Summary of the PhD thesis

"Steps toward the implementation of precision medicine in the diagnosis and monitoring of dyslipidemias"

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Precision medicine has gained significant attention over the past decade. Given the lack of a universally accepted definition, this work seeks to define it as follows:

> *Precision medicine aims to enhance healthcare by tailoring it to the unique characteristics of each individual. Through the integration of clinical and imaging data with information from genetics, epigenetics, and omics, precision medicine has the potential to improve the prevention, prediction, diagnosis, risk stratification, treatment, monitoring, and prognosis of diseases at the individual level.*

The integration of precision medicine into all areas of healthcare is a key objective of 21st-century medicine. However, few medical fields have fully realized the benefits of precision medicine. Cardiovascular medicine holds particular significance, as cardiovascular diseases are the leading cause of mortality worldwide [1]. Dyslipidemia, a major risk factor for atherosclerotic cardiovascular diseases, is alarmingly prevalent, affecting approximately 39% of adults over the age of 25 globally [2]. This contributes to over one-third of deaths caused by ischemic heart and cerebral diseases [2]. In Romania, the situation is even more concerning, with around 67% of adults exhibiting at least one major dyslipidemic trait: hypertriglyceridemia, elevated LDL-cholesterol, or reduced HDL-cholesterol [3]. Given its widespread impact both globally and in Romania - a country with a notably high cardiovascular risk - dyslipidemia represents a critical area of focus. The application of precision medicine in this field holds great potential to advance the prevention, diagnosis, risk stratification, and monitoring of dyslipidemia-related cardiovascular diseases.

In this PhD work, we explored the cardiovascular domain, particularly dyslipidemias, through a multimodal approach. The outcomes of our research have culminated in six *in extenso* scientific articles. After reviewing both conventional [4] and emerging [5] humoral markers, we delved into the cellular landscape to examine the impact of hyperlipidemia on monocyte phenotype [6]. Subsequently, we assessed the effect of statins on plasma matrix metalloproteinases in individuals without cardiovascular disease [7]. Our work also involved rigorous statistical analyses aimed at elucidating the strengths and limitations of LDL-cholesterol estimation [8]. Finally, we directly addressed a key aspect of precision medicine: the molecular diagnosis of familial hypercholesterolemia [9]. In the following sections, we will highlight the key findings from these studies.

By all current medical standards, biomarkers, regardless of their type, are fundamental to the prevention, prediction, diagnosis, risk stratification, monitoring, and prognosis of diseases. Precision medicine cannot be discussed without acknowledging the crucial role of biomarkers. Thus, this PhD work began with a review of biomarkers relevant to cardiovascular diseases, focusing on both well-established, conventional markers and novel, underexplored ones [4, 5]. This comprehensive analysis of biomarkers with predictive potential for cardiovascular patients resulted in the publication of two review articles [4, 5]. The key insight from this review is that, despite the abundance of candidate biomarkers, only a few form the

foundation of cardiovascular risk assessment. For example, in the context of dyslipidemias and atherosclerotic cardiovascular diseases, few biomarkers rival the clinical utility of LDL-cholesterol.

However, we sought to explore new and diverse possibilities for cardiovascular biomarkers. Our efforts were therefore divided across several avenues, including the investigation of monocytes as a potential source of cellular biomarkers relevant to atherosclerosis, matrix metalloproteinases as components of the plasma proteome involved in various cardiovascular diseases, LDL-cholesterol as the most critical representative of plasma lipids, and, lastly, a selection of single nucleotide polymorphisms (SNPs) carefully chosen as genetic biomarkers.

In the realm of cellular biomarkers, we conducted and published an original experimental research study [6]. In summary, we demonstrated that both temporary postprandial (in vivo) and prolonged experimental (in vitro) hyperlipidemia induce distinct inflammatory phenotypic changes in monocyte populations [6]. Furthermore, this small study allowed us to establish several techniques and protocols, serving as a foundation for future experimental studies on cell cultures [6]. This research direction is particularly significant for us, as such experiments can be directed toward the in vitro functional characterization of the LDL receptor in patients with familial hypercholesterolemia.

In the second original research study, we demonstrated that atorvastatin treatment does not affect plasma levels of matrix metalloproteinases 2, 7, and 9 in apparently healthy individuals [7]. Additionally, we identified an association between MMP-7 concentrations and several atherogenic parameters, an association that appeared to be disrupted by atorvastatin through a mechanism likely independent of inflammation, potentially pleiotropic in nature [7]. While extensive literature shows that these enzyme levels are altered by statin treatment in patients with cardiovascular diseases, studies on the modulation of MMPs by statins in healthy individuals are virtually nonexistent. In this context, our findings are valuable, as they address this gap and contribute to a deeper understanding of how plasma MMPs respond to statins in the absence of cardiovascular comorbidities [7].

The next study, also original research, addressed an eminently practical issue [8]. Although measuring LDL-cholesterol may appear to be a straightforward task, laboratory professionals are well aware of the technical, financial, and analytical limitations of this seemingly simple test. The focus of this article lies in a niche area, grounded primarily in technical details and statistical analysis. In this study, we evaluated the performance of the most widely used equations for estimating LDL-cholesterol, with particular attention to the Martin-Hopkins equation, which can be considered a precision medicine tool [8]. While the Martin-Hopkins equation is regarded as the most accurate according to the literature, we explored its limitations through a series of innovative statistical analyses. As a result, we identified specific patterns in the standard lipid profile associated with unacceptably high bias in the Martin-Hopkins equation [8]. Additionally, we proposed an algorithm for clinical laboratories to implement in order to personalize lipid management for patients [8]. Unlike the earlier original research on monocyte phenotype and plasma matrix metalloproteinases, which has a more theoretical and long-term research impact, this study offers a practical, immediately applicable solution to address the limitations of LDL-cholesterol testing in clinical practice.

In the final original research study, we investigated the relationship between an LDL-cholesterol polygenic score and the biological, phenotypic, and pathological characteristics of a central Romanian cohort [9]. We found significant associations between the polygenic score and key phenotypic traits such as the DLCN score, body mass index, and plasma lipoprotein concentrations [9]. Additionally, individuals with higher polygenic scores had a greater prevalence of premature coronary heart disease [9]. A high polygenic score was also linked to the clinical phenotype of familial hypercholesterolemia [9]. In clinical practice, these findings advocate for the use of an LDL-cholesterol polygenic score as a valuable tool for screening polygenic familial hypercholesterolemia, a condition notoriously difficult to diagnose due to the multitude of potential mutations in key genes (LDLR, APOB, PCSK9) and their variability across different populations. Implementing such a score would represent a significant advancement in the diagnosis and management of dyslipidemias, particularly as approximately 50% of familial hypercholesterolemia cases are of polygenic origin. Moreover, the adoption of such a polygenic score offers further benefits, which were outlined at the end of the study as a strong case for its large-scale implementation as a precision medicine tool [9].

A distinctive feature of this work is its hybrid nature, situated at the intersection of cardiology and laboratory medicine. Another noteworthy aspect is its multimodal approach, all while maintaining a focused exploration of dyslipidemias. The essence of the studies presented can be encapsulated in a guiding principle: "Let us advance cardiovascular precision medicine!". This objective has been a primary focus of the research team led by Professor Minodora Dobreanu, whose engagement in this field spans over 25 years. Conducted under her supervision, this work represents one of the few initiatives aimed at studying familial hypercholesterolemia in Romania. Furthermore, to the best of our knowledge, this research is the first of its kind in Romania to explore the feasibility and implications of implementing an LDL-cholesterol polygenic score. Thus, we have tested and validated important observations in a central Romanian population that were previously available only for foreign cohorts. Collectively, these factors establish this PhD thesis as a pioneering contribution to precision medicine in Romania.

References

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