Present and Future Perspectives on the Role of Biomarkers in Atherosclerotic Cardiovascular Disease Risk Stratification

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Atherosclerotic cardiovascular diseases (ASCVD) remain the leading cause of death in Europe, with more than 4 million deaths in 2017.[1] Ischaemic heart disease and stroke account for 82% of disability-adjusted life years (DALYs) in European Society of Cardiology member countries, even though ASCVD incidence has declined during the past 27 years.[1]

ASCVDs are highly heterogeneous in pathogenesis, clinical presentation and susceptibility to treatment. Risk scores based on common established cardiovascular (CV) risk factors (age, sex, smoking, diabetes, high cholesterol and blood pressure) have been developed to estimate an individual's likelihood of fatal and non-fatal ASCVD over the next 10 years. They have the advantage of being easily understood and readily used in clinical practice. On the other hand, risk scores do not reflect ASCVD heterogeneity because they provide a summary estimate of CV risk in populations.

The addition of biomarkers to risk scores does not dramatically improve the measures of calibration and discrimination.[2] The main reason is that biomarkers marginally change risk estimates in large and unselected populations when compared to common and established CV risk factors.[2] On the contrary, biomarkers can help to nail down risk profiles. They may be of use to improve risk classification across risk categories, for example, an individual classified at intermediate risk of having a CV event in the next 10 years can be more correctly assigned to a low- or high-risk group depending on a certain biomarker level. Implementation in clinical practice could reduce the use of medications in people who have more accurately been classified as having a low CV risk, while it will prompt aggressive prevention measures in those correctly classified as having a high CV risk. In this regard, biomarkers may contribute to the progress in precision medicine in complex and heterogenous diseases.

The availability of panel tests or array technology to detect genetic variants, metabolites and proteins has made possible the simultaneous measurement of thousands of biomarkers in each study participant in epidemiological studies. Having access to large amounts of data prompts the question of how to use this knowledge in a sensible way and, at the same time, how we can bridge the gap between molecular epidemiology and clinical practice.

It may seem reductive to use a classical statistical epidemiological approach to identify novel risk predictors when a large number of circulating biomarkers could be used at the same time to provide a more accurate risk assessment.

In this *European Cardiology Review* special collection, Chiarito et al. have reviewed how machine learning-based approaches may overcome some of the limitations imposed by statistical models when dealing with a large dataset.[3] Statistical models, mainly based on regression analyses, are driven by *a priori* hypothesis as a fixed number of variables can be included in the regression model. Risk estimates assess the strength of an association between one or several independent variables, such as smoking or serum levels of a cytokine and occurrence of cardiovascular outcomes. Machine learning-based approaches help to identify risk profiles based on patterns of different predictors. One trusts the strength of an algorithm to find patterns that cannot be seen by the human eye, which may be able to differentiate risk profiles, drug response and patients' characteristics.

Patterns of biomarkers and clinical variables label risk profiles regardless of which variables are included in the analytical pipeline and how strong the association is between the variables included in the model and the disease under investigation. As Chiarito et al. discuss in their review, machine learning is still in its infancy when it comes to risk prediction. Several analytical issues must be resolved, such as external validation of the results obtained, the possibility to perform *post hoc* analyses and the feasibility of this approach and implementation in clinical practice. The sensitive issue is how to interpret results derived from a hypothesis-free analysis with an immense computational strength that operates above and beyond the limits of our comprehension based on our current, still incomplete, knowledge of disease mechanisms. Even if the road ahead is long, we do not have to forget that artificial intelligence-based methods are not entirely new in medicine. The automatic interpretation of ECGs is an example of how we use predefined algorithms in clinical practice. Powerful analytical algorithms working on hypothesis-free analyses generate results that we need to critically interpret considering our previous knowledge of disease mechanisms and natural history to avoid classifying a biomarker as a novel risk marker when it is, in fact, a confounder by indication.

Among the emergent biomarkers, measurement of circulating levels of plasma microRNAs has attracted substantial attention.[4] MicroRNAs are small circulating molecules whose biological role is to fine tune gene expression. Their mechanism of action is complex: one microRNA usually regulates the expression of several genes and, at the same time, several genes may be regulated by a single microRNA. The complexity of their mechanism of action represents both a strength and a limitation for their use as biomarkers. The strength of microRNA as biomarkers rely on their stability in the circulation, consistent expression in different species making translational studies feasible, and their tissue specificity. On the other hand, as atherosclerosis is a dynamic process, pleiotropy and redundancy of microRNA make it difficult to interpret the association of single microRNA with ASCVD risk.

In this collection, Vavassori C et al. have discussed the role of circulating microRNA as emergent predictive biomarkers in the general population and as prognostic biomarkers in patients with established coronary heart disease (CHD).[5] In particular, the authors discuss the challenges in using microRNA as biomarkers. Studies performed so far in cardiovascular cohorts show low reproducibility of association between microRNAs and the risk for ASCVD. Even if several microRNAs recur as associated with the risk of ASCVD or cardiovascular mortality, the signatures of microRNAs associated with ASCVD risk are hardly reproducible and a single microRNA may show an opposite association with ASCVD risk in different studies. A review of the methods used in original research articles identifies pre-analytical and analytical differences that not only limit the possibility to replicate findings of biomarkers as risk predictors in primary CV prevention and as prognostic biomarkers in patients with chronic coronary syndromes.

MicroRNA are detected and their expression measured in plasma/serum through reverse transcription and quantitative polymerase chain reaction. A central step is the normalisation of the microRNA expression. As different methods for normalisation have been used, the relative increase or decrease in microRNAs may change, thus limiting the reproducibility of the observed association. The way forward to implement microRNA in clinical practice is to overcome the relatively low generalisability of the studies published so far. Standardisation of pre-analytical and analytical protocols, including standardisation of normalisation of the microRNA expression and replication in independent populations with a similar study design and outcome definition is the way forward.

Under the umbrella of ASCVD, lower extremity artery disease (LEAD) is seldom considered as a disease entity in observational studies. Consequently, prevalence and incidence of LEAD is underestimated. It represents a serious condition associated with a high risk of cardiovascular mortality, critical limb ischaemia and need to amputate, thus contributing to ASCVD morbidity and mortality. LEAD is asymptomatic in the early stages of the disease and as symptoms appear the question to be answered is the indication for percutaneous or surgical invasive treatment. Recently, two large randomised clinical trials have shown that anti-thrombotic treatment may improve prognosis and reduce the risk of acute limb ischaemia and amputation in patients with LEAD even after limb revascularisation.[6,7] Biomarkers are not currently used to predict the risk of LEAD or diagnose LEAD.

However, as Ziegler et al. report in this collection, biomarkers mirroring inflammation, lipid metabolism and coagulation associate with prognosis and to a certain extent may predict the risk of LEAD.[8] In particular, in line with the results from clinical trials, Mendelian randomisation studies indicate hypercoagulability as a causal factor in the development of LEAD.[9] The diagnosis of LEAD is mainly based on clinical evaluation of patients and the assessment of circulating biomarkers may improve our understanding of its pathophysiology and identify patients who would benefit from medical treatment. As Ziegler et al. point out, implementation of screening using the ankle brachial index in primary care and eventually assessment of circulating levels of biomarkers of relevance for the progression of LEAD are key steps for prevention and treatment.

In summary, biomarkers have multiple roles in cardiovascular medicine. It is high time to merge omics data with clinical and imaging data to enable improved personalised care of patients. Implementation of biomarkers in clinical practice requires the development of point-of-care instruments able to provide reliable and fast answers to the clinician to identify those who are at high risk of adverse events. Novel analytical platforms and emergent biomarkers will probably serve this scope as they give the opportunity to explore molecular pathways not covered by the known CV risk factors. At the same time, efforts to identify biomarkers causally related to single ASCVD clinical entities as well as a deep definition of clinical phenotypes are highly advocated. The novel CV drugs are mostly monoclonal antibodies or recombinant forms of naturally occurring peptides. These drugs are expensive, highly specific and they are going to improve prognosis in a selected patient population in a similar way to the biological drugs used to treat cancer. Therefore, we have to use all our knowledge and possibilities to use biomarkers in the right and most effective way to improve the prevention and treatment of ASCVD. ❏

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