digital future in health will look like. It explores the key leadership aspects and workforce challenges in delivering a digitally transformed health and care service.
- Big Data, AI, Blockchain and Analytics: ML and AI, Data capture, Data analytics and Blockchain have driven improved outcomes in the digital transformation of healthcare. The ability to gather, safely store and analyze very large data sets fast, endows informed predicting health outcomes.
- Integration and Interoperability: Looking forward to fully integrated care, interoperability will enable systems and staff to seamless share information on patients, alongside the growing need to improve alignment of services and systems to reduce duplication and error, streamline clinician and patient experience whilst improving outcomes across Primary Care, Local Authority, and Hospital Trust putting the citizen in the centre of its own health data.
- Cybersecurity: Developing an agile and strong cybersecurity infrastructure is critical in the continuous move towards a fully digitalized system, prepared as individuals and organisations in terms of systems, human factors, physical security, changing regulation and data storage concerning that patient data is absolutely valuable.
- Personalized health and Care with Digital Empowerment: Put the citizen the owner of his own health data. Digital tools and personalized care collectively improve health care experience and outcomes, reducing pressure on the system and provide value for money. Digital services, platforms, infrastructure and standards are put in place to enable interoperability across health settings and create an environment to enable a new relationship between people, professionals and the health and care system.
- Citizen-centred Prevention, Patient Health Data and Wellbeing: Prevention starts in the advent of increasing pressures of patient demand alongside budgetary and workforce constraints. Opportunities, risks and ethics in

broadening the use of patient data, and look to the expansion of Citizen Health Management, using data and analysis to drive change in the way health and care services are delivered for a better citizen-centered care and health outcomes in a sustainable system.

- Innovation through Cloud and Mobile: The cloud is a major catalyst for co-creation and collaboration innovation, and the digital transformation in Health is changing the way we work. The cloud offers flexible and dynamic solutions and delivers significant opportunities to provide digital services and servitization to improve the connected patient journey, optimize the delivery of personalized care larger populations, enable the great potential of AI and evidence-based decision making.

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COMMUNICATION STRATEGIES IN HEALTHCARE

Helena Serra

1. INTRODUCTION: WHY HEALTH COMMUNICATION(S) MATTERS?

One of the key goals of Health Communication (HC) is to engage in power and influence individuals and communities, in order to improve health outcomes by sharing health related information. Thus, the notion of HC implies the use of communication strategies to inform an influence individual and community decision to enhance health. The word "influence" is crucial in the definition of HC as the art and technique of informing, influencing and motivating individuals, institutions and the public about health issues [1].

HC is intrinsically related to its potential impact on vulnerable populations that include groups with higher risk for poor physical, psychological or social health in the absence of equated conditions that are supportive of positive outcomes, for instance children, the elderly people living with disability, migrant populations, groups affected by stigma and social discrimination and also groups who do not have adequate access to health or community services. The use of health communication strategies aims to achieve health care quality and health equity. Health equity is providing every person with the same opportunity to stay healthy or to effectively cope with disease regardless ethnicity, gender, age, economic conditions, social status, environment and other socially determined factors. Only by creating a receptive and favorable environment where information can be effectively shared, understood and discuss by different communities and sectors, health equity can be achieved. That is the only way to guarantee the inclusiveness and representativeness of vulnerable groups. This demands an in depth understanding of needs, beliefs, attitudes, lifestyles, socio economics, social norms of all key groups and sectors involved in the communication process.

Understanding the context of communication means to become familiar with intended audiences. This increases the chances that all meanings are shared and understood in the way communicators intended them. Hence, communication about health and illness, or life and death, matters in public health and healthcare and must be seen as a long-term strategic process which implies in depth understanding of the key groups and communities, but also the capacity to engage as well as to be able to adapt and redefine goals, strategies and actions of communication interventions, on the basis of audience participation.

Several authors have noted that HC draws from numerous disciplines and theoretical fields including health education, social and behavioral sciences, community development, mass communication, marketing, psychology, anthropology

and sociology [2, 3, 4, 5]. It means that HC is a multidisciplinary field of research, theory and practice, concerned with reaching different groups to exchange health-related information in order to influence, engage, empower and support individuals and communities, healthcare professionals, patients, policymakers, organisations, so that they will embrace a health or social behavior, practice or policy that will improve individual community and public health outcomes.

2. A BRIEF STATE OF THE ART REVIEW AND HC DESCRIBING FEATURES

HC is about improving health outcomes by encouraging behavior modification and social change and increasingly considered a primary part of most people health interventions. It is a comprehensive approach that relies on the in depth understanding and participation of its intended audiences [2]. HC is a progressive and dynamic subject area of research, which is receiving huge popularity because of its applicability and widely acceptance among health professionals [6, 7]. Its wider acceptance among health, social and data scientist, has been drawing the attention among researchers of various fields to collaborate for an in-depth and inclusive research [8]. Several health communication models along with theories have emerged as key definitions and describing features, making this an interdisciplinary area of research.

Sharma, Nahak and Kanozia [9] present a systematic literature review of 20 different research outcomes of HC, emphasizing their key strategic features in order to achieve its goals: communicate the health values by using effective means of communication to generate collective consensus among masses [6, 8]. HC mainly implies a dialogue between health professionals and health communicators and media professionals with a purpose: to make echo for health rights, health education and health awareness in general [10, 11, 12]. The mainly aim is to boost public health campaigns, starting health favorable narratives. The key strategy of the HC is to inform the public about health issues, challenges and opportunities [13, 14].

Improving health literacy is critical to the dissemination of health information and knowledge among individuals as well as society. In the literature we find four approaches in HC Strategies, which include information, education, persuasive and prompting [9]. HC can be defined as encompassing the study and use of communication strategies to inform and influence individual and community decision

that enhance health. It means that being a practitioner of HC involves being also a strategist, raising health issues, health risks and health solutions along with experts. In practice, HC implies to frame community point of view, to established public health agenda, to advocate policies and programs in order to spread public health and health care services [11, 15, 16].

Listing HC issues is an evolving process and always under construction. It should seek to integrate lessons from the field as well to use a multidisciplinary approach to all interventions [1]. Although, literature point out several key features that should guide and inform HC planning and management.

People-centered
Evidence-based
Multidisciplinary
Strategic
Process-oriented

Table 1 – Key Features of Health Communication

People-centered

People's needs and preferences are in the center of HC strategies. It is a long-term process where intentional audiences are not merely a target, even if the aim is that a communication intervention focus on benefit and engage a specific group of people that shares similar characteristics (e.g., age, ethnicity). Several key groups and stakeholders from different segments of society, including professional sectors, assume a crucial position in the communication process. Communities, teachers, parents, healthcare professionals, religious and community leaders, are some of the examples of these key groups.

Engaging communities and different stakeholders it is a necessary but not a sufficient step to implement the people-centered approach. This is because the effectiveness and sustainability of most interventions. Saying this, in HC practice is often accomplished by working together with organisations and/or leaders who represent key groups or by directly involving members of a specific community since the kickoff of program design. It is really important that all the key groups feel invested and well represent in the process. They must act as key protagonists of the action-oriented process that will lead to behavioral and social change.

Evidence-based

HC is based in research. To be effective, HC interventions are based on a true knowledge and understanding not only of those key groups but also of the context, real situations and sociopolitical environments. The main principle of HC is that behavioral and social change is conditioned by the context in which people live and work, as well as by those who influenced them. There are socially determined factors, referred as social determinants of health, that influence and are influenced by HC. They include social economic conditions, ethnicity, culture, access conditions to health care services, accessible, and affordable nutritious food health information culturally appropriate (which truly reflects literacy levels).

Multidisciplinary

HC draws on multiple disciplines, recognizing the complexity of accomplishing behavioral and social changes. HC uses a multidimensional approach that is based in the application of several theoretical frameworks and disciplines as health education, social marketing, behavioral sciences, social change theories and medical and clinical studies. Disciplines such as psychology, sociology and anthropology have commonly constituted the basis to draw a people-centered approach [5]. Four different periods or eras, concerning HC were identified [17]: i) the *clinic era*, built on a medical care model and the idea that if people knew the services locations they would find their way to clinics; ii) the *field era*, which involve a more proactive approach highlighting outreach workers, community based distribution and different kinds of information, education and communication products; iii) the *social marketing era*, established from the commercial concepts that consumers will buy the products they want and subsidized prices; iv) the *strategic behavior communications era,* established on behavioral science models that stress the need to influence social norms and policy contexts to enable an empower the iterative and dynamic process of both individual and social change [1]. A 5th period, the *strategic communication for behavioral and social change era*, emerge lately, linking behavioral in social science disciplines along with marketing, medical based models and emphasize the need to achieve long-lasting behavior and social results.

Strategic

A very sound strategy and plan of action must be displayed when talking about HC programs. All the activities need to be carefully planned and respond to a specific audience-related need. All the activities should serve the communication strategies

which need to be research-grounded in order to meet the communication objectives. That means that the content and format of workshops, press releases, brochures, videos or another support to provide effective communication, must be in line with the strategy selected. It is a priority that HC strategies respond to the needs that have been identified by preliminary research and confirmed by intended audience.

Process-oriented

As already stand, HC is a long-term process, as implies to influence people and their behaviors. It involves an ongoing commitment to the health issues and its solutions. This is deep-rooted in a deep knowledge of key groups, communities and their contexts, and aims to build consensus among individuals, community members and key stakeholders about the plan of action.

HC programs constitute a process under construction. Its dynamic nature implies a continuous change from what communication experts may have initially anticipated due to the input and participation of the community members, key opinion leaders, patient groups, professional associations, policymakers and other key stakeholders. To engage key groups on relevant health issues and explore suitable ways to approach them, is crucial to guarantee the success of a long-term people-centered process.

3. RELEVANT CONTRIBUTIONS FROM NOVA

A broad picture of the research activity and relevant contributions from NOVA University of Lisbon (NOVA) related to HC issues was carried out. The information has been taken from NOVA Research Portal and RUN (NOVA's Repository), which provides a public view on the NOVA's research activity. All categories of research activities were used (research outputs: articles, chapters, books, master and doctoral thesis, profiles, prizes and press/media) and related to the key concepts: health communication; health communication strategies; and health literacy.

Concerning the contributions to journals, were located 36 outputs, distributed by twenty articles [18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37]; one systematic literature review [38] and two editorials [39, 40]. Two chapters in books [41, 42] and one meeting abstract [43] from conference proceedings were also located. Regarding master and doctoral thesis, we located eight master [44, 45] and two doctoral thesis [52, 53], all of them associating both key concepts, health communication and health literacy.

4. IMPACTS IN SCIENCE AND SOCIETY

Despite fast improvements in health and healthcare, a wide variety of problems still exist in terms of the capacity to provide adequate care for everyone and in terms of prevention and control of diseases and other health problems. It seems that the severity of many of these problems could potentially be reduced by improving communication among providers, between providers and patients, between health researchers, and between public health leaders and the public.

A number of health issues are related to communication. Global issues such as lake of access to adequate healthcare, war, poverty, hunger, environmental injustice and lack of education about health issues, still problems for people around the world. Unfortunately, many of these issues have had the greatest impact among the underserved populations. HC researchers are currently working to better understand these issues so that they can offer suggestions for improvement and it is clear that communication is an important underlying factor for most issues mentioned above. A better understanding of how communication is related to health problems may ultimately help to reduce incidents of disease, human suffering, and mortality rates while increasing physical and psychological well-being and satisfaction among society.

When looking at what we learned from COVID-19 pandemics, risk communication emerged again as a key intervention area. This is not new as communication has been recognized in previous public health emergencies as a core discipline, along with a variety of types of responses (intervention-based, specific programs, clinical, resources and supplies responses, and others) [54]. The pandemic exposed important gaps in communication systems as associated to readiness, processes, and consistency that is necessary to ensure that communication is evidence-based and effective in reaching and engaging its many publics [55].

A main feature of communication systems strategies should be the capacity to build social support networks, so that people are encouraged to speak about their health problems and feel supported in their decisions, for example, to comprise COVID-19 protection measures or to promote vaccine acceptance in their community. Myths and misinformation about COVID-19 promoted the so called infodemics [43], creating global panic and risks to people's lives and slowing down the efforts of public health bodies to contain the pandemic. Then, the role of public health bodies in communicating the right message and in the right way to the public is fundamental [43]. Critically important is also the clarification of the behavioral results we seek to achieve, our ability to acknowledge uncertainty [8], as well as

a strong emphasis on addressing communication inequities such as those related with low health or media literacy and digital redlining.

5. ONGOING HEALTH COMMUNICATION RESEARCH

Despite the term Health Communication as only been more visible since the middle of 1970s, scholars have taken a scientific approach for studying communication within health contexts for decades. Research in the area of HC as grow exponentially over the last 40 years [56]. In the Social Sciences, scholars who were interested in the study of communication began to examine the healthcare systems in the late 60s, encouraging several scholars to follow. Korsch and Negrete's [57] "Doctor-Patient communication" published in Scientific American is still considered as the foundation of the field. Also, other works came from medical researchers, focusing particularly the study of provider-patient interactions. The prevalent interest in HC leads to the creation of two important publication for HC researchers. The first, the Journal Health Communication appeared in early 1989, followed in 1996 by the Journal of Health Communication. These two journals have been played a crucial role in the increment of HC as an area, by circulating HC research to a wider audience within and outside of the communication field.

The area of HC is now widely recognised as lively, theoretical driven and a key contributor to National Health Policies [58]. Most of the research in this field has been conducted outside the academic settings. In fact, many of the research studies are interventions designed to improve physical and mental health outcomes in a number of contexts, such as health campaigns, provider-patient relationships, organisations and the use of new technologies in healthcare. Presently, the field of HC still growing and diverse. New areas of research emerge and have been expanded HC domain, such as palliative care, spirituality and health, online support groups, and telemedicine.

Though, a number of HC topics have remained as prominent themes in HC literature over the past years. Thompson and his colleagues [56] in an analysis of the topics that appeared in the Journal of Health Communication between 1989 and 2005, show that over 20% of the articles dealt with provider-patient interaction, followed by health campaigns, risk communication, health and ageing, language and health media, and social support in health. Most of the prominent theories on the field have their origins in communication, social psychology, sociology and anthropology [59], reflecting the ways in which theory has developed in the various contexts

of HC research. For instance, several theories of provider-patient interaction have their roots in the interpersonal communication research; a number of the theories used to understand intercultural health issues have their origins in sociology and anthropology; and many of the theories of social influence that are associated with health campaigns have been borrowed from sociology. Communications scholars are continuing to refine these early theories as well as developing new HC theories. As stand above, the COVID-19 pandemics context came to emphasize the critical point of addressing communication strategies to inform the public on the new corona virus disease risks and prevention. This topic definitively marks the research agenda in the field of HC at present and in the coming times.

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HEALTHCARE QUALITY AND PATIENT SAFETY

Paulo Sousa
José Fragata

INTRODUCTION

The global movement to improve quality and safer care has galvanised significant interest and developed momentum in health systems. Quality and Safety can be seen as two sides of the same coin. Quality tries to optimize the effectiveness of health care. Safety addresses the minimization of risks that come with the delivery of health care. As such, they are complementary, and methods to address them all have in common the fact that they consist of a combination of change, measurement and improvement. Therefore, it seems wise not to separate quality and safety in the organizational arrangements and structures that are set up to address them.[1]

Quality of care is *the degree to which health services for individuals and populations increase the likelihood of desired health outcomes and are consistent with current professional knowledge*[2]. This definition implies that quality of care can be measured and it is ultimately aimed at health improvements rather than simply increasing service inputs or refining system processes. Quality of care should reflect the desires of key stakeholders, including service users and communities. By including health services in general, this definition of quality of care spans both curative and preventive care, and facility and community-based care for individuals and populations.[3]

At its core, patient safety is the prevention of errors associated with healthcare and the mitigation of their effects. It is both the processes used to reduce harm, and the state that arises from the actions taken to prevent patients from harm.[4]

Quality and safety is not an automatic process, it requires planning and it should be a clearly identified priority in the health agenda of all countries, along with access, coverage and financial protection.

This chapter aims to highlight the importance of quality and safety as a centre piece of the health agenda worldwide and their impact in terms of reducing waste and harm at the same time that improves clinical, economic and social outcomes. The alignment of healthcare quality and safety with the United Nations 2030 agenda for sustainable development and the universal health coverage principles, together with the growing safety movements lead by some of the most important international organizations such as World Health Organization (WHO), Organisation for Economic Co-operation and Development (OECD), World Bank, Institute for Healthcare Improvement (IHI) and the International Society for Quality in Healthcare (ISQua), are a strong signal of the importance of this issue nowadays.

Throughout this chapter, together with some of the most recent evidence and national and international reports that have been published, we will briefly present some of the most relevant contributions from NOVA giving particular emphasis to the collaborative research that has been developed. The core of this research addresses existing quality and patient safety challenges that have been prominent in health systems and services over the last years across a range of healthcare settings with impact on the point of care, science and society.

QUALITY OF CARE AND PATIENT SAFETY: THEIR RELATION WITH MDGs, SDGs AND UNIVERSAL HEALTH COVERAGE

Quality of care is one of the most frequently quoted principles of health policy, and it is currently high up on the agenda of policy-makers at national, European and international levels.[4] At the national level, addressing the issue of healthcare quality and safety may be motivated by various reasons – ranging from a general commitment to high-quality healthcare provision as a public good or the renewed focus on patient outcomes in the context of popular value-based healthcare ideas to the identification of specific healthcare quality problems.[5]

Health care systems worldwide share common goals in order to improve the quality and safety of care, despite some differences in structure, resources, accountabilities and priorities. Patient safety is widely recognized as an essential component of health care. For that reason, improving patient safety, and broadly the quality of health care, has become a core issue for many countries.

The twenty-first century began in a promising way for health, public health and their relation with other social, economic and environmental areas – *health in all policies*. Leaders of 191 nations signed the Millennium Declaration in 2000 and committed themselves to reach its eight goals (the Millennium Development Goals – MDGs) by 2015.[6] In 2015, the United Nations General Assembly adopted a new development agenda: *Transforming our world: the 2030 Agenda for Sustainable Development*. The Sustainable Development Goals (SDGs) comprise a broader range of economic, social and environmental objectives than the MDGs and set a new health goal in order to "ensure healthy lives and promote well-being for all at all ages". Its agenda is based on a plan of action for people, the planet and prosperity, and aims to promote a transition to more productive and equitable societies and economies.[6]

Figure 1 – Sustainable Development Goals
Source: https://www.unido.org/2030-agenda-and-sustainable-development-goals[7]

By explicitly focusing on the quality of health care services, the 2030 Agenda for Sustainable Development recognizes the urgent need to place quality of care in the fabric of national, regional, and global action towards promoting well-being for all.

Another important goal for the beginning of this century is related with the Universal Health Coverage (UHC). Universal health coverage is an important and noble objective which means that all people (individuals, families and communities) have access to the health services they need, when and where they need them, without financial hardship. It includes the full range of essential health services, from health promotion to prevention, treatment, rehabilitation, and palliative care[8].

But universal health coverage could not be discussed and planned, let alone implemented, without a focus on quality and safety in healthcare. It is essential to ensure that care is effective, safe, and it keeps within the preferences and needs of the individuals and communities being served. Usually, the progresses in UHC have been measured through effective coverage of essential health services and financial protection.[9] But even if a country achieved essential health coverage and financial protection, health outcomes would still be poor if services were of low-quality and unsafe. For that reason, delivering quality and safe health services are crucial to help to achieve UHC.[3]

High-quality health services involve the right care, at the right time, responding to the service users' needs and preferences, while minimizing harm and resource waste. Quality healthcare increases the likelihood of desired health outcomes and is consistent with six measurable dimensions: effectiveness, safety, people-centeredness, timeliness, equity, and efficiency.[2] A handful of analytic frameworks for quality assessment has guided measure development initiatives in the public and private sectors showing that there is not one single definition of quality of care.

Nevertheless, one of the most influential definition is the framework put forward by the Institute of Medicine (IOM), which includes the following six aims for the healthcare system.[10]

- **Safe**: Avoiding harm to patients from the care that is intended to help them.
- **Effective**: Providing services based on scientific knowledge to all who could benefit and refraining from providing services to those not likely to benefit (avoiding underuse and misuse, respectively).
- **Patient-centered**: Providing care that is respectful of and responsive to individual patient preferences, needs, and values and ensuring that patient values guide all clinical decisions.
- **Timely**: Reducing waits and sometimes harmful delays for both those who receive and those who give care.
- **Efficient**: Avoiding waste, including waste of equipment, supplies, ideas, and energy.
- **Equitable**: Providing care that does not vary in quality because of personal characteristics such as gender, ethnicity, geographic location, and socio-economic status.

Frameworks like the IOM domains also make it easier for consumers to grasp the meaning and relevance of quality measures. Studies have shown that providing consumers with a framework for understanding quality helps them value a broader range of quality indicators. For example, when consumers are given a brief, understandable explanation of safe, effective, and patient-centered care, they view all three categories as important. Further, when measures are grouped into user-friendly versions of those three IOM domains, consumers can understand the meaning of the measures more clearly and apprehend how they relate to their own concerns about their care.[11]

By including the six domains defined by IOM in the triple aim scheme developed by Institute for Healthcare Improvement (IHI), we can have a more comprehensive and integrated understanding of what patient-centered care means and how important it is to align all these dimensions towards improving quality and safer care. By reducing the per capita cost of health care – efficiency – and improving the patient experience of care – by delivering safe, effective, timely and equitable care – we contribute to achieve one of the most important goals of public health – improving health for individuals and populations.

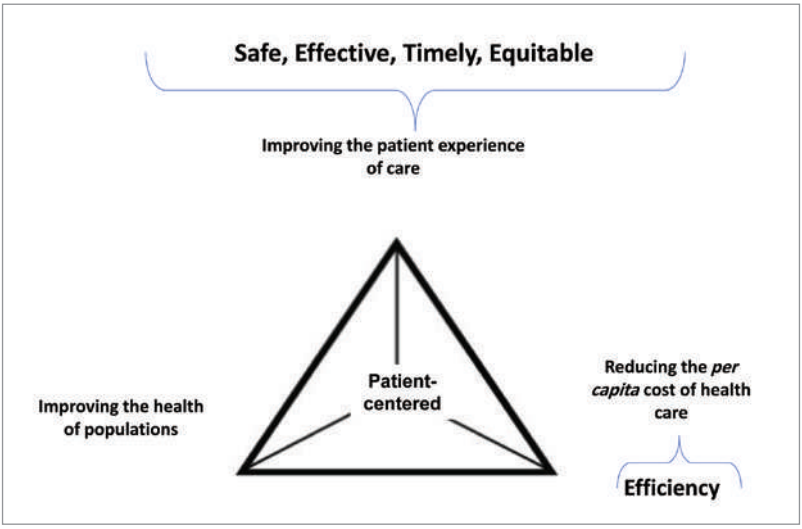

Figure 2 – Triple Aim
Adapted from: Stiefel M, Nolan K. *A Guide to Measuring the Triple Aim: Population Health, Experience of Care, and Per Capita Cost*. IHI Innovation Series white paper. Cambridge, Massachusetts: Institute for Healthcare Improvement; 2012. (Available on www.IHI.org)[12]

QUALITY OF CARE AND PATIENT SAFETY: A PUBLIC HEALTH ISSUE THAT NEEDS TO BE TACKLING

Besides the negative effects on people's lives, poor-quality of care results in waste of time and money. Making quality an integral part of universal health coverage is both a matter of striving for longer and better lives and an economic necessity. Building quality in health systems is affordable for countries at all levels of economic development. In fact, the lack of quality is an enormous cost, especially for the poorest countries. Substandard quality of care not only contributes to the burden of global disease and unmet health needs, but also exerts a substantial economic impact with considerable cost implications for health systems and communities across the world. Approximately 15% of hospital expenditure in high-income countries is used to correct preventable complications of care and patient harm.[13] Poor-quality care disproportionately affects the more vulnerable groups in society, together with the broader economic and social costs of patient harm caused by long-term disability, impairment and lost productivity amounting to trillions of dollars each year.[14] In addition, it duplicates services, ineffective care

and avoidable hospital admissions – features of many health systems – generating considerable waste.[14]

In the last twenty-five years, several studies have been published suggesting that substandard care wastes significant resources, reducing productivity and, in some cases, harming patients. Quality of care, especially patient safety, is essential to create trust in health services and in healthcare professionals.[6,14,15]

Relevant research have shown that an average of one in 10 patients is subject to an adverse event while receiving hospital care in high-income countries.[15] The estimate for low- and middle-income countries (LMICs) suggests that up to one in four patients is harmed, with 134 million adverse events occurring annually due to unsafe care in hospitals, contributing to around 2.6 million deaths.[15] Overall, 60% of deaths in LMICs from conditions amenable to health care are due to unsafe and poor-quality care. Half of the global disease burden arising from patient harm originates in primary and ambulatory care.[16]

Globally, the cost associated with medication errors has been estimated at US$ 42 billion annually, not counting lost wages and productivity or increased health care costs. This represents almost 1% of global expenditure on health. Unsafe and poor-quality care leads to US$ 1.4-1.6 trillion worth lost in productivity each year in LMICs.[15]

Available evidence estimates the direct costs of harm, such as additional tests, treatments and health care, in the primary and ambulatory setting to be around 2.5% of total health expenditure although this probably underestimates the true figure.[16] Harm in primary and ambulatory care often results in hospitalizations. The health burden of harm is estimated at 64 million Disability-Adjusted Life Years (DALYs) a year, similar to that of HIV/AIDS. Each year, these may account for over 6% of hospital bed days and more than 7 million admissions among member countries of the Organisation for Economic Co-operation and Development. [15]

It is well established that health care services around the world occasionally and unintentionally harm patients. In recent years, different studies have estimated that around 4% to 17% of hospital admissions have resulted in an adverse event and that up to half of these events were preventable.[17-18] As result, addressing patient safety represents an important challenge that is receiving attention in the public health domain. However, no matter what systems and precautions are put into place, it should be recognized that health care will always involve risks and the consequence of accepting those risks will have strong clinical, social and economic impacts.[19]

A study developed in acute public hospital centres in Portugal, by a group of research from the NOVA National School of Public Health, identified a total rate of AEs of 12.5%.[18] The majority of AEs (66.1%) occurred in patients aged 65 or older. Of all AEs, 39.7% were related to hospital-acquired infections, followed by 26.7% associated with surgical procedures and 9.8% related to medication. The majority of AEs (67.4%) did not result in any significant physical impairment or disability, and were resolved during the in- hospital period. However, a small but significant proportion of patients died or experienced a permanent disability as a result of their AE (12.5% and 3.0%, respectively). The majority of patients (60.8%) who experienced AEs prolonged their length of stay in hospital by on average 9.6 days, with an estimation of additional costs of €1.9 million.[18] Hospital adverse events continue to be an important public health issue. They constitute a burden in terms of clinical, economic and social impact and, for that reason, they are a challenge for the health system not only in Portugal but also worldwide.

We must have in mind that total safe health care is an objective that may never be achieved. However, the creation of a healthcare system that is aware and systematically reflects, learns and acts to reduce unintended patient harm is a reasonable and achievable aim.[19]

Clearly, patient safety issues result from various combinations of individual, team, organization, system and patient factors. A systemic and integrated approach to promote patient safety must acknowledge and strive to understand the complexity of work systems and processes in health care, including the interactions between people, technology, and the environment. As a result, the Public Health approach to patient safety should use a systemic and integrated approach (Figure 3) aiming to implement and sustain a comprehensive risk control system, instead of focusing on only errors and fails arise from human mistake or system failures.

As show in Figure 3 a systemic and integrated approach to patient safety should be patient-centred; promoting a culture of learning and openness; taking into consideration good hospital design and ergonomics; supporting strong leadership; encouraging good reporting systems and epidemiologic knowledge of adverse events and; be based on research and innovation and developing capacity and knowledge.

The value of education, training and research on patient safety is widely recognized. Recently, The OECD has produced evidence which can be applied on an international level regarding the “best buys” in patient safety.[13,14] Their work demonstrated the overall importance of investing in safety improvements to ameliorate the overall economic burden of harm, but it goes further to suggest which interventions

are most cost-effective. Experts surveyed in the OECD study prioritised both system-level and organisational-level improvements as best buys, including, *build and reinforce capacity and* educational, training *programs,* as one of the most relevant.[13,14]

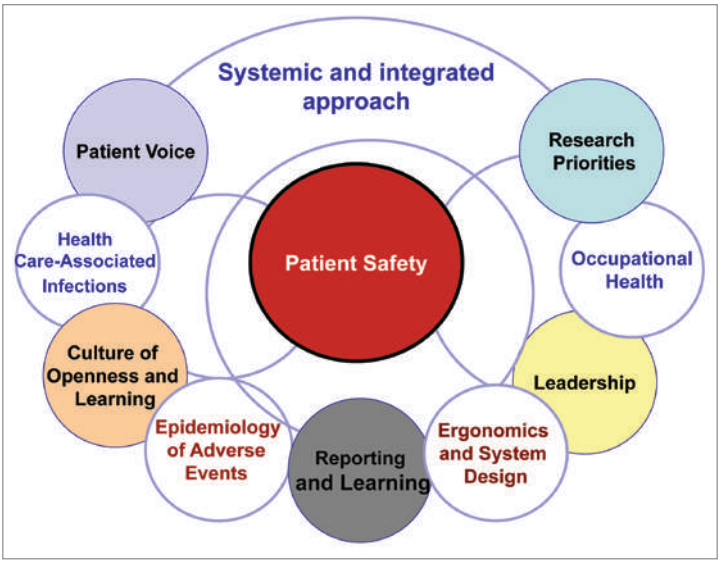

Figure 3 – Systemic and Integrated Approach to Patient Safety[20]

The inclusion of patient safety issues on the curricula of different under-graduate and post-graduate courses, such as master and PhD degrees should be priority. Because their multidisciplinary nature, public health researchers, teachers and schools can have a central role in creating capacity through educational courses and researches programmes and by fostering national and international networks on health care quality and patient safety. Furthermore, it will be important to go beyond research and educational developments to translate knowledge gained to policies and practices. We believe that NOVA university, by having the National School of Public Health, a Medical School, a School of Social and Human Sciences, an Engineering School, a Business and Economics School, and an Information Management School, is in a great position to lead training, educational programs and research, in the area of quality in healthcare and patient safety. To reinforce this (as a sign of this strategic position), was created in 2019 the NOVA Quality Improvement and Patient Safety multidisciplinary group.

This group, includes researchers and professors from different faculties and institutes of NOVA University, is committed to develop research, knowledge and

innovation in the field of quality and safety of care and related areas and aims at ensuring that the research developed addresses existing quality and patient safety challenges that are and will be prominent in health systems and services over the next years and across a range of healthcare settings with impact at the point of care.

Patient safety has become a core issue for many modern healthcare systems. All healthcare systems around the world occasionally and unintentionally harm patients whom they are seeking to help. The effects of harming a patient are widespread. There can be devastating emotional and physical consequence for patients and their families. For the staff involved too, incidents can be distressing, while members of their clinical teams can become demoralised and disaffected. [21]

As mentioned before, clinical and economic burden of unsafe care is only the tip of the iceberg. The personal impact is traumatic and enduring; experiencing harm as a patient or as a member of staff has consequences in terms of psychological damage, fear, degradation of trust and loss of confidence.[21,22]

A study involved different Ibero-american countries, where Portugal participates, conclude that the majority of the hospitals of these countries have not protocolized how to act after an AE and they have not in place a support programme to help healthcare professionals after an AE occurs.[21]

Since November 2020, researchers from NOVA National School of Public Health, together with colleagues from 14 European countries, integrate an international research project, financed by the European Union – COST Action (https://cost-ernst.eu/about/) The European Researchers' Network on Second Victim – ERNST – that aimed to create and share scientific knowledge and best practices concerning adverse events in healthcare institutions to implement joint efforts to support second victims and to introduce an open dialogue among stakeholders about the consequences of this phenomenon based on a cross-national collaboration that integrates different disciplines and approaches.

We strongly believe that, in the years ahead, this international collaboration will contribute with evidence to help healthcare professionals, managers and decision-makers to respond to this imperative safety challenge.

CONCLUSIONS / MAIN REFLECTIONS

Too often, quality and safety are perceived as a luxury that only rich countries can afford. This is not true at all. Building quality health services requires

strong leadership, a culture of transparency, engagement, and openness about outcomes, which are possible in all societies – regardless of their income and development level.

Universal health coverage is not a dream for the future. It is already a reality in many countries. However, without quality health services, it can remain an empty promise.

In the last decades, Portugal has gone a long way with some slow but consistent progresses towards improving quality and safety of care.

The fact that NOVA National School of Public Health is now a World Health Organization Collaborating Center for education, research and evaluation of safety and quality in healthcare, together with the circumstance that some of NOVA University researchers, research centers and Faculties/Schools integrate the Comprehensive Health Research Centre (CHRC) and the existence of the NOVA Quality Improvement and Patient Safety group (QI&PS), places us as an important player and partner at the national and international level contributing with research, educational programs and evidence to support decision makers towards the improvement of quality and safety in healthcare.

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I

STUDIES OF ARTIFICIAL INTELLIGENCE IN HEALTHCARE

Leonardo Vanneschi

Abstract. The availability of vast amounts of data has fostered the development of a large amount of research, aimed at using artificial intelligence to support or improve healthcare in several possible different ways. The developed technolo- gies and obtained results seem to point towards a future in which data science and machine learning will provide better information to clinicians, so that they will be able to make more informed decisions, for instance, about patient diagnoses and treatment options, while understanding the possible outcomes and costs. The value of machine learning in healthcare stands in its ability to process vast amounts of data, beyond the scope of human capability, and then reliably convert them into clinical insights. The Nova Information Management School of the Universidade Nova de Lisboa has conspicuously contributed to the field in the last decade. In this chapter, we summarize and critically discuss some of the most recent and important studies, including the automatic development of predictive models for pharmacokinetic parameters, for the unified Parkinson's disease rating scale assessment and for the pathological complete response in breast and axilla cancer. Besides their inherent value, those applications are particularly interesting because they have been tackled using novel and promising machine learning methods, such as several improvements of genetic programming, and a regularization strategy called priority-lasso. These methods have outperformed the state of the art in the studied applications. Furthermore, they are general purpose, and thus they can successfully be employed also to problems of different nature.

1. INTRODUCTION

Artificial intelligence (AI) is arguably going to be one of the biggest transformative technologies for human society in this century, influencing global productivity, working habits and lifestyles, and creating enormous wealth. It is a well-established opinion that this transformation will be, to a large extent, fueled by Machine Learning (ML) [33]. In simple terms, ML represents the set of AI methods aimed at programming computers to learn information without human intervention. In ML, computers are provided with data and they learn from data. ML algorithms extract knowledge from data, by dis- covering their complex patterns and hidden information, and contribute to make them usable and in some cases interpretable to human beings. Traditional business and tech- nology sectors are not the only fields being impacted by ML. Healthcare is one of the areas that is more deeply influenced

by ML. Actually, ML is becoming widely used in healthcare and is nowadays helping patients and clinicians in many different ways, with great potential to vastly increase its influence in the future. The complexity and rise of data in healthcare can be identified as the main reason for the establishment of ML in the area. Thanks to the many recent technical and scientific advances, high throughput screening, genomics, metabolomics, proteomics, and recently even radiomics, amounts of data that were not even remotely presumable until two decades ago are nowadays available, promoting ML as the ideal future state-of-the-art technology in healthcare. The most common healthcare use cases for ML include the automation of the diagnosis and prognosis processes, clinical decision support and the development of clinical care guidelines. For these applications, as well as for many others, the automatic develop- ment of predictive models, that is one of the most lively ML hot topics, play a crucial role.

The Nova Information Management School (NOVA IMS) has a vast experience in areas such as Data Science and ML, and a long tradition of applications and case studies of ML in the healthcare sector. Examples include, but are not limited to:

- the development of predictive models for important pharmacokinetic parameters in the drug discovery process [1,52,64,9,63,67,62];
- the development of predictive models for the unified Parkinson's disease rating scale assessment [12];
- the development of predictive models for the pathological complete response to neoadjuvant chemotherapy of breast and axilla cancer patients [6];
- the study of radiomics in rectal cancer [35];
- the development of predictive models for the relative position of computerized to- mography slices [11];
- the development of a system for supporting medical decisions for the treatment of rare diseases [2];
- the study of multiobjective metaheuristic to design RNA sequences [40] and of various methods for optimizing the parameters of multiple sequence align- ers [7,45,41,46,42,43,44];
- the development of predictive models for the effectiveness of music therapy [39];
- the development of gene regulatory networks reconstruction systems from time series datasets [16,66];
- the development of predictive models for anticoagulation levels in pharmacogenet- ics [8].

In this chapter, we summarize and critically revise the first three contributions of the previous list, consisting in the development of predictive models for pharmacokinetic parameters in drug discovery, for the unified Parkinson's disease rating scale assessment and for the pathological complete response to neoadjuvant chemotherapy of breast and axilla cancer patients. Besides the importance of the aforementioned applications in healthcare, our choice of those contributions is also motivated by the particular com- putational ML methods that were used: several novel developments of Genetic Programming (GP) [26,38] concerning the first two, and a novel regularization strategy, called priority-lasso [25] for the third one. Even though different between each other, these computational methods share the fact of being new and show extremely promising properties and results. Being general purpose algorithms, these methods can be used for a vast range of different applications.

The continuation of the paper is organized as follows: in Section 2, we briefly revise the ML methods used in the studies presented next. Section 3 summarizes our previous work on the prediction of pharmacokinetic parameters in drug discovery. In Section 4, we present our approach for predicting the unified Parkinson's disease rating scale. In Section 5, we discuss our study on the prediction of pathological complete response in breast and axilla tumours. Finally, Section 6 concludes the chapter.

2. A SHORT INTRODUCTION TO THE USED COMPUTATIONAL METHODS

2.1. Genetic Programming Improvements

Models lie in the core of any technology in any industry, be it finance, healthcare, man- ufacturing, services, mining, or information technology. The task of data-driven modeling lies in using a limited number of observations of system variables for inferring relationships among these variables. The design of reliable learning machines for data-driven modeling tasks is of strategic importance, as there are many systems that can- not be accurately modeled by classical mathematical or statistical techniques. Reliable learning in ML revolves around the notion of generalization, which is the ability of a learned model to correctly explain data that have not been used during the training pro- cess. Genetic Programming (GP) [38,27] is one of the youngest paradigms inside the computational intelligence research area called evolutionary computation and consists in the automated learning of computer programs by means of a process mimicking Dar- winian evolution. In GP a population of computer programs is evolved and, generation by generation,

stochastically transformed into a population composed by new, hopefully better, programs. After a random initialization of a population of computer programs, an iterative application of selection-variation-replacement operators is employed, aimed at improving the programs quality in a stepwise refinement way. The original definition of GP suffers from two major problems: bloat [52], i.e. the progressive growth in size of the models without a corresponding improvement in their quality, and the fact that GP search spaces are usually extremely complex [67].

The GP configurations discussed here are aimed at couteracting these two flaws. They are: DynOpEq and MutOpEq [52,64,9] and GS-GP [63,67]. All of them have been shown to enhance the "canonic" version of GP (i.e. the original GP formulation introduced by Koza in [26], referred to as standard GP, or ST-GP, from now on), even though each method does it differently and presents different pros and cons.

DynOpEq and MutOpEq. These GP versions are two different, although related, im- plementations of the Operator Equalisation bloat control technique [14,50]. Developed alongside the crossover bias theory, operator equalisation is one of the few bloat con- trol methods based on a precise theoretical study. By filtering which individuals are accepted in each new generation, this technique allows accurate control of the distribu- tion of program lengths inside the population, easily biasing the search towards smaller or larger programs. In the first published version of OpEq [14], the user had to specify the desired length distribution, called target, as well as a maximum allowed program length, and both remained static throughout the entire run. Each newly created individ- ual was accepted or rejected for the new generation based on its length and the target distribution. In [52], two more sophisticated implementations of the operator equal- ization technique were used for predicting the value of pharmacokinetic parameters in drug discovery. In the first one, called DynOpEq and first introduced in [50], both the target and the maximum allowed program length are self adapted along the run. Also, in DynOpEq the acceptance or rejection of the newly created individuals is based not only on their length, but also on their fitness. In the second variant of the operator equalization, called MutOpEq and first introduced in [51], no individual is ever rejected, but instead programs that are not acceptable are transformed by slightly mutating their genotype until they reach a length that fits the target distribution. Both these variants of the operator equalization have proven to be more than just a bloat control method, revealing novel evolutionary dynamics that allow, for the first time since the beginning of GP, a successful search without code growth.

GS-GP. In the last few years, several contributions have appeared focusing on the importance of integrating semantic awareness in the GP process. A rather complete survey can be found in [65]. Particularly relevant for the work presented here is contribution [34], where new genetic operators have been defined that, although acting on the genotype of individuals, have very precise consequences on their semantics. These operators, called geometric semantic operators, are particularly interesting because it is possible to prove that they induce a unimodal fitness landscape for any supervised learning problem (including regression). Nevertheless, these operators also present an important limitation: by construction, they produce larger offspring than their parents. As a consequence, after few generations the population is unmanageable because fitness evaluation becomes unbearably slow. In order to overcome this limitation, a new imple- mentation of GP using geometric semantic operators, identified with the name GS-GP (which stands for Geometric Semantic GP), was proposed in [63,67,10]. Nevertheless, also this approach leaves an important open issue: in order to reconstruct the individ- uals, an "unwinding" of the compact representation used by the algorithm is needed. Although this step can be done offline, after termination of a GP run, and only once for the best generated individual, given the large size of the individuals this step can be very computationally expensive, or even impossible. For this reason, so far, GS-GP has only been seen as a "black-box" system.

2.2. Priority Lasso

Logistic regression is a popular classification method that describes the relationship between one or more independent variables and a binary outcome variable *Y,* given by the logistic function:

$$p_i = P(y_i = 1|\mathbf{x}_i) = \frac{\exp(\mathbf{x}_i^T \boldsymbol{\beta})}{1 + \exp(\mathbf{x}_i^T \boldsymbol{\beta})}$$

where *xi* corresponds to the feature value for observation *i* (*p* is the number of features and *n* is the number of observations), *pi* is the probability of success for observation *i* and β = (β1, β2, … β*p*) are the unknown regression coefficients. The β parameters are estimated by maximizing the log likelihood function of the logistic model given by:

$$l(\boldsymbol{\beta}) = \sum_{i=1}^{n} \left\{ y_i \log p_i + (1 - y_i) \log[1 - p_i] \right\} + F(\boldsymbol{\beta})$$

with *F* (β) denoting the regularization term, which for the elastic net penalty takes the form:

$$F(\boldsymbol{\beta}) = \lambda\{\alpha\|\boldsymbol{\beta}\|_1 + (1-\alpha)\|\boldsymbol{\beta}\|_2^2\}$$

with λ > 0, which controls the penalization of the weights and 0 ≤ α ≤ 1 gives the balance between *L*1 and *L*2 norms, with the *L*1 part being responsible for achieving sparsity. For α = 0, leads to the ridge regression, for α = 1 it corresponds to the lasso regression. As previously mentioned, multiple types of data can improve the prediction accuracy. In order to incorporate all different types of data, different groups of features (*blocks*) should be considered. The extension of lasso, the priority lasso [25] builds a predictive model, based on different blocks of features. Let π = (π1, ..., π*M*) be the permutation of (1, ..., *M*) indicating the priority order, where *M* is the number of blocks. At a first step, the features from π1 are used to fit the lasso regression model with the highest priority. Following the notation of Equation (2), the regression coefficients β can be estimated by minimizing:

$$l(\boldsymbol{\beta}) = \sum_{i=1}^{n} \{y_i \log p_i + (1-y_i)\log[1-p_i]\} + \lambda^{\pi_1}\|\boldsymbol{\beta}^{\pi_1}\|_1$$

Secondly, lasso is applied to the block with second highest priority. In this step, a linear score obtained from the previous step is used to force the model with coefficient fixed to 1 (*offset*). Finally, in the third step, lasso is applied to the block with third highest priority, using the linear score of the previous step as an *offset*. This process is repeated until all blocks have been considered. The priority-lasso is a hierarchical approach, since blocks with higher priority tend to explain the largest part of the variability in the outcome.

3. PREDICTION OF PHARMACOKINETIC PARAMETERS IN DRUG DISCOVERY

The progress of pharmaceutical research in the last fifteen years has been very fast. Thanks to the technical and scientific advances in combinatorial chemistry, high throughput screening, genomics, metabolomics and proteomics, amounts of data that were not even remotely presumable until two decades ago

are nowadays available. This completely changes the picture of the drug discovery process, generating an impressive number of previously unknown target molecular structures. In modern drug discovery, after a phase of design of a structural fragment with therapeutic potency, libraries of millions of chemical compounds are tested and ranked according to their specific bio- logical activities. In this scenario, biological validation is, even more than in the past, an hazardous task which can, as indeed has happened recently, lead to failures [55,21]. This has lead to an increasing and tangible demand of new, reliable and sophisticated computational methods for the automatic assessment of drug discovery [18,36]. In par- ticular, it is clear that it is necessary to deeply characterize the behaviors of the phar- macological molecules in terms of Adsorption, Distribution, Metabolism and Excre- tion Toxicity (ADMET) [22]. The availability of reliable pharmacokinetics prediction tools permit to reduce the risk of late-stage research failures and enable to decrease the number of experiments and cavies used in pharmacological research, by optimiz- ing the screening assays. Furthermore, predictive ADMET models are of critical rele- vance for preventing adverse drug reactions like for instance those involved in the recent Lipobay-Baycol (cerivastatin) toxicity [59], that can be very dangerous for patients. Ge- netic Programming (GP) [26,38] has been a popular Computational Intelligent method in pharmacokinetics [30,18,36].

In this section, some published contributions investigating the usefulness of GP in predicting three important pharmacokinetic parameters are reviewed and dis- cussed [1,52,64,9,63,67]. Those three parameters are human oral bioavailability, protein binding level and median oral lethal dose.

- Oral bioavailability (%F from now on) is the parameter that measures the percent- age of the initial orally submitted drug dose that effectively reaches the systemic blood circulation after being filtered by the liver.
- Plasma protein binding level (%PPB) consists in the percentage of the initial drug dose which binds plasma proteins.
- Median oral lethal dose (LD50) refers to the amount of compound required to kill 50% of the test organisms (cavies), and it is currently one of the most used param- eters to quantify the toxicity of a candidate new drug.

3.1. Data

The datasets studied in this work consist in a collection of molecular struc- tures (used as inputs, or features) and the corresponding %F, LD50 and %PPB values (used as target values). The method that was used to build those datasets

was inpired by [15]. More in particular, the following steps were implemented to construct the datasets:

(1) A set of drugs and drug-like compounds with known values of the target param- eter (%F, LD50 or %PPB, according to the dataset that was going to be constructed) was considered; (2) for each one of these compounds, an encoding of its 2D struc- ture was used; (3) these encodings were successively transformed into vectors of num- bers, called molecular descriptors, used as features. The objective of the studied regres- sion problems is now to find a function that, applied to the molecular descriptors of a given compound, returns as output a reliable approximation of the target parameter for that compound. The set of compounds that was used has been extracted from a public database of Food and Drug Administration (FDA) approved drugs and drug-like com- pounds, presented in [47]. More in particular, in this dataset we have been able to find (and we have used) 260 compounds with known %F, 234 compounds with known LD50 and 662 compounds with known %PPB. The used encoding of the 2D structure of these compounds was a linear encoding called Simplified Molecular Input Line Entry Specification (SMILES) [47]. SMILES strings representing the 2D structures of the compounds with known %F have been transformed into vectors of bi-dimensional de- scriptors using ADMET Predictor (a software produced by Simulation Plus Inc.[53]). This software automatically produced vectors of 241 values for representing these com- pounds. The features for molecules with known values of LD50 and %PPB have in- stead been calculated using the on-line DRAGON software [56], which returned 626 bi-dimensional molecular descriptors. Thus, data have been gathered in matrices of 260 (respectively 234 and 662) rows and 242 (respectively 627 and 627) columns. Each row is a vector of molecular descriptors identifying a drug; each column represents a molecular descriptor, except for the last one, that contains the known values of %F (re- spectively LD50 and %PPB). In the discussed experiments, training and test sets have been obtained by randomly splitting the datasets. For each dataset, and for each different execution, 70% of the molecules have been randomly selected with uniform probability and inserted into the training set, while the remaining 30% form the test set.

3.2. Experimental Results in a Nutshell

DynOpEq and MutOpEq. In [52,64,9], DynOpEq and MutOpEq have been compared between each other and with ST-GP on the %F, LD50 and %PPB datasets. In all cases, both DynOpEq and MutOpEq were proven to outperform ST-GP. DynOpEq

was shown to have some overfitting, due to the fact that it tends to generate models that, although small (in terms of size) present a relatively high degree of functional complexity. On the other hand, although MutOpEq has a lower optimization speed, its behavior is more reliable and generalizes better compared to the other studied methods, probably because it is able to produce models that are, at the same time, small (in terms of size) and simple (in terms of computational complexity).

GS-GP. The results presented in [63,67], comparing GS-GP with ST-GP, are definitely the best ones that have been obtained so far on the studied pharmacokinetic problems from the viewpoint of the quality (in terms of RMSE) of the model returned by GP on unseen data. Not only GS-GP presents a regular and progressive improvement of fitness on the training set, as it was expected since it induces a unimodal fitness landscape. It also consistently outperforms ST-GP on test data for all the studied problems. Never- theless, GS-GP also has a very important open issue: the models it generates are very large (in terms of program size), and thus hardly readable. If on the one hand this is not a problem from the viewpoint of computational complexity (an extremely efficient implementation of GS-GP was presented in [10]), on the other hand turning GP into a sort of "black-box" system is undesirable and may be an issue. Investigating solutions for this problem is one of the main open issue of our future research.

4. PREDICTION OF THE UNIFIED PARKINSON'S DISEASE RATING SCALE ASSESSMENT

Neurological disorders, including Parkinson's disease (PD), affect profoundly the lives of patients and their families [5]. PD is a disorder of the central nervous system that leads to severe difficulties with body motions. It is the second most common neurode- generative disorder after Alzheimer's disease [13] and it is estimated that more than one million people in North America alone are affected by it [29]. Moreover, as explained in [31], because of the rapid increase in the average population age in several countries, and since the risk of contracting PD increases after the age of 60 [60], this number is expected to rise in the next few years. As a direct consequence, the medical care costs for patients with PD is estimated to rise in the future [23]. The currently available therapies aim at improving the functional capacity of the patient for as much time as possible; however they are not

able to modify the progression of the neurodegenerative process [54]. Most people affected by PD will therefore be substantially dependent on clinical intervention.

The process of tracking PD symptoms progression is a complex task. It often uses a system of measurement of the intensity of the symptoms called Unified Parkinson's Disease Rating Scale (UPDRS). The UPDRS is a scale that was developed as an effort to incorporate elements from existing scales to provide a comprehensive, efficient and flexible way of measuring and monitoring PD-related disability and impairment [37]. It assesses both motor disability and motor impairment. It reflects the presence and severity of symptoms, expressing it in a range from 0 to 176, with 0 representing a healthy state and 176 total disability. The UPDRS contains three sections:

- Mentation, Behavior and Mood.
- Activities of daily living.
- Motor.

The motor section of the UPDRS encompasses tasks such as speech, facial expres- sion, tremor and rigidity and expresses the severity of the related symptoms in a range from 0 to 108, where 0 represents a symptom free state and 108 denotes severe mo- tor impairment. For many persons affected by PD, the necessary specialized medical examinations to estimate the severity of their symptoms are difficult and invasive and they have to be performed by trained medical staff. Thus, as described in [58], symptom monitoring is costly and logistically inconvenient for patients and clinicians. All these critical aspects highlight the need of reliable and accurate computational techniques that allow estimating the UPDRS automatically and effectively. In [12], we present a comparative study of a set of computational methods aimed at predicting the severity of the PD symptoms in their entirety (i.e. including all of the three sections of the UPDRS) and the severity of the symptoms considered in the motor section of the UPDRS.

4.1. Data

This study makes use of the recordings described in [17] and in [58], where 52 sub- jects with idiopathic PD were recruited. A subject was diagnosed with PD if he/she had at least two of the following: rest tremor, bradykinesia (slow movement) or rigid- ity, without evidence of other forms of parkinsonism. The study was supervised by six US medical centers: Georgia Institute of Technology (7 subjects), National Institutes of Health (10 subjects), Oregon Health and Science University (14 subjects), Rush Uni- versity Medical Center (11 subjects), Southern Illinois University (6 subjects)

and Uni- versity of California Los Angeles (4 subjects). The selected subjects had at least 20 valid study sessions during the trial period. We used data from the remaining 42 per- sons affected by PD (28 of which males) with diagnosis within the previous five years at trial onset, with an age range expressed as mean ± std. equal to 64.4 ± 9.24, min. 36, max. 85, median 65 years. All subjects remained un-medicated for the six-month dura- tion of the study. The UPDRS was assessed at baseline (onset of trial) and after three and six months. At baseline the scores were 19.42 ± 8.12, min. 6, max. 36, median 18 points for motor-UPDRS and 26.39 ± 10.80, min. 8, max. 54, median 25.5 points for total-UPDRS. After three months: 21.69 ± 9.18, min. 6, max. 38, median 21 points for motor-UPDRS and 29.36 ± 11.82, min. 7, max. 55, median 28 points for total-UPDRS and after six months: 29.57±9.17, min. 5, max. 41, median 20 points for motor-UPDRS and 29.57 ± 11.92, min. 7, max. 54, median 26 points for total-UPDRS.

Feature	Description
Age	Subject Age
Sex	Subject gender "0" – male, "1" – female
MDVP:Jitter(ABS)	Kay Pentax MDVP absolute jitter in microseconds [3] Kay Pentax MDVP
MDVP:RAP	relative amplitude perturbation [3]
MDVP:PPQ	Kay Pentax MDVP five-point period perturbation quotient [3]
Jitter:DDP	Average absolute difference of differences between cycles, divided by the aver- age period [3]
MDVP:Shimmer	Kay Pentax MDVP local shimmer [3]
MDVP:Shimmer(dB)	Kay Pentax MDVP local shimmer in decibels [3] Three-point amplitude
Shimmer:APQ3	perturbation quotient [3] Five-point amplitude perturbation quotient [3]
Shimmer:APQ5	Kay Pentax MDVP 11 point amplitude perturbation quotient [3]
MDVP:APQ	Average absolute difference between consecutive differences between
Shimmer:DDA	the am- plitudes of consecutive periods [3]
NHR HNR RPDE	Noise to harmonies ratio [3] Harmonies to noise ratio [3] Recurrence period density entropy [32] Detrended fluctuation analysis [32]
DFA D2 PPE	Correlation dimension [24]
	Pitch period entropy [31]

Table 1 – Features in the considered dataset. MDVP stands for (Kay Pentax) Multidimensional Voice Program.

As described in [17], the data was collected using the Intel At-Home Testing De- vice (AHTD), which is a telemonitoring system designed to facilitate remote, Internet- enabled measurement of a variety of PD-related motor impairment symptoms. The data were collected at the patient's home, transmitted over the internet and processed ap- propriately in the clinic to predict the UPDRS score. The AHTD

contains a docking station for measuring tremor, paddles and pegboards for assessing upper body dexter- ity, a high-quality microphone headset for recording patient voice signals and a USB data stick to store test data. A liquid-crystal display (LCD) displays instructions for tak- ing the tests. Typical audible prompts instruct the patient to undertake tasks to measure tremor, bradykinesia, complex coordinated motor function, speech and voice. As part of a trial to test the effectiveness of the AHTD system in practice, people affected by PD were recruited and trained to use the device. Subsequently, an AHTD was installed in their home and they performed tests on a weekly basis. Each patient specified a day and time of the week during which they had to complete the protocol, prompted with an au- tomatic alarm reminder on the device. The collected data was encrypted and transmitted to a dedicated server automatically when the USB stick was inserted in a computer with internet connection. The audio recordings are of two types: sustained phonations and running speech tests in which the subject is instructed to describe static photographs displayed on the AHTD's screen. They were recorded using a head-mounted micro- phone placed 5 cm from the patient's lips. The AHTD software was devised such that an initial audible, spoken instruction followed by a "beep" prompted the subject to be- gin phonation: an audio amplitude threshold detector triggered the capture of audio and subsequently the capture was stopped one second after the detected signal amplitude dropped below that threshold, or 30 seconds of audio had been captured. Further details about the AHTD system and trial protocol can be found in [17].

4.2. Experimental Results in a Nutshell

Besides standard GP and GSGP, that have been discussed previously, several other ML algorithms have been tested on this application, among which linear regression [68], least square regression [49], radial basis function network [19], isotonic regression [20], and support vector machines [48] with first and second degree polynomial kernels. The results of the experimental comparison among these ML algorithms for the prediction of the UPDRS are summarized in Figures 1 and 2. More in particular, Figure 1 reports the training and testing results obtained for the motor part of the UPDRS dataset, while Figure 2 reports the analogous results for the total dataset. In both figures LIN stands for linear regression, SQ stands for least square regression, RBF stands for radial basis function network, ISO stands for isotonic regression, SVM-1 refers to the support vector machines with polynomial kernel of first degree and SVM-2 refers to the support vector machines with polynomial kernel of second degree.

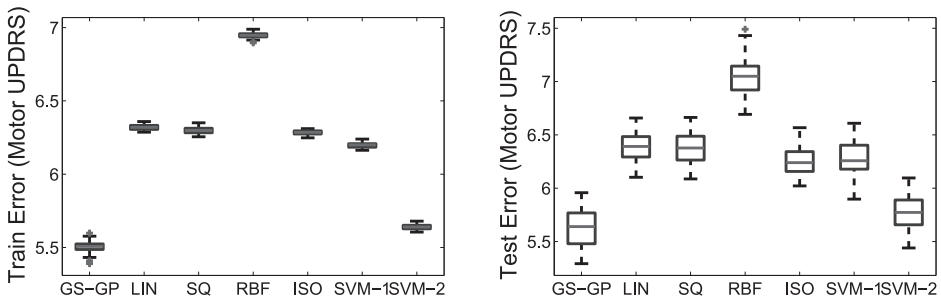

Figure 1 – GS-GP vs other state-of-the-art ML methods. Results obtained for the Motor-UPDRS. Boxplots of (a) training and (b) test fitness for 50 independent runs of the studied ML techniques.

From these figures it is possible to see that GS-GP performs better than all the other considered ML methods. These results confirm that GS-GP is able to outperform the state-of-the-art in the prediction of PD symptoms progression, thus fostering GS-GP as a new concrete support tool for PD treatments. The comparatively small errors returned by GS-GP with respect to other state-of-the-art methods is notable and demonstrates that the sustained vowel phonations convey sufficient information to predict UPDRS to clinically useful accuracy. The predictions obtained by GS-GP are comparable to, and in some conditions even better than the clinicians' observations and pave the way to relevant practical implications in the future, which, for instance, may involve the development of an embedded system working on the currently used At-Home Testing Devices (AHTD).

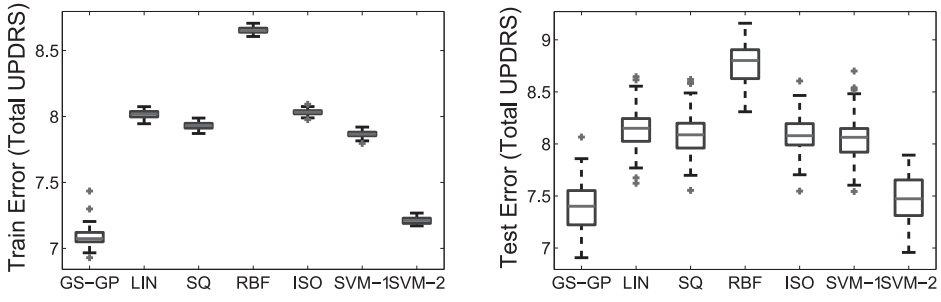

Figure 2 – GS-GP vs other state-of-the-art ML methods. Results obtained for the Total-UPDRS. Box- plots of (a) training and (b) test fitness for 50 independent runs of the considered ML techniques.

5. REGULARIZATION TECHNIQUES IN RADIOMICS: PREDICTION OF PATHOLOGICAL COMPLETE RESPONSE IN BREAST TUMOURS AND THE AXILLA

Radiomics, recently proposed in [28], is a recent development of medical imaging, partcularly used in oncology, based on the extraction of a large number of imaging fea- tures, that could contain complementary information and provide a comprehensive view of a tumour. In this sense, the objective of radiomics is to convert imaging data into a high-dimensional mineable features. Due to the high-dimensionality of this type of data, a natural choice is the use of ML algorithms to extract knowledge from data, possibly without any assumption on the shape and characteristics of the model [4]. Some ML approaches have been considered so far. However, due to the nature of the data, regu- larized optimization techniques have revealed their importance [57,69]. Regularization techniques usually work by adding constraints to the cost function, with the objective of improving the generalization ability of the model. Ridge, lasso, and elastic net are the most common examples of regularization techniques [69]. In the context of can- cer diseases, heterogeneous types of information (genomic, radiomic and clinical) are very common, and the inclusion of all information may improve the prediction accu- racy of the model. Nevertheless, each type of data has generally a different structure. To overcome this problem, [25] proposed a hierarchical approach to predict a clinical out- come using different types of omics data, called priority-lasso. This method is similar to the lasso regularization technique, but takes into account groups of variables (blocks). The idea is to prioritize blocks that explain the largest possible part of the variability in the outcome. In this section, the work in [6] is revised. In that work, we evaluated the predictive performance of logistic regression in predicting pathological Complete Response (pCR) of breast cancer patience, using different regularization techniques, namely, the ridge, lasso and priority-lasso.

5.1. Data

131 patients diagnosed with early breast cancer underwent breast Magnetic Resonance Imaging (MRI) examination at the Breast Unit of the Champalimaud Clinical Center before receiving neoadjuvant chemotherapy (NAC). After NAC, at the time of breast surgery, 58 patients presented with pCR and 73 without

pCR, while for the axilla 31 pa- tients had pCR and 36 had no pCR. Notice that in the axilla pCR dataset patients without nodes were removed. Apart from the radiomic features, the dataset was composed of demographic, clinical and histopathological data. The clinical variables were nodal clin- ical status, tumour stage and menopausal status. For the histopathological features, four tumour variables were considered (tumour morphology, histological grade, subtypes and biological subtypes); age was the only demographic feature considered and for the radiomics features 4300 variables were used (2150 extracted from the tumour and 2150 extracted from a 5mm peritumoural zone). The Pyradiomics, was used for the extraction of the radiomics features from medical images [61]. Arterial phase from the dynamic contrast enhanced MRI sequence was used to delineate the tumour by two radiologists (M.L. with 11 years of experience and A.U., a last year radiology resident). A second region of interest (ROI) comprising peritumoural tissue was created automatically from the tumour segmentation, comprising a zone with 5mm of thickness. Both tumoural and peritumoural ROIs were used to extract radiomic features (first order, shape, gray- level co-occurrence matrix (GLCM), gray-level run length matrix (GLRLM), gray-level size zone matrix (GLSZM), neighbouring gray-tone difference matrix (NGTDM), and gray-level dependence matrix (GLDM) of both original and filtered images – Lapla- cian of Gaussian, LoG, with sigma 1mm, 2mm, 3mm; wavelets – level 1 and 2) using Pyradiomics. Recursive feature elimination (RFE) was used to find the most important features. A stability analysis for the radiomic features was conducted, using the intra- class correlation coefficient (ICC) with a threshold of 0.81, selecting 3023 out of 4300 radiomic features. Based on the correlation matrix, the absolute values of pair-wise correlations were obtained. Only features with low correlation were considered, using a threshold of 0.95. All radiomic features were normalized based on the z-score, and zero-variance and near-zero-variance features were removed from the analysis.

5.2. Results in a Nutshell

Breast pCR. Table 2 reports the results obtained using logistic regression for tumour pCR, without grouping features. The first line shows the results achieved with recursive feature elimination (RFE), that selected 10 most important features;"CV" stands for the mean score obtained on the validation set, while "Testing" is the score obtained on the test set, both averaged over 100 executions. From Table 2, at least two facts can be seen:

	lasso	ridge	No regularization
With RFE	ROC cv: 0.84(+/-0.03) ROC testing: 0.85(+/-0.06) PR cv: 0.86(+/-0.02) PR testing: 0.87(+/-0.06)	ROC cv: 0.76(+/-0.06) ROC testing: 0.77(+/-0.09) PR cv: 0.80(+/-0.05) PR testing: 0.79(+/-0.09)	ROC cv: 0.70(+/-0.06) ROC testing: 0.69(+/-0.07) PR cv: 0.76(+/-0.05) PR testing: 0.77(+/-0.10)
No RFE	ROC cv: 0.84(+/-0.03) ROC testing: 0.84(+/-0.06) PR cv: 0.86(+/-0.03) PR testing: 0.86(+/-0.06)	ROC cv: 0.57(+/-0.05) ROC testing: 0.58(+/-0.07) PR cv: 0.66(+/-0.05) PR testing: 0.66(+/-0.07)	ROC cv: 0.58(+/-0.05) ROC testing: 0.57(+/-0.08) PR cv: 0.64(+/-0.04) PR testing: 0.63(+/-0.10)

Table 2 – AUC scores on breast tumour for lasso, ridge and no regularization of logistic regression, for the Receiver Operating Characteristics (ROC) and the Precision Recall (PR)

first, lasso is the method that provides the highest performance, regardless the use or not of RFE. Secondly, in practically all the presented experiments, the results obtained on the validation set are very similar to the ones obtained on the (hold-out) test set, which indicates no overfitting. When considering different types of features (age, clin- ical/pathological, radiomic), using priority-lasso, the results show that the ROC AUC values for train and test depend on the block that is prioritized. For this analysis, two blocks were considered: one block for the age, clinical/pathological features and a sec- ond block for the radiomic features. If demographic, clinical/ pathological features were prioritized, the ROC AUC values for train and test were 89.17% and 78.57%, respec- tively. When priorities were switched and radiomic features block had the highest pri- ority, the ROC AUC values were 87.79% and 74.6%, for the training and test cohorts, respectively.

5.3. Axilla pCR

Table 3 reports the results obtained for axilla pCR. This table confirms that penalization based on *L*1 is preferable for the studied data and modifications based on lasso are appropriate. PR AUC is not added to the table as data is well balanced and ROC AUC is more representative. For the priority-lasso, the blocks considered were the same as the ones mentioned in the previous section. The results show that if the block with the highest priority was regarding the demographic and clinical/ pathological features, the ROC AUC values for training and test cohorts were 87.01% and 87.5%, respectively. When priorities were switched and radiomic features block had the highest priority, ROC AUC values were 93.83% and 86.11%, for training and test cohorts, respectively.

	lasso	ridge	No regularization
With RFE	ROC cv: 0.67(+/-0.06) ROC testing: 0.70(+/-0.09)	ROC cv: 0.60(+/-0.06) ROC testing: 0.64(+/-0.10)	ROC cv: 0.59(+/-0.07) ROC testing: 0.57(+/-0.09)
No RFE	ROC cv: 0.66(+/-0.05) ROC testing: 0.70(+/-0.08)	ROC cv: 0.58(+/-0.04) ROC testing: 0.60(+/-0.08))	ROC cv: 0.58(+/-0.05) ROC testing: 0.57(+/-0.09)

Table 3 – AUC scores on axilla dataset for lasso, ridge and no regularization of logistic regression, for the Receiver Operating Characteristics (ROC)

6. CONCLUSIONS AND FUTURE PERSPECTIVES

A set of studies on the application of machine learning to healthcare problems, re- cently developed at the Nova Information Management School, have been presented in this chapter. In particular, we have presented the development of predictive models for pharmacokinetic parameters in drug discovery, for the unified Parkinson's disease rating scale assessment and for the pathological complete response in breast and axilla cancer. These three research tracks have been characterized by the successful employment of novel and sophisticated machine learning algorithms, such as three improvements of the standard version of genetic programming, and a regularization strategy called priority- lasso. These algorithms have been demonstrated to consistently outperform the state of the art in the studied applications. Furthermore, being general purpose algorithms, they all can be applied also to other applications in the future.

Thanks to studies and results like the ones presented in this chapter, it is nowadays clear that artificial intelligence is destined to play an important role in healthcare in the future. In the form of machine learning, it is our most promising tool for the develop- ment of precision and personalized medicine, widely agreed to be a much in demand advance in care. The greatest challenge to artificial intelligence, in particular in healthcare, is not whether the technologies will be capable enough to be useful, but rather ensuring their adoption in daily clinical practice. For these technologies to be widely adopted in clinics and hospitals, in fact, for instance artificial intelligence systems and models must be approved by regulators, integrated within existing platforms, standardised to a sufficient degree that similar methods work in a similar fashion, taught to clinicians, paid for by public or private organisations and updated over time, consistently with the research advancements in the field. These challenges will eventually be overcome, but they

will take much longer than it will take for the technologies themselves to mature. As a result, and despite the potential clearly demonstrated by the current state of the art of research, we expect to see limited use of artificial intelligence in clinical practice within 5 years, and hopefully a more extensive use within 10. Last but not least, it also seems increasingly clear that artificial intelligence will never replace human clinicians, but rather integrate and support their work, thus improving their efforts to care for patients.

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WHICH MEDICAL PROFESSIONAL?

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INTRODUCTION

The 2020-2021 SARS-CoV-2 pandemic has shown it clearer than ever that the world is infinitively more complex than it appeared[1]. The more complex the world becomes, the most difficult it is to complete something without cooperation, supporting the holistic and conjunctive perspective described by Ludwig von Bertalanffy that the *whole is greater than the sum of the parts*[2].

In recent years, medical attention has shifted from acute-illness hospital-based care towards a bigger stand on prevention and management of chronic non-communicable diseases by primary care facilities. Hospital-based care also aimed to reduce inpatients' stay durations. All of a sudden, in the midst of a global pandemic of unforeseen dimensions, all objectives were let go in face of a crisis that put health systems to a complex and extreme test. Overnight, medical professionals were faced with an acute increase of a transmissible disease that affected more severely patients with chronic co-morbidities.

The increase in complexity was even more evident with the consequent direct intervention of national and international politicians in medical activity. Thus, if there were doubts that the classic paradigm of medical professionals' autonomy, authority and expertise was shifting to that of interdependency, collaboration or change management[3] within complex contexts, they have completely vanished.

In addition to this change, the pandemic also exposed the impact of decisions that are not based on science, the need for training to produce the best scientific evidence, the dependence on basic scientists to develop diagnostic tests and vaccines, as well as the importance of physicians being simultaneously a public communicator and opinion leader in a digital world with no limits on access to information and no filter to available resources, whether they are accurate or not.

This context emphasized the relevance of the discussion initiated by the NOVAhealth platform at NOVA University Lisbon in 2017, based on the premise that universities have a duty to be ahead of time in thinking about what will be relevant in terms of the training of health professionals.

ONGOING RESEARCH

NOVA University Lisbon's scientific competences in the health field range from biomedical research to health policies through technologies and clinical research, with an important focus in medical professionals.

A session launched in 2017 by NOVAhealth intended to bring to NOVA University Lisbon the discussion on strengthening research training in the area of health sciences, a debate that is also being carried out at a European level. In addition to strengthening research skills, it was also assumed that medical professionals would have to develop new skills, concerning new health-related professions and with mixed professional careers shared between different institutions (e.g., research, healthcare units, industry, etc.).

The unique characteristics of research and training in health at NOVA University Lisbon give it greater responsibility in anticipating the training needs of health professionals, and in particular, medical doctors.

In this sense, the NOVAhealth platform organized a workforce for the production of recommendations, currently led by Vice-Rector Professor José Fragata. The workforce, including its Advisory Board, brings together representatives from all areas of research/training in healthcare of NOVA as well as employers from health units affiliated with NOVA University Lisbon.

The work program defined two phases: a first one consisted in a research project aimed at identifying emerging areas in medical education (NOVA AREMED) and a second sequential step, to incorporate these areas, not only in medical education (pre and post-graduated) but also foreseeing new health professionals as well as new skills and capabilities.

A qualitative research methodology was designed to obtain information from society (not just specialists in medical education) in a bottom-up process using NOVA's community, including alumni. Focus groups were the chosen qualitative method to explore and understand NOVA's community experiences and reported satisfaction with several healthcare services dimensions. The purpose of choosing this method was firstly because it is highly recommended for educational research and produces in-depth interactional data[4], being able to identify less evident concepts and topics, which is the main advantage of this method. Additionally, this method[5] also enabled to evaluate actions to be developed within the scope of the NOVA AREMED project.

The study featured 15 focus groups with a total of 115 participants that were divided in two main groups: internal to medical school stakeholders (IMSS) and external to medical school stakeholders (EMSS). The first group included medical students, residents, medical faculty and specialists with 5 to 10 years of experience. The second one contained biomedical researchers, private and public health employers, medical school/other schools academics (not physicians), university employees, and also health managers, technologies and pharmaceutical industry.

Questions in the focus groups inquired about *experiences and reported value, criteria to assess the quality of a medical act*, *criteria for choosing a doctor, thoughts about how a doctor could improve*, *how to prepare future doctors and what are the main emergent fields in medical education and training*.

In this chapter, we present the model that emerged from the analysis of the data obtained in the first sub-project that answers the following question: *how does health beneficiary experiences and reported satisfaction contribute to new strategies of medical education*? We also established a comparison between these results and two previous seminal works, both based on the perspectives of doctors and academics with interests in medical education – CanMEDS 2015 Framework and Pennsylvania State Medical School Model[7].

Data analysis was performed following thematic coding analysis[5], considering two different levels. The first level was data-driven (attaching labels to groups of words) and the second level followed a deductive approach (coding groups into a smaller number of themes, then into aggregate dimensions). Themes and aggregate dimensions were based on a theoretical approach that requires a comparison with theoretical research, called deductive perspective[5,8]. This encounter between inductive and deductive is called abduction[8].

STATE OF THE ART REVIEW

Healthcare organizations are considered one of the most complex human systems in today's societies[9,10], being shaped by the diversity of perspectives of its professionals and their dynamic relationships inside the systems[11,12] defined. work schedules or interactions with patients are an example of such relationships[3]. Accordingly, complexity is an inevitable feature of professional services[12] with its inner tensions and contradictory perspectives[9,12] between society, organizations and professionals[12] described as highly differentiated healthcare worlds of cure, care, community and control[9,10].

Healthcare systems are known as complex adaptive systems (CAS)[11,13,14]. These systems are composed of a) diverse agents that learn, b) nonlinear interdependencies, c) self-organizing agents, d) emergence, and e) co-evolution[11]. These characteristics call for a paradoxical lens of "the one and the many"[2] rather than a view of "either-or thinking"[2]. Additionally, healthcare systems are CAS once things are often "unique" and may "not proceed as predicted"[11]. Together, the loosely precarious coordination between the parts[14-16] and the dynamism and unfolding of things

in unpredictable ways[11,14] result in an emerging organizing that highlights the social nature[12,16] and the complexity of healthcare organizations[11] beyond the appealing metaphor of systems depicted as "rational machine" or "heart as a pump"[2,17] uphold on additive (e.g., sum of its parts) and predictability (e.g., certain input always result in an expected output)[11,13,14,17] assumptions.

In light of the complexity science approach[11,14], physicians and healthcare systems are challenged to embrace complexity[2,18] through the need of a complexification of professionals' vocabulary, procedures and thoughts[18] in order to further capture unexpected phenomena[16,17,19] and act accordingly. Additionally, literature also points to the need for practitioners to embrace new professional values and expectations (e.g., interprofessionalism, lifelong learning, change management) beyond the traditional principles of autonomy, authority and expertise[3]. Complexity science approach contrasts with the prevailing Newtonian-style of thinking in medical training, characterized by simplification[2,14,15,17] of the "reduce-and-resolve"[17,14] assumption that is often translated into procedural rules and controlled experiences that in association with strict case studies may limit situational learning and intuitive skills[20], essential to interpret (i.e., sense and make sense)[16] weak signals[19] that may not fit the expected patterns[20]. Future doctors need a range of diverse responses (i.e., past experiences) intuition and attention to unspecific signals that add up with her/his capabilities (e.g., doubt, inquiry, argumentation and deliberation)[16]. Such combinations help physicians to predict and adapt to situations that may involve failure[16,20].

Complementarily, the objectives of the present work also aim to use health beneficiaries' experiences to frame and broaden the repertoire of competences and capabilities in medical education, considering the literature co-production concept[21,22] within the service management perspective. Co-production is in line with the need of healthcare delivery services to change from a predominant provider-focus and disease-driven approach towards a more patient-centered care[24,23] highlighting that care must be respectful and open to patients' expectations, needs and values through a clinician-patient indispensable collaboration.

A pivotal work on medical curricula based on Canadian reality and subscribed by the Royal College of Physicians and Surgeons of Canada, called CanMEDS[6] together with another relevant and innovative work developed in the United States of America, within the area of medical education curriculum from the Pennsylvania State University[7], support a comparison with the NOVA AREMED study findings.

The CanMEDS 2015, currently on its third edition, used a multi-level approach through a National Advisory Committee, an Integration Committee, several Expert

Groups, an International Advisory Committee and a Royal College French Advisory Committee to achieve a general framework for medical curricula. Using this collaborative model, the framework defined 6 dimensions that a Medical Expert (a CanMeds role itself) should feature: Professional, Scholar, Health Advocate, Leader, and Collaborator, Communicator[6]. The work from Dr. Gonzalo and colleagues, from the Pennsylvania State University, defined a third pillar for medical education called "system science"[7] beyond the two classical ones: the clinical and biomedical science. Thus, system science encompasses seven different modules to frame medical curricula, namely: 1) patient navigation principles, 2) healthcare delivery, 3) healthcare reform, 4) population health management and public health, 5) socio-ecological medicine and determinants of health, 6) high-value care delivery, and lastly 7) delivery and change agency[7]. The work also identified two threads to be embedded within said modules – teamwork and collaboration and evidence-based medicine[7].

RELEVANT CONTRIBUTION FROM NOVA

The focus groups discourses grounded on beneficiaries' reported experiences provided several insights for understanding the opposing challenges that medicine faces at the operational level during the process of service delivery. Based on the concepts of complexity and co-production paradigms a model was produced that frames the four emerged dimensions in two contrasting pairs, as depicted in Figure 1: (1) clinical and integrated structures (focusing routines and guidelines) *versus* holism and diversity (making sense of the entire phenomena) and (2) expertise vigilance (emphasizing clinical specialization) *versus* medical proximity (humanity and collaboration).

Medical proximity refers to physicians' need to co-produce with patients regarding their needs, expectations and roles (intuitions, skills, knowledge). These are visible in empathy and humanizing. Internally, among colleagues, physicians need to adjust, communicate and have open skills in teamwork. In sum, it boils down biomedical variables together with biosocial overlapping system.

Expertise vigilance means that physicians need to tolerate ambivalence and lack of order using complex capabilities and strategies to be vigilant towards patients' co-evolution. Other core competences of this dimensions base themselves on doctors' expertise knowledge, proficient and effective vigilance training as well as technical and technological update and clinical knowledge from undergraduate to lifelong learning.

Holism and diversity dimension practitioners need to tolerate ambivalence and unpredictability using complex capabilities such as intuition, attuning to distortions in patterns or to slow down when things do not fit the expected pattern. Patients' feedback-loop can give practitioners the control over unpredictable evolution of symptoms and therapies.

Clinical and integrated structures routines rationalize the approach to the patient. Protocols and guidelines are deployed in a rather linear way, considering the patients' needs, mostly from an integrated clinical perspective. Clinicians need to manage the multiple distracting stimuli in a thoughtful way which is intellectually and emotionally absorbing.

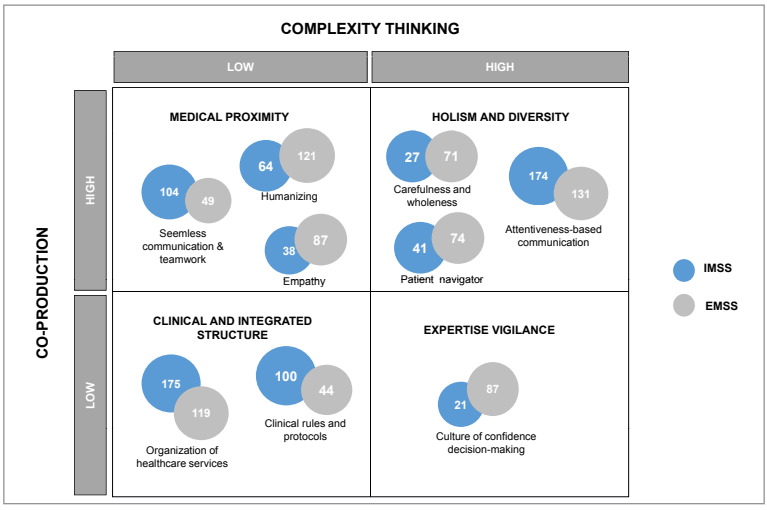

Figure 1 – Model for medical education that depicts the four emerged dimensions in two contrasting pairs framing data (number of records) obtained from focus groups on health beneficiary experiences and reported satisfaction.

In a glance, the distribution of data (number of records) shown in Figure 1 reveled a variability among the relevance of each dimension and its components (second-order themes, e.g., empathy). Accordingly, the dimensions in the high co--production quadrant were those most frequently identified by the participants, suggesting that they need to be improved. In contrast, regarding the dimension of high complexity thinking but low co-production (vigilance decision-making), only one component was identified (e.g., culture of confidence decision-making). Although it seems to be better covered by clinicians and medical curricula, there is openness to improve the patient collaboration in decision-making process.

Additionally, Figure 1 also shows that medical participants (IMSS) value dimensions differently from others (EMSS, mostly outside the NOVA Medical School faculty). For example, humanization is much more valued by external people than by internals, while the existence of clinical rules and protocols is much more highlighted by doctors. These different perspectives emphasize that medical viewpoint and patients' needs and expectations are both important. Thus, the design of a medical curriculum that properly addresses contemporary society challenges needs to incorporate overlapping social systems contributions beyond medical education expertise.

In summary, based on 15 focus group dedicated to health beneficiary experiences an innovative paradoxical model was drawn for complexity and co-production on the light of competing needs and demands that healthcare services require.

IMPACTS TO SCIENCE AND SOCIETY

To emphasize the added value and estimate the impact of the model presented above, we performed a comparison between NOVA AREMED dimensions and the two other previous works about medical curricula innovation, above referred, resulting from two distinct realities: the USA[7] and Canada[6] (Figure 2).

The topics identified in the present work through the account of the beneficiaries' experiences that were exclusive of NOVA AREMED are: 1) carefulness and wholeness, 2) empathy and 3) patients' protocols and pathways. Those emerged only in the NOVA AREMED study and have not been identified by medical educators as they correspond mainly to a patient centered-perspective brought by the inquired health beneficiaries. This contribution illustrates the importance of the co-production[21,22] concept in the training of health professionals regarding patient's needs, expectations and values to the process of service delivery.

Moreover, the results of focus groups were based on individual reported experiences and expectations of 115 participants with a mean age of 38 years old. Once more, they did not identify needs of health management and public health most probably due to a low prevalence of chronic diseases in this young sample, and also because public health specialty is upstream from the medical-patient relationship. Moreover, the study was performed before the COVID-19 pandemic. It may be expected that if it was repeated nowadays, this dimension would have been marked by the impact that public health issues have had in the last year.

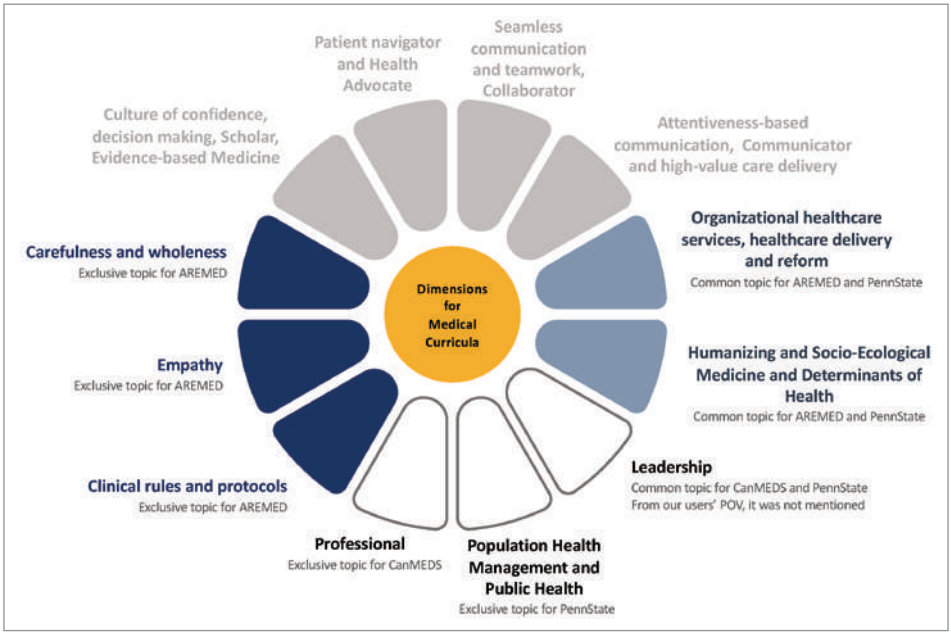

Figure 2 – Comparison between medical education components previously described in CanMEDs[6] and Penn State[7] studies and those identified in the Model proposed by the present work. Color code: **grey**: common to all studies; **light blue**: common to NOVA AREMED and other study; **dark blue**: identified by NOVA AREMED; **white**: identified by other studies but not acknowledge in NOVA AREMED.

It is also interesting to note that leadership and professionalism have not directly emerged from the experiences of the beneficiaries, although they appear in the perspectives of medical educators[6,7]. It may be assumed that in the context of the beneficiaries' sample experiences, the lack of such characteristics, leadership and the professional, are systemic, multilevel and distributed over other identified dimensions (e.g., organizational health care delivery, patients' protocols and pathways, expertise decision-making). Although leadership and professional role were not perceived from the beneficiaries' point of view, these dimensions should be integrated in the medical curriculum. Once the literature points that the medical system[10,12,16] is the most influential one in healthcare organizations, practitioners must ethically behave (e.g., conflict of interest) enable the process of dynamic interactions between all autonomous and self-organizing agents, mainly colleagues, patients, stakeholders, and also be a reliable source of information.

CONCLUSION

The analysis of beneficiaries' reported experiences and the participation of external elements to medical schools constituted an innovative and patient-centered method that allowed for the identification of new dimensions to be incorporated in lifelong medical education.

In the present work, the concepts of complexity, uncertainty and ambiguity were introduced by NOVA AREMED, as well as the co-production dimension that is translated into patient carefulness and wholeness, empathy and patients' protocols and pathways, as important components of medical expertise. The NOVA AREMED particularly reinforced innovative components of medical curriculum related with high levels of complexity and co-production mainly, the patient navigator, the seamless communication and teamwork, and finally the attentiveness-based communication towards patients.

Globally, new and already known dimensions were organized in a new conceptual model that unveils complexity and co-production assumptions and contributes to forthcoming doctors enlarging their professional values, expectations and capabilities towards healthcare dualities.

Further research will look into additional concepts on how to better plan, enrich and prepare future doctors by taking into account their needs as well as patients and the system in which they will be included. Analysis of two additional order of questions already addressed in the focus groups about expectations and preferences about the ideal medical act and future areas of health professionals is ongoing.

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MEDICAL EDUCATION OR THE CONFLUENCE OF TWO RELATED AREAS

Patrícia Rosado Pinto

INTRODUCTION

The links between medicine and education have often been considered natural and mutually beneficial. In fact, several values and practices are common to the two domains: a caring and reliable interaction with others (patients, students, parents, caretakers, other professionals working in the same field); a holistic approach towards the patient or the student; special adequate and aligned communication with diversified target audiences (in age, gender, life experiences, socio-economic and cultural backgrounds); professional training in an on-the-job format, just to mention a few examples.

Furthermore, both professions exhibit a specific "praxis" deeply rooted in sound conceptual frameworks, most of the times emerging from traditional, "basic", sciences or disciplines. Moreover, the nature of these professional practices, based on sustained and solid reasoning, has led to countless debates about the core of the medical and teaching professions, with an endless search for balance between art and science.(1)

Several examples of cross fertilization between medicine and education can be pin-pointed such as the concept of medical education itself, related to the education and professional development of a competent medical practitioner, from the initial training (medical school and internship) to the additional training thereafter (residency, fellowship and continuing medical education).(2) Another example is the notion of Best Evidence Medical Education (BEME) taken from the concept of "evidence-based medicine" and defined in the first BEME Guide as "the need to move from opinion-based education to (...), the implementation, by teachers in their practice, of methods and approaches to education based on the best evidence available".(3 p.3) On the other hand, one can identify numerous contributions to medical education and medicine, from educational research on several domains, namely human cognition and human behaviour.(4)

It will never be too much, however, and for the sake of rigor, to deepen this taken for granted relationship. Above all, and in what concerns medical education research, referential concepts and methodology in both fields are still far from harmonization, namely because, as it is stated by some authors "research designs that many in the clinical sciences often perceive as 'weak' are entirely appropriate in education research fields".(5 p.150)

The generous invitation from Professor Fragata offers me the opportunity to further reflect on the subject and to share the experience of the creation and

development of the Medical Education Office of the Faculty of Medical Sciences (FCM)/ Nova Medical School (NMS), as a tangible example of a bridge between medicine and education.

Moreover, it seems of the most elementary justice, to pay tribute to Universidade Nova de Lisboa and to those who had the vision to start at NMS (FCM at the time) an institutional structure, aiming to enhance teaching and learning. In fact, this was the first structure of its kind to be formally created at NOVA, due to the vision and initiative of Professors Nuno Cordeiro Ferreira and António Rendas and to the involvement of Professors Ramiro d'Ávila and Graça Morais. I accepted to collaborate from the first hour, and later to coordinate this office and today, a little further away from the NMS due to other functions performed at the University, I witness with great pride the vitality of the current office and the professional quality of all those who are part of it.

HIGHER EDUCATION TODAY

Higher Education (HE) has evolved in the last decades, living an increasing focus shift, from teaching to learning. As it is stated by Vieira(6), there is a broader movement to build new meanings for higher education, rooted in profound changes in the role that universities play today. According to Cross(7), this new situation is essentially due to specific factors, such as the growing demand on the part of the community, in what concerns the diversification and flexibility of the graduates' and post-graduates' skills; the significant advances in research on cognition and learning, including how adults and young adults learn and, finally, the diversity of students and of their learning sources and resources.

Consequently, and in what concerns teaching capacity, HE professors are asked to exhibit a multiplicity of competences, in addition to those resulting from the scientific or professional area in which they perform their activity – deep knowledge of the objectives and concepts of their own discipline; the ability to transform them into learning contents, using pedagogically consistent formats; the ability to use appropriate forms of assessment and, finally, the capacity to critically review and reconstruct their teaching.(8)

According to Biggs and Tang(9), only the professional development of the HE professor will enhance these competences, in areas that are unknown to him. In fact, complementary to their scientific capacities (teachers should always be experts

in the scientific areas they teach), HE professors are supposed to be proficient in planning their own courses, selecting the most appropriate teaching methods, as well as the most adequate evaluation tools to assess learning and offer feedback to their students.(10) Finally, professors are still required to assume a scholar attitude of reflection and evaluation of their own pedagogical practice.(11)

And yet, due to constraints of various kinds and specific academic cultures, still exclusively oriented towards research in the scientific fields, HE professors seem to be in the middle of two opposing forces – one that pulls towards their scientific domain, their "barricade", and another that pushes them to the need to properly teach the content to target audiences increasingly more diverse and demanding.(12)

In the case of medical education, additional challenges can be identified. Dent and Harden(13) underline several societal variables – patients' expectations; evolution of healthcare delivery; deeper and more diversified medical knowledge; doctors' availability and workload and, finally, students' requirements. These new challenges had their consequences in medical schools and in medical education, namely in western countries. Medical schools responded by developing:

- new curricular models – integrated systems-based, outcome-based, problem-based, task-based, team-based;
- new curricular themes – communication skills, attitudinal and ethical skills, the use of information technology, peer-assisted learning, team work and inter-professional collaboration;
- consistent approaches to science and to scientific behavior, enhancing students' experiences in basic scientific and ethical principles of clinical and translational research, including the ways in which such research is conducted, evaluated, explained to patients, and applied to patient care;
- new learning situations – self-directed learning experiences and time for independent study, new tools and aids (like all kinds of simulations);
- new methods and tools of learning assessment, like Objective Structured Clinical Examinations (OSCE) or learning portfolios;
- new staff development structures as it is the case of medical education units/offices in the medical schools.(13,14)

The existence of a medical education structure inside a medical school is no longer a novelty in Europe. In general, these departments are well implanted in their schools and integrate professionals from various areas, namely professionals from medical origin (professors and researchers) and from educational sciences (psychologists, curriculum developers). The option to recruit educationalists seems

to indicate the recognition that there are specific knowledge and skills that these professionals can bring to medical schools. The functions of the medical education departments/offices/units are varied, being closely linked to the culture of the institution, on the one hand, and to the defined institutional goals, on the other. From the point of view of the institution organization, they are transversal structures, called to co-work or even coordinate curricular committees and being responsible for the pedagogical training of professors, as well as for institutional evaluation.

THE CASE OF NOVA MEDICAL SCHOOL

In the case of NMS, besides the international trend of supporting medical training with inputs from education, several internal factors were in the origin of the launching of a medical education office. In the first place, the existence of several institutional evaluations (national and international) with recommendations for curricular change, integrating new approaches to teaching and learning. Secondly, the need for internationalization and consequent requests for curricular harmonization, namely with the curricula of other European medical schools, aiming at promoting students' mobility. Finally, the necessity to develop consistent and aligned teachers' training programs to support innovation and educational change.

The NOVA Medical Education Unit was created in 1994. Its objectives and functions were not clearly defined at first. To help designing what could be the role of this new office, a large consultation process took place with in-depth interviews to representatives of the several "bodies" of the school (presidents of scientific and pedagogical councils; heads of departments; representatives of the students). Three main areas of concern and need emerged from these interviews:

- Curriculum issues (standardization of learning outcomes; horizontal and vertical content harmonization, appropriate syllabus sequencing; core disciplines and elective ones; integration of formal knowledge and clinical experience);
- Evaluation models and tools (of programmes; of teaching; of learning);
- Faculty pedagogical training (targeting the academic staff as a whole).

This process led to the definition of the main goals for this new office:

- To support faculty in their educational activities by promoting academic staff development activities;
- To collaborate in the analysis and evaluation of programs and courses;

- To foster transversal discussions on educational themes, within the institution;
- To participate, in all the institutional evaluation programs.

Finally, several activities were launched:

- Dissemination of educational support materials (e.g. how to define program and learning objectives, how to select teaching techniques, how to design adequate assessment tools, how to align all these teaching elements in a coherent educational approach);
- Implementation of transversal teachers' training activities – educational workshops targeting, on one hand, new lecturers and, on the other hand, experienced professors;
- Developing journal club sessions, aiming to foster educational discussion and to share teaching practices;
- Observing teaching performances and providing feedback and pedagogical support;
- Collaborating in the design and application of assessment tools (multiple choice tests; observation grids; short answer written tests, for instance);
- Collecting and analysing data on faculty and students' degree of satisfaction about courses and programmes and helping to introduce changes, if necessary;
- Participating in the academic audit processes (at an institutional level).

Far from being random activities, these actions followed a strategy that obeyed to basic institutional innovation rules:

Firstly, the importance of knowing and understanding the school culture, values and routines and the acceptance that any innovation, as it was the case of a medical education unit, should be based on the reality of the institution. The interviews performed in the beginning of the process were crucial to understand the needs and the expectations of professors and students and to establish a sort of educational contract with the different stakeholders.

Secondly the validation by the institution leadership was vital to the process. The activities of the new office were always strongly supported by the leaders of NMS.

Thirdly the implementation of all innovative actions was grounded in solid educational knowledge, namely theories on adult learning and research on teaching.

Lastly, the establishment of an atmosphere of collaboration. In fact, faculty involvement was volunteer but the process was implemented following a progressive and enlarging ownership approach.(15)

After this first period specially dedicated to academic staff development, a second period took place. This new phase was triggered by specific pedagogical problems raised from entire departments or from individual professors. Thus, the medical education unit evolved from organising workshops and training activities, to collaborating in solving specific educational problems and to giving expert advice, as well as to monitoring educational innovations.

From the regular questionnaires passed on to professors on the advantages of having a medical education office in place, several positive characteristics were identified:

- The monitoring of pedagogical practice and timely feedback to the academic staff;
- Pedagogical training, based on real pedagogical challenges and the opportunity to discuss educational themes and to share practices;
- The creation and sharing of educational support materials and resources;
- The development of transversal spaces for analysis and discussion of pedagogical themes and the construction of institutional pedagogical networks and communities of practice, independently from the scientific areas or departments of origin.

In a third moment, the medical education unit started a close and fruitful collaboration with the students' union, both promoting the discussion on educational themes and inviting them to the workshops and training sessions.

FROM THE MEDICAL SCHOOL TO THE WHOLE UNIVERSITY

The main impact of the experience of NMS was, without doubt, its transferability to other educational settings. In fact, the creation, at NOVA Rectorate, of a similar structure for the whole university was the natural corollary of the experience at NMS.

Today, NOVA offers a pedagogical training course for its academic staff, with participants from the nine Academic Units. Its objectives are very similar to those designed for the course at NMS.(16)

Two other projects have been developed: the GIP (Grupo de Inovação Pedagógica) and the PIN (Programa de Interobservação da NOVA).

The GIP, integrating professors from all the AU, meets regularly and aims at discussing educational techniques and technologies and at sharing good pedagogical practices.

The PIN is a multidisciplinary and voluntary program in which observers and observed give and receive feedback on given classes. To this end, trios of Professors from different Academic Units observe and give feedback on concrete aspects of their peers' classes. Previously, participants receive training in observation and feedback techniques. The goals are strictly formative and are not intended for any kind of evaluation. This program is available to all NOVA professors.

Furthermore, an educational resource hub was created, called NOVA Teach. It is a digital library to help professors (and students) to find books, articles and videos related to teaching and learning. In the different tabs, there are different topics, namely guidelines and broad conceptual approaches about teaching and learning in HE; teaching materials and teaching tips and, finally, collaborative sharing of resources, for example, real cases, testimonials of experiences and projects.

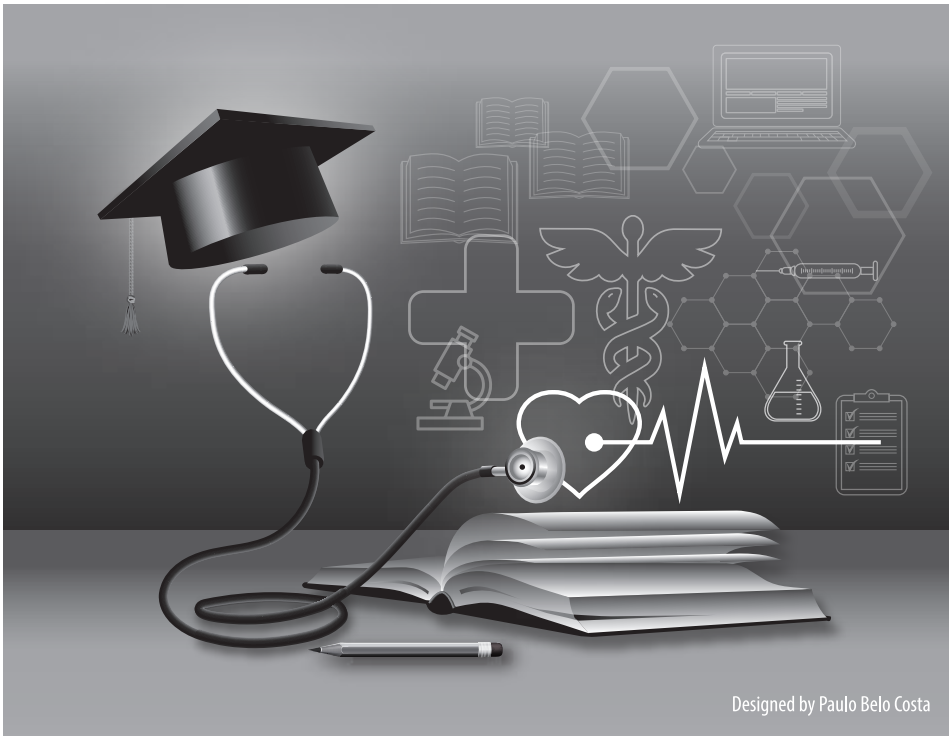
There is also, like in NMS, an educational consulting service for academic staff.

FINAL REMARKS

As final remarks, we underline the following key ideas:

- Faculty pedagogical development is still a rare reality in Portuguese HE institutions. Medical schools represent a refreshing exception in this field, as they have been leaders on innovations in the way professors teach and in the transformation of their curricula. NOVA Medical School accompanied this movement to adapt to new challenges in medical education;
- Pedagogical training can be one of the privileged forms of promoting institutional innovation, being evident that change has to be made collaboratively;
- The validation, on the part of the institutional leaders, and valorization in terms of incentives, constitute an added value to the process;
- The most accepted training programs are directly linked to the needs of the institution and /or to the individual needs of professors;
- HE professors value targeted pedagogical training, based on credible theoretical references and articulated with their own practice;
- Such training must respect what is known about adult learning and the professional circumstances of academic staff;

- The training programs must be based on the assumption that the pedagogical activity stems, like the scientific one, on a rigorous and investigative attitude on the part of the teacher;
- Pedagogical training can provide the opportunity to create common ground among scientific departments, even though they were previously separated;
- Training can have diverse and complementary formats and must be flexible and adapted to the needs and pace of the institution and of its academic staff;
- It is essential to develop educational research at HE level. This requires professors to analyze their own practices, in order to understand and innovate them;
- HE institutions should encourage the dissemination of solid and scientifically grounded educational practices, in order to mitigate the gap between education and research.



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HEALTHCARE MANAGEMENT AND LEADERSHIP

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1. INTRODUCTION

Healthcare delivery is increasingly complex considering technological, scientific, technical and epidemiological challenges in todays' world[1]. The rising interdependence between different medical specialties[1], the scarcity of resources[2], the demand for more knowledge in each clinical case[3], and the management of uncertainty[4] and of surprise[5] seems to support the study of leadership styles and organizational effectiveness[6] towards a better cope with complex healthcare environments[7].

Healthcare organizations (HCOs) are challenging mainly due to its highly differentiated operational center with its technical-scientific requests and the autonomy of their professionals (functions and markets)[8] that in association with the need of decision-making, connected to unexpected and extreme situations[9] complexifies the management of organizational behavior.

Additionally, HCOs are known as complex adaptive systems (CAS) composed of diverse self-organizing agents in dynamic interaction that learn and co-evolve[5]. CAS are dynamic once it can unfold deterministically over time yet be wholly unpredictable and, as a result, plans and forecasts should be better seen as "distributions of probabilities than as exact predictions"[5]. Based on the complexity science, leadership should account for the complex adaptive needs of these organizations. Therefore, leadership should go beyond the role of a leader, based on a position or authority, depicted as a complex and dynamic process that emerges from the interaction between people and ideas[10,11] in order to achieve creative solutions, adaptability and learning out of collective heterogeneous agents[12].

The HCOs are also considered professional bureaucracies[8] and loosely coupled systems[13] where its operational center includes expert-workers previously trained in health schools to know what to do and what to expect from others[14]. Professional's qualifications enable them to work autonomously, from colleagues and hierarchies[8], organizing themselves in almost "automatic way"[14]. Accordingly, professionals usually coordinate through mechanism of "standardization of skills and knowledge"[8,14]. During extreme situations (e.g., medical-emergences; outbreaks) two other forms also arise: mutual adjustment (i.e., informal communication) and standardization of norms (i.e., values from strong culture)[14]. Nevertheless, clinical practice usually follows the standardize mechanism of relationship. Moreover, healthcare systems organize on a functional and market-bases by specialty department where patients receive care in different places according

to medical specialties locations[8,14,15,16]. The co-occurrence of standardized skills and knowledge, and the clinical operations that are organized around isolated specialties, has been contribute to higher "efficiency" (higher number of acts) and "smoothness" of the working system (semi-automatic coordination). However, the process of service delivery still remains provider-focus and disease-driven moving aside from the expected patient-centered care[14,16,17]. Since the 90s, the pioneer report Crossing the Quality Chasm (Institute of Medicine, 2001)[17] underscored that care must be respectful and open to patient's expectations, needs, and values through an indispensable clinician-patient collaboration[17]. More than two decades afterwards, the delivery process is still highly differentiated with most healthcare professionals working in silos, disconnected from each other[14,16]. Such challenges require a complex approach from leaders but essentially from leadership, perceived as a process[10,11]. Thus, the complex leadership approach, mainly the Complexity Leadership Theory (CLT)[12] framework, seems to contribute to better cope with HCOs, defined as CAS[5], with its extensive challenges. Both, CAS and CLT will be described in further detail, in the following sections.

