Challenges and opportunities for biomarker discovery to predict imminent myocardial infarction

Biomarkers

Challenges and opportunities for biomarker discovery to predict imminent myocardial infarction

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Standfirst

In coronary artery disease the transition from an apparently stable state to a life-threatening acute cardiac event is challenging to predict. As such, a recent study applied proteomic and metabolomic approaches to discover novel biomarkers that herald imminent myocardial infarction.

Coronary artery disease may evolve over many decades from asymptomatic endothelial dysfunction to atherosclