Arteriosclerosis, Thrombosis, and Vascular Biology

EDITORIAL

Hunt for the (Multi)-Marker Grail in the Diverse Landscape of Atherosclerosis

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The search for biomarkers related to atherosclerosis progression and prediction of subsequent adverse events is ongoing with multiple potential applications including, but not limited to, well-known objectives such as prediction, prognosis, diagnosis, cause, and efficacy testing of (experimental) medication.

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This search is anything but a straight road toward success and knows many hurdles. In the current issue of *ATVB*, Lind et al[1] faced several of these challenges after applying a multiplex biomarker panel to assess the association of 86 preselected proteins with intima-media thickness (IMT) and plaque occurrence in the carotid artery and incident cardiovascular events during follow-up. The availability of larger targeted biomarker discovery platforms using aptamer and Proximity Extension Assay technology enables determination of large numbers of proteins in small sample volumes. These analyses are often performed centrally, allowing for a certain degree of standardization and renders replication studies more reliable, also with good interchangeability between techniques, for example, Somascan (aptamers) and OLINK (Proximity Extension Assay).[2]

Finding a causal relationship between a biomarker and destabilization of the atherosclerotic process is an extra impulse for further development of diagnostic technology. Therefore, significant effort is made to understand mechanisms that lead to biomarker expression changes related to the disease. Multiplex biomarker platforms have been used for the discovery of hundreds of genetic variants affecting circulating protein biomarkers levels.[2–4] These large-scale genetic studies enable the investigation of complex disorders where the underlying molecular pathways often remain elusive. Here, Mendelian randomization (MR) studies come into play to infer a causal relation between putative biomarkers and disease. MR studies harness the invariant nature of the genome, thus genetics is not influenced by confounders or disease status. Recent MR studies on cardiovascular biomarkers have already led to surprising results and debates.[3,4] Lind et al used MR analyses in this issue of *ATVB* to find causal relationships between the measured biomarkers and their chosen (surrogate) end points of atherosclerotic disease.

The report by Lind et al reveals very strong associations of several biomarkers with measures of atherosclerotic disease. Maybe even more interesting though, the study also unfolds the enormous complexity and diversity of the disease with sometimes apparently counterintuitive observations.

A limitation in research into novel biomarkers of atherosclerosis progression is the variability of the chosen primary end point and that subsequent outcomes may not be exchangeable. Hard clinical end points, such as myocardial infarction, require large prospective studies. Smaller cohorts mostly rely on surrogate end points, often imaging-related, such as IMT of the carotid artery. The report by Lind et al reveals that associations of biomarkers with IMT and binary measured plaque presence differ significantly. For example, comparing associations of CXCL6 after adjustment for sex and age revealed an association (P=10^{-27})

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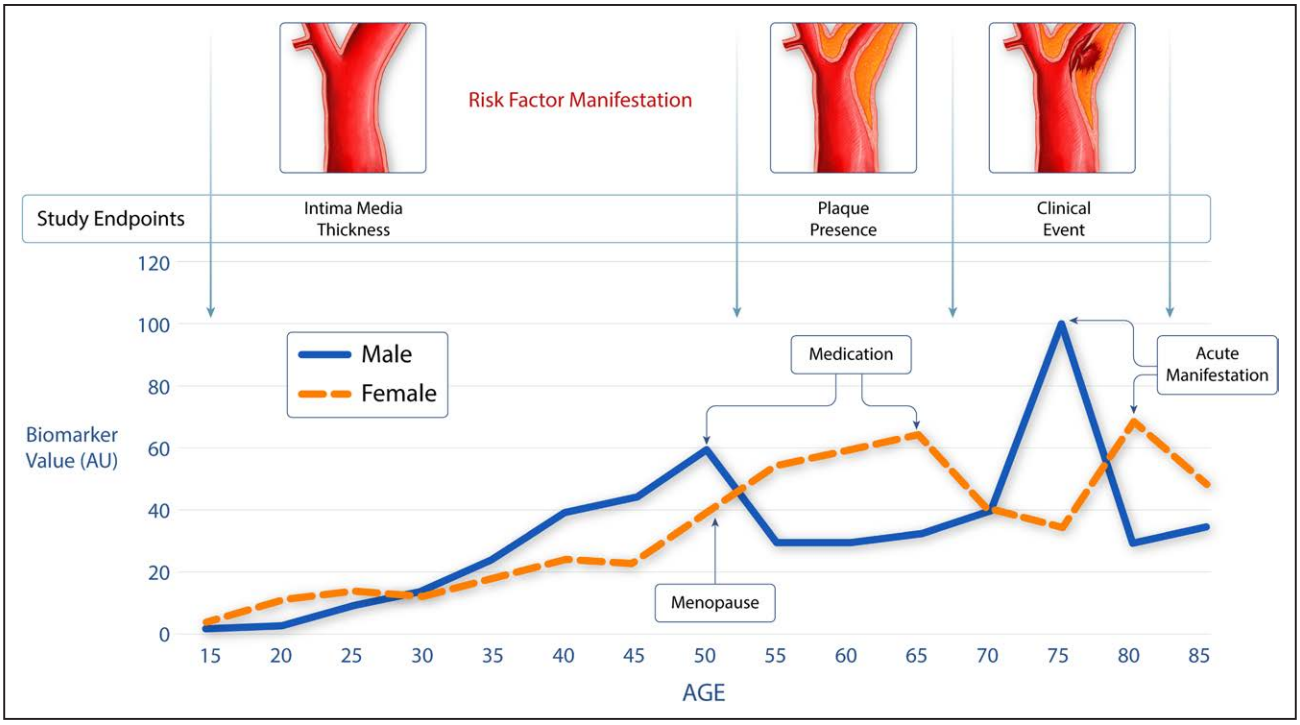

Figure. Fictional biomarker expression levels that may vary significantly depending on many determinants such as age, state of disease (early or advanced with or without clinical symptoms; phase of disease stabilization or destabilization), risk factors such as sex, use of medication, hormonal status (menopause), anatomic location, etc.
Subsequently, associations between biomarker levels and atherosclerotic disease may differ significantly based on the chosen (surrogate) end point.

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with IMT but did not associate with plaque presence. Atherosclerosis is a disease that can stabilize and destabilize over time, locally and systemically, resulting in significant expression level fluctuations over time that do not necessarily parallel the clinical presentation (Figure). IMT as a continuous measure varies in every age-stratum, whereas plaque presence as a binary measure likely reflects a more advanced disease stage. The latter could also explain the stronger overlap in biomarker associations between plaque presence and incident events.

Another interesting observation is the diversity of effect direction in the MR analysis. While MMP12 and NT-proBNP (N-terminal pro-B-type natriuretic peptide) protein levels were positively associated with chosen end points, the MR analyses outcomes counterintuitively suggested protective effects. Genetic variants near the MMP12 gene have previously been associated with large artery atherosclerotic stroke with a strong associations between the variant and RNA levels (eQTL),[7] a result that may seem conflicting with the current observations.

The selection of a biomarker is often based on a scientific result that represents an activity of a specific cell population. However, as with any organ system, detrimental and beneficial processes that progress and stabilize disease (such as in atherosclerotic disease) are subsequent or even simultaneous events. For example, proteolytic activators produced by inflammatory cells are essential for matrix degradation and may ultimately lead to a fragile fibrous cap. On the contrary, the matrix degradation is evenly important to allow migration of stabilizing smooth muscle cells. Before the foundations of a house can be reinforced, the demolition hammer advances the constructors, notwithstanding potential collateral damage. Furthermore, downstream genes and proteins can be simultaneously expressed in an active disease, pointing to both damage and repair, and have different directions of effect in MR analyses which may also depend on the timing of measurements (Figure).

Targeted proteomics panels are mostly comprised of preselected biomarkers that were found to be relevant in the pathogenesis and diagnosis of a given disease (ie, CVD in the current study) and pathway analyses. The low number of biomarkers with significant associations in the MR analyses may trigger re-evaluation of presumedly causally related genes for atherosclerosis development. Supportive evidence for causality mostly originates from animal studies and previous extrapolations of atherosclerotic mice studies to human GWAS outcomes already showed that confirmation in human disease is often difficult to find.[8]

There are other, often neglected, challenges that complicate interpretation of large biomarkers studies. We would like to point to the following 2: First, biobank samples of larger population cohorts with sometimes 10 to 20 years of follow-up are of great value. However, the results of these studies merit careful consideration.

Especially, given that blood was drawn several decades ago (potential affecting protein stability), clinical presentation, lifestyle, medical treatment have changed enormously. The past years and decades, the incidence of ST-segment–elevation myocardial infarction has decreased and the underlying plaques, therefore, appear to be subject to change in composition.[9] The samples frozen >20 years ago thus represent a different era with a different disease manifestation and prevalence of risk factors.

The second challenge is more conceptual. Atherosclerosis is not a disease that meets the one size fits all criterium. Advanced arteriosclerotic lesions that are the pathological substrate of a clinical event have many different characteristics, with plaque rupture and plaque erosion being the best-known examples. There are also evident differences in plaque characteristics between men and women, young and old, differences related to geographic origin,[10] and anatomic locations in the body.[11] Therefore, a successful hunt for biomarkers that reflect specific plaque characteristics will ask for appropriate stratification.

ARTICLE INFORMATION

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Disclosures

None.

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