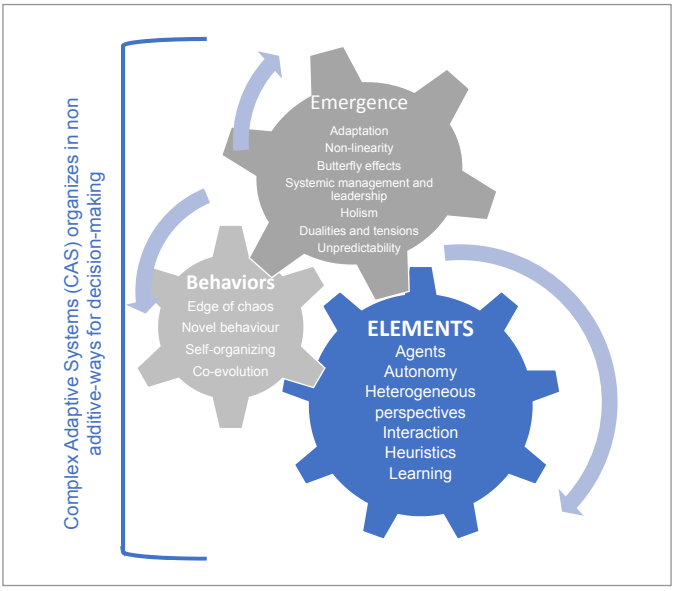
The present chapter develops the complexity leadership approach within the scope of complexity science applied to HCOs as CAS with its features of loosely coordination, autonomous and self-organizing agents that learn, adapt and co-evolve nonlinearly, and produce emergent outcomes and not in an additive way. In the next sections HCOs are described, followed by the state-of-the-art review regarding the concepts of CAS and complexity leadership theory. Two study findings elaborated within the NOVA and an ongoing research project are also presented. At the end, a conclusion and impact to society is discussed.

2. STATE OF THE ART REVIEW

Complex adaptive systems

Healthcare is considered one of the most complex human systems in today's societies[14,23] shaped by professionals' diversity of perspectives and relationships[24,5] defined for example by their differentiated roles, their relationships with their patients, or work schedules[24]. Differentiation is so extensive that some authors referred to it as the worlds of cure, care, control and community each of which with its activities, ways of organizing, and mindsets[15]. Considering the distribution of resources, the variety of activities, and the singularity of mental models and rules

of each agent [25] HCOs are social complex systems composed of diverse, interconnected and adaptive agents that co-evolve[5,23,25,42] (Figure 1).



Additionally, things are often "unique", and may "not proceed as predicted"[5,23]. Considering it all, healthcare systems are CAS[5,25,42] which encompasses the essential following concepts:

a) **diverse agents that learn** including providers, patients and stakeholders are guided by their mental models (i.e., schemata) that adapt and evolve. The "schemata" enables communication between parts as they use the same organizational grammar (aiming at uncertainty reduction and absorption)[5,26,27]. However, as learning is not-linear and changes in internal models occur, communication difficulties can emerge[5,26].

b) **nonlinear interdependencies between agents** implies that outcomes may be disproportional to inputs [5]. In other words, "small difference in the initial variables leads to huge differences in outcomes"[25];

c) **self-organization** behaviour emerges from non-directive or centrally-imposed interaction within a complex system. Agents with its inner rules self-organizes in stable yet dynamic and even in innovative ways[5,25];

d) **emergence** is a property of complex systems regarding two main ideas: 1) behaviour emerges from agents' interaction, and 2) observable outcomes

are not merely the sum of the parts. Thus, properties that exist at one level cannot be explained by understanding properties at other levels[5,25]. Examples in HCOs can be different levels of trust among medical specialists or communication patterns at patient-doctor relationship[5];

e) **co-evolution** of CAS with their environments or systems, in which CAS are embedded, occurs when organizations are influenced but also influence the systems with that they relate to. Thus, co-evolving is not just reacting (response) it is an enacting (alters) either of the environment and systems[5,25].

In sum, HCOs are CAS with their mental models and rule-based routines (i.e., guidelines, protocols) that together with their learning agents, loosely coupled in nonlinear interactions, guide itselves towards self-organizing, emergence and co-evolution in unpredictable ways. Such raises the need for complex thinking[28] headed by a holistic[18] and diffuse leadership[7,43] to cope with the increase complexity of healthcare environments[7] towards organizational outcomes[18].

Complexity leadership approach

HCO as CAS have dynamic capabilities[29] described as a transformation process (obtain, integrate, reconfigure and release) leading to new resources and configurations[30] that leadership should take advantage of[10,11]. Considering the CAS concept, Uhl-Bien, Marion and McKelvey (2007)[12] proposed leadership as an emergent, iterative, dynamic and complex interaction of diverse interdependent forces rather than a simple act of one or more individuals[31] with position or authority[10,11,12]. Based upon the science of complexity, the complexity leadership assumptions fit the dynamics of social, managerial, and organizational behaviour[12] in the postmodernism era. It is through complex interaction, using communication and influence[32] that natural tensions of HCOs[25] can self-organize enabling innovation, optimization, creativity and creation of new insights to deal with its complex organizational issues[12]. Regarding to HCOs, complexity leadership does not need detailed targets and specifications but rather seeks to mobilize "natural creativity and organizing ability of its staff and stakeholders"[7]. Thus, the complexity leadership role is more associated to the disclose of good practices and information[7] allowing others to adapt them in the most meaningful way to each and every one[10,11,12]. Complexity leadership has been approached from two different literature viewpoints[7]: 1) a more comprehensive and holistic one, situated at all organizational levels (top, middle and line management) seeking to manage the

dynamism and entanglement of interactions[10,11,12] *versus* 2) a narrower leadership view, that is further oriented to the operational level and to day-to-day routines that includes non-linear, iterative and reciprocal interaction between patients and doctors[7]. In line with the more holistic perspective Uhl-Bien et al. (2007)[12] proposed one of the most encompassing leadership perspectives called CLT a true overarching framework. The CLT comprises three leadership components: administrative, enabling and adaptive that includes the interactions among diverse, self-organizing and adaptive agents that co-evolve and explores how order therefore emerges[12] defined as follows:

> "*Adaptive leadership is an emergent, interactive dynamic that is the primary source by which adaptive outcomes are produced in a firm. Administrative leadership is the actions of individuals and groups in formal managerial roles who plan and coordinate organizational activities (the bureaucratic function). Enabling leadership serves to enable (catalyse) adaptive dynamics and help to manage the entanglement between administrative and adaptive leadership (by fostering enabling conditions and managing the innovation-to-organization interface). These roles are entangled within and across people and actions.*"[12]

Lichtenstein and Plowman (2009)[11] highlighted that "leadership is not only incremental influence of a boss toward subordinates, but most important it is the collective incremental influence of leaders in and around the system (…) as agents are in constant interaction exchanging information, learning, and adapting" (2009:618)[11]. Accordingly, by co-evolving, agents expand their own behavioural repertoire therefore expanding the behavioural repertoire of the whole CAS, itself[10,11].

In sum, CLT by focusing in the system (and not the leader alone)[12] captures the view of diffused power[7], also called "constellation leadership"[7,43] considered beneficial for complex organizations, as HCOs.

3. RECENT CONTRIBUTIONS FROM NOVA

AREMED – Emergent areas in medical education: NOVA case study

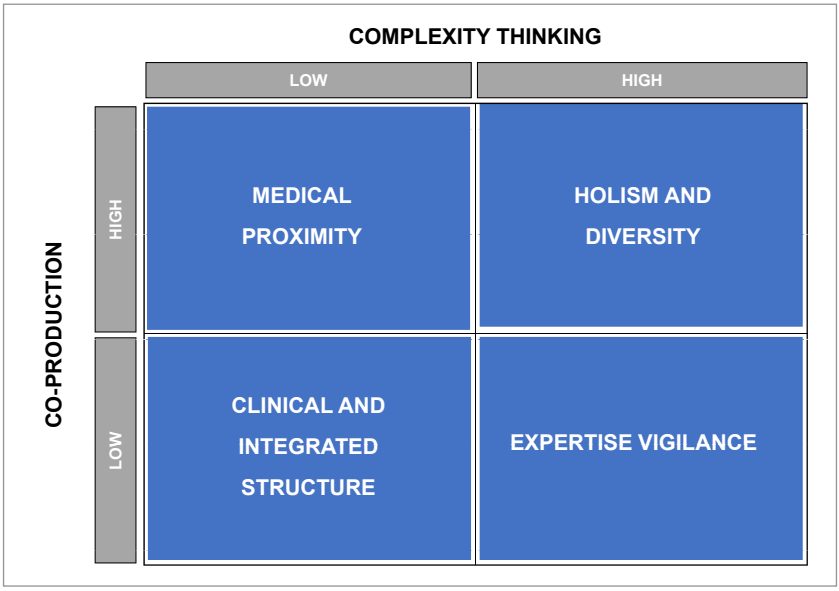
Aiming to understand future areas for medical education a qualitative study was conducted with 15 focus groups and 115 participants. Through healthcare beneficiary experiences and their reported satisfaction, the research uses concepts

from two streams of literature brought together in this analysis in order to identify new medical education policies.

The concepts are firstly complexity thinking within the scope of complexity science[28,23,25] and secondly co-production, framed by the service management perspective[38,39]. Regarding the first, medical education and healthcare systems with its contrasting healthcare worlds are usually framed from a dominant Newtonian-style of thinking[28], uphold in orderly and predictable assumptions linked to the reduce-and-resolve metaphor[23,25] and also to the search for the correct label and categories of diagnosis[9], considering patients symptoms, in order to define plausible treatments. Such viewpoint contrasts with the science of complexity perspective, defined as a conjunctive thinking[28] that tolerates ambivalence (i.e., knowing and doubting)[9] emergence of interactions (i.e., greater than the sum of its parts)[5,23,25] and unpredictability (i.e., inputs do not linearly lead to predicted outcomes)[5,23,25] more suited to cope with challenges of HCOs as CAS[5,23,25].

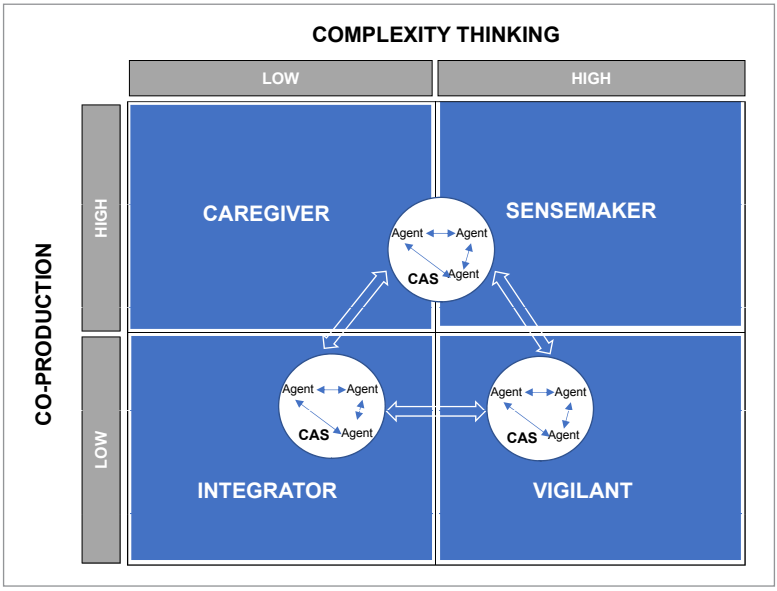
Embracing complexity encompasses the need of complexify doctors' education to life-long learning through complex language, acts and thoughts[40] in order that agents can be better equipped for the interpretation of disturbances, but also to deal with unexpected situations that cannot be forecasted[9]. Secondly, healthcare delivery process must adjust towards the service perspective[38] which includes the respectfulness and openness to patient's expectations, needs, and values through an indispensable clinician-patient collaboration called co-production[38,39]. Opposing to the new public management approach that maintains co-production as something optional, the service literature viewpoint highlights that user involvement in service should not be seen for design and planning purposes, but rather contributing to good performance and effectiveness, at operational level (patient-doctor relationship)[38,39]. Therefore, co-production requires the management of relationships between practitioners and beneficiaries. Additionally, the service co-production approach highlights the influence of medicine either for systems and users once the chosen co-productive strategy (i.e., optional/indispensable) will impact on doctors' comprehension of users' needs, expectations and participation, being co-production phenomenological determined by beneficiary's experiences in interaction with complex providers.

The main contributions of the study are a paradoxical model to integrate emergent healthcare dualities to guide medical education, preventing the overfocus in one pole and achieving a balance between the four views of healthcare (Figure 2) and also a grammar to operationalize the model. Further details about the model and the grammar are presented in the chapter "*Which medical professional?"* of the present book.



The four emerged dualities of the model are the following: *medical proximity* (biosocial skills to a better co-production between patients and doctors and also between healthcare professionals) *expertise vigilance* (expertise vigilance training to cope with patients unexpected conditions) *clinical and integrated structure* (integrated protocols and guidelines with healthcare service organization) and *holism and diversity* (skills to manage patients' holism and feedback loop, a minimal structure that captures patient needs and records the whole, for clinical adjustments).

The *AREMED* project helped to identify the main dimensions that should be included in medical curricula of future doctors. Although it was not a primary outcome of the study the findings also highlighted future directions to follow, regarding leadership archetypes to cope with healthcare challenges of CAS[5,23,25]. Traditional concepts of leadership emphasize a role of position and authority to organize activities and actions in lower levels (middle and line)[10,11]. Such conventional notions fail to notice that HCOs as CAS are composed of members that interact, interchange knowledge, learn and adapt, without the need of a hierarchical coordination[10,11]. Framed by the complexity science, leadership has been introduced as a process, namely a process of influence, where each interaction can be perceived by every collaborator as more or less "meaningful"[11] leading agents to adjust their behaviors to the perceived meaning, thus promoting the system to change[11] (Figure 3).



The study findings suggest that future doctors can be educated to embrace complexity and co-production as a process of interaction and influence which could be achieved through leadership architypes like the *caregiver* (medical proximity) the *vigilant* (expertise vigilance) the *integrator* (clinical and integrated structure) and the *sensemaker* (holism and diversity).

Management models and types of organizational culture – The profile of portuguese hospital administrators

A recent work developed by Mateus and colleagues[41] which aimed to understand the profile of management and leadership skills of Portuguese hospital administrators (HA) reveals the respective management models and types of culture. The study was based on the Competing Values Framework (CVF)[44] which is defined by two pairs of dualities: the first pair encompassing Flexibility versus Stable Structure, and the second one including the Internal versus External Focus.

The CVF is a paradoxical model developed by Quinn and Rohrbaugh (1981)[45] used broadenlly to guide organizational effectiveness but also to anchor the specific leadership roles in each of the four emergent quadrants (re)called by Cameron et al (2014)[46]: Compete, Control, Collaborate and Create. The CVF is also an instrument that through analysis of management and leadership skills most used in the professional practice of managers allows to identify the most frequent management

models and types of culture in the organizations. Based on this framework Mateus' study presents important conclusions: 1) healthcare organizations have strong hierarchies and remain focused on planning, 2) responsibility and capacity for programming and organization, 3) task distribution, 4) compliance with rules and objectives, in other words, rigid cultures from a structural point of view. These results confirm a hierarchical and market cultures as dominants which mainly correspond to coordinator, producer, director and monitor roles. These dynamics are centered on control and rules models of the CVF.

Following a recent line of inquiry aiming to comprehend the success of the managerial leaders in complex and dynamic setting, Mateus' work did not find the "behavioral complexity" defined as the "individual's capacity to exhibit a broad array of contrasting behaviors (...)" essential to cope with the healthcare paradoxicalities of "roles and constituencies" managerial leaders face, in daily bases. Although these models are essential for HCOs structured care, managers and policies should also develop management skills, leadership roles and management models, promoting types of culture that integrate flexibility and complexity aiming to cope with co-evolving, emergent and non-linear cases, as referred in earlier literature review of this chapter.

The leadership dimensions in healthcare organizations: how to deal with unexpected and extreme events?

Previous studies have focused on the ambiguity and uncertainty of HCOs as CAS and recognized that they are not prepared for the unexpected due to the dominance of orderliness paradigm, which uphold on assumptions of plan and control[9]. Thus, leadership should involve dynamics, dimensions or practices that allows interactions among heterogeneous agents and across agent networks[10,11] to manage challenges associated with uncertainty, messiness and emergent situations of CAS. This type of leadership seeks to recognize that leadership transcends the individual, assuming it as system phenomenon[10].

As relationships are not defined hierarchically but rather by interactions, they require mainly three leadership frameworks to lead complex healthcare environments: transformational, humble and distributed leadership. The first is connected with vision and has been related with positive impacts as job satisfaction, trust, loyalty of members and better performance. Respectively, transformational leadership[20] with it charismatic and visionary attitude, inspires professionals by communicating a clear vision[20] transferring authority to the team and encouraging

creativity in solutions[21]. Humble leadership[22] refers to leaders with three qualities, the willingness to view oneself accurately, appreciation of others' strengths and contributions, and teachability[22]. Additionally, it includes feedback, openness and relationship. Finally, distributed leadership[36] is pictured as a group quality a set of functions which must be carried out by the group[36], encompassing the need to develop a culture of shared ideas, shared accountability and shared decision-making. Based on these challenges, this ongoing study aims to identify the leadership dimensions in healthcare organizations present in unexpected and extreme events as COVID-19 outbreak, specifically in a hospital intensive care unit.

Regarding methodology, the study will follow a qualitative approach using semi-structured interviews with healthcare professionals considering dimensions of transformational, distributed and humble leadership. In order to identify common themes, topics, ideas and patterns of meaning, thematic analysis method will be applied. This research is expected to contribute to develop complex leadership theory in extreme events, specifically in healthcare organizations. It is also expected to represent a multilevel framework in which healthcare leaders, policy and decision-makers can support themselves in managing individuals, teams and organizational challenges proper of unexpected and extreme situations. Additionally, the study may also contribute to the advancement of CLT in healthcare by encouraging further studies.

4. CONCLUSION AND IMPACT TO SOCIETY

The complexity science paradigm helped to reframe the approach towards complex and dynamic social systems, as contemporary healthcare organizations, which can no longer be managed like machines, according to order and control assumptions. On the contrary, HCOs are well study as CAS which cannot be described in terms of its individual elements (complicated view), differing from the systems perspective. Accordingly CAS are organic, unpredictable and the whole emerges not additively but from self-organizing, non-linear learning and adaptive agents that interact and co-evolve, leading the systems to change.

A significant body of knowledge was able to shift attention away from the leadership models of the last century, mainly described as top-down, bureaucratic, individual-centered with positions or authority roles towards a complex interactive dynamic system described as of diffuse power[7], like a collective constellation

leadership[7,43] and more recently as holistically[18] once it considers the whole system[11]. The CLT defines the leadership construct as a "pattern"[18] of dynamic and influence interactions, where each relational interaction can be perceived by every agent as more or less "meaningful"[11] leading the "autonomous heterogeneous individuals"[18] of CAS to adjust their behaviors, as they sense and make sense of the meaning conducting systems to change[11]. In sum, CLT as a holistically view (not the leader alone)[12] captures the emergences resulting from interactions between agents but also their behaviors, expanding their behavioural repertoire of the whole CAS[10,11]. CLT is considered more beneficial for CAS as HCOs that become more agile, resilient and effective to unexpected situations that cannot be forecast.

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HEALTH INFORMATICS: MACHINE LEARNING AND MULTIMODAL INTERACTION IN HEALTH DOMAINS

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1. INTRODUCTION

This paper describes research work and contributions of NOVA LINCS (NOVA Laboratory for Computer Science and Informations) in the health domain. Main aspects that were targeted over the years consider how data is processed and multimodal interaction. Health related projects were carried out in topics including machine learning, image processing, user experience design, interaction and serious games for health. Security and privacy of health data are also very important aspects where NOVA LINCS has expertise and contributions. NOVA LINCS is a leading research unit in the area of Computer Science and Informatics Engineering, associated to the Departamento de Informática of Faculdade de Ciências e Tecnologia of Universidade NOVA de Lisboa (FCT NOVA), a pioneering national institution in the field. The mission of NOVA LINCS is to develop cutting edge scientific research in key areas of Computer Science and Informatics, contribute to advanced education in the field, and share the produced knowledge, results, and innovation with users and communities within society.

The emerging software ecosystems and their supporting technologies are radically changing human activities in all areas and healthcare is a main example of this shift. From a range of diverse contributions we chose to highlight work in the areas of clinical decision support, conversational assistants, and user experience design for personal health records. Other relevant work includes serious games for rehabilitation [7, 11] where gaming strategies were used for speech therapy.

The paper is organized to present work regarding the highlights mentioned above and it concludes with ongoing research on the FrailCare.AI [10] project done in collaboration with NOVA Medical School, Value4Health CoLAB and SPMS.

2. CLINICAL DECISION SUPPORT

In making clinical decisions, physicians often discover diseases or health patterns in their patient population. In parallel, years or months of patient observation generate large amounts of data that can be explored by health-care data analysis algorithms. Combining health-care professionals' knowledge with the collected data is key to leverage big data mining and analytics. Such a combination of factors creates a series of opportunities to improve the value of health-care processes. Hence, we advocate a new breed of medical and clinical data mining techniques and

clinical decision support algorithms that favours a translational reasoning process that crosses different dimensions of health-care sectors.

Case-based medical retrieval systems can empower healthcare experts by allowing them to find the most relevant publications or explore cases with similar symptoms or conditions in medical information repositories. These tools can include search by image [4] based on a medical images classification scheme, region-of-interest, image captioning [5], or medical terminologies. FCT NOVA has developed a system to improve case-based search in the medical domain.

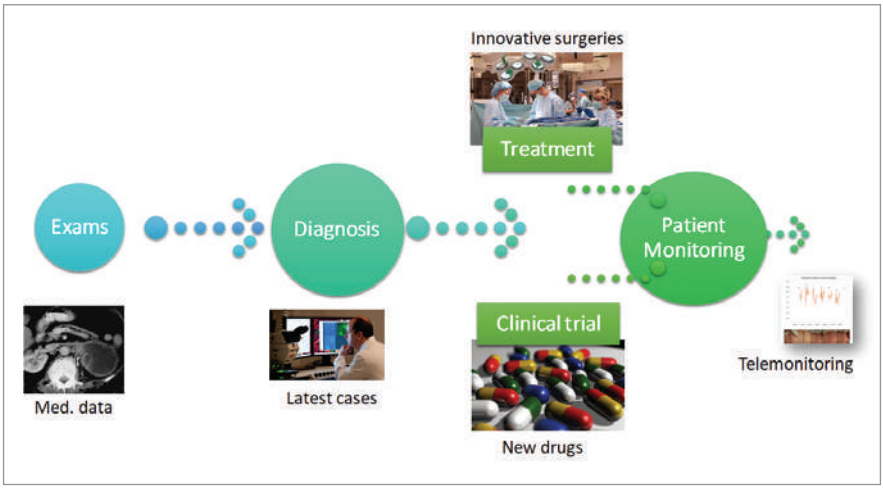

Figure 1 – The clinical decision support workflow addressed by the research conducted at NOVA-LINCS.

The opportunities to use such decision support technologies in health-care processes range from exams, diagnosis and treatment to the simple patient follow-up processes. The system is particularly suited to support decisions in the following tasks:

- Suggest **exams** to be carried-out by the patient;
- Support the **diagnosis** decision;
- Suggest possible **treatments**;
- Identify **clinical trials** where the patient can be enrolled.

All these decisions are based on the patient data and the most up-to-date bio-medical literature and clinical trial data. The NovaMedSearch retrieval architecture is designed to exploit the bulk of medical articles and clinical trial data [2, 6] available at PubMed and ClinicalGov using images and text, both independently

or combined, by leveraging on their implicit correlation (images to captions and article text to images).

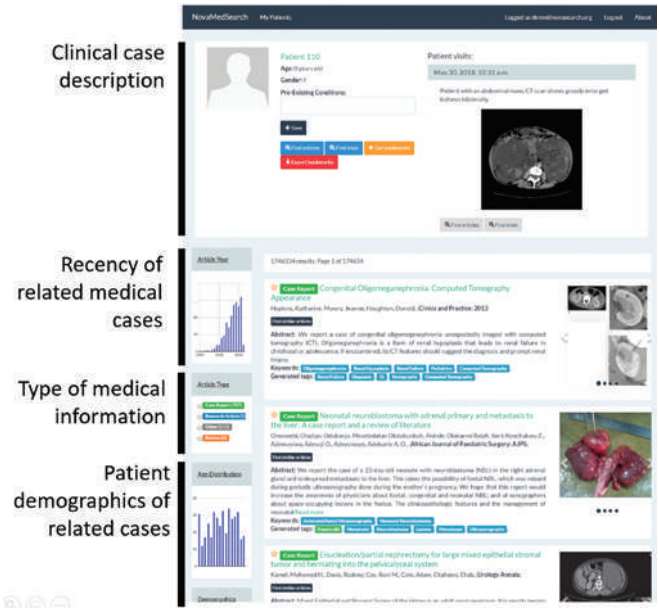

Figure 2 – An example of personalized activity notifications and feedback (simple phone-based (left) and detailed browser view (right)), based on activity data captured using a smartwatch (https://medical.novasearch.org/).

On its core, NovaMedSearch is a multimodal (text and image) medical search engine that can retrieve either medical images or cases. It builds on advanced retrieval techniques and a simple design that helps users build their queries with interactive query expansion. To support rich search in the medical domain, the system provides health professionals with enough tools to best capture the information needed by the expert. Multimodal queries can contain a textual description of the case, an image textual description, and a set of relevant query images.

The top section of Figure 2 illustrates a query containing a clinical case description and its corresponding images. In this figure the query text was parsed to detect medical terms and by leveraging a medical thesaurus, alternative terms are suggested on-the-fly (terms ct-scan and computed tomography scan are both recognized and expanded). The bottom section of the figure highlights how query expansion helped matching the query term ("CT scan") to a document term ("computed tomography scan"). Users can also submit multiple images in the same query.

This system has been evaluated by healthcare providers. It scored among the top systems in terms of text-only searches and was the best system for multimodal searches due to two novel methods: a multimodal retrieval method [3] and a query expansion based on medical terminology [12]. With this system, healthcare professionals can search medical literature by supplying a case description and image exams concerning the patient's clinical history [1].

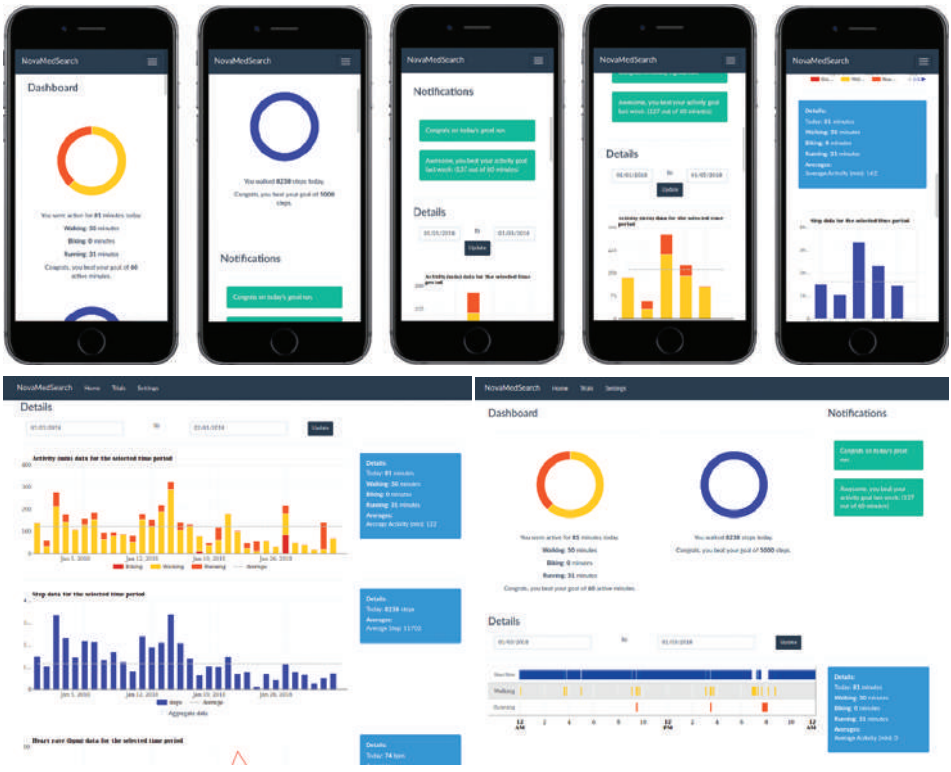

Figure 3 – An example of personalized activity notifications and feedback (simple phone-based (top) and detailed browser view (bottom)), based on activity data captured using a smartwatch (https://medical.novasearch.org/wear).

3. HEALTH MOBILE MONITORING

With the ubiquity of smartphones, its biosensors and the ability to obtain knowledge from it, the search for more efficient data mining methods has become very important. In the medical area there are series of data obtained from monitoring

and/or wearable devices. In this line of work, we have investigated the challenge of exploring such activity data for preventive health-care. With the data collected by multiple sensors, it is possible to relate patient behavior (e.g. sleeping patterns, daily walking activity, exam results) with the clinical condition or history of the patient. NOVA LINCS has explored this task through the discovery of high level events in the time series and the use of these events (behavior outliers) as features. The profile of each patient was enriched with other data (patient records, location, type of environment) and used for various tasks (patient clustering, clinical condition forecasting). Association and sequence mining can also be used to discover/construct interesting features for patient profiling.

Hence, smartphones and activity tracking devices have been used to passively collect activity and other wellness metrics, and are key tools to provide specialized notifications and feedback tailored to individual patients (Figure 3).

4. PERSONAL HEALTH RECORDS: USER EXPERIENCE AND VISUALIZATION

This section describes work on designing and assessing platforms for Integrated Personal Health Records (PHR) [5,6]. It was done in collaboration with the Portuguese Ministry of Health SPMS (Shared Health Services of Portuguese Ministry for Health) and National Patient Portal Linha de Saúde 24 (24h first contact health care for all portuguese citizens) and carried out in the scope of Inês Rodolfo PhD dissertation. PHRs combine, represent and visualize health data from different sources into meaningful summary health care records that create a valuable user experience to engage people to feel in control of their own health (as represented in the image above). Innovative HCI techniques employed in this platform include methods for participative interface design, mobile computing, and experience design. The overall goal is to provide citizens with control over their personal health information allowing them to reflect and act upon the accomplishment of their good health and wellbeing.

The Integrated PHR was then the framework for this research on exploring how communication and collaboration between patients and providers can be improved, thus requiring contributions from the field of Human Computer Interaction. An underlying principle of this research is that patients are the ones who own their health data, leading to a new model. a design strategy and design

outcomes in the scope of this research. Patients can have more control of their health over time, through a patient-centered system, which has the ability to combine multiple sources of data both from the patient and provider side.

This new type of PHR fosters the creation of integrated data networks, achieved in this research by interacting with cross-channel user experiences that took part of nationwide healthcare ecosystems. This work has demonstrated through the analysis and development of two use cases in cooperation with organizations connected to the Portuguese Ministry of Health, how an Integrated PHR can be a powerful tool, to be used by the citizens with undeniable value to the demands of an aging society.

Figure 4 – Integrated PHR dashboard.

The first use case was done in collaboration with the Portuguese National Patient Portal, combines an Integrated PHR and incorporates the Portuguese Data Sharing Platform (PDS), which can be used by any Portuguese citizen. This use case study led to a proposal of the portal by also creating a foundational model for designing Integrated PHRs.

The second use case, in collaboration with the Portuguese National Senior Telehealth Program (Saúde 24 Sénior), led to another proposal for an Integrated PHR, applying the outcomes from the previous one and the requirements that derived from the findings explored in this second use case study. The proposed solution has the potential to be used by the Portuguese senior community in the scope of home assistive care.

Both proposals applied user experience design methods leading to the development of two prototypes. The engagement of the stakeholders during

the two case studies was accomplished with participatory design methods to create solutions that would meet the human, politics and behavior interdependencies that were inherent to the process of working with large healthcare organizations.

5. ONGOING RESEARCH

This section describes current research on two topics: modeling, predicting and visualizing frailty risks and clinical history and a new interface paradigm based on conversational assistants, to better motivate the patients.

5.1. FrailCare

Current work in this area is partially done in the scope of the FrailCare.AI FCT funded project [10]. The partners of the project are the NOVA Medical School, NOVA LINCS, Value4Health.CoLab and SPMS/SNS24. SPMS 's mission is to provide specific shared services in the health area in the scope of the Ministry of Health and ensures the operation of the Contact Center of the National Health Service – SNS 24. SNS 24 promoted the Senior Proximity project, a tele-care service, created to help and accompany the elderly population.

Old citizens are at greater risk of frailty and multiple chronic health conditions, as well also higher risk of poverty and social isolation, with negative consequences for their independence, safety and quality of life. In 2018, SNS 24 started a pilot program called Senior Proximity (SNS24|SP), with collaboration of primary healthcare institutions to target and support elderly population with frailty. The main objectives were to follow in a telecare service, a group of elderly citizens with frailty to: prevent health occurrences; early detect needs; promote integrated care in health, social and safety dimensions; and to contribute for a healthy and active ageing.

FrailCare.AI addresses challenges regarding the outcomes and possible followups of the SNS24 pilot program. It targets the construction of predictive models using the labeled data obtained in the SNS24|SP results together with related information from aggregated patients' health record databases. The models are then used to identify citizens at a risk of frailty given their demographics and clinical history. The model can then be used for frailty risk prediction to further select participants for an evaluation at a national level.

The models and tools will be applied to find the most adequate interventions for a given participant by finding similar cases from similar subjects. A visual tool to represent frailty pathways is being developed, where interventions, routes, outcomes and costs are represented. The developed tools will be tested by a group of health professionals that deliver the telecare service and a user population.

5.2. Conversational Assistants in the Health Domain

The growth in the mobile health and wellbeing market has been accompanied by a rapid increase in the number of health-related software applications for mobile devices (or 'apps'). Such scenarios potentially offer health-information enabling diagnostic tools and many possibilities to 'self-quantify' as well as new modalities of care. The evolution towards digital services in primary health care demand for approaches that steps-up the perception of a mobile application and presents them as a companion with conversational skills to better follow the patient mood and establish goals to motivate the patient.

This research is leveraged by current trends in conversational assistants, a more human user interface for interacting with services. In healthcare, the use of conversational assistants is a natural tool since they can be a convenient and quick way to interact with medical institutions or obtain relevant information. Conversational assistants can be the first point of contact with a healthcare provider, and with an automated assistant dealing with simple requests, in non-critical situations, more human resources can be allocated to more complex cases that require expert medical knowledge. Moving beyond these more administrative-based interactions, conversational agents can also be used to monitor a patient in a number of ways and provide adequate advice of when to contact a health professional.

Currently, the goal of this ongoing project is to explore the design of conversational assistants in patient follow-up and as a companion to detect and prevent depression.

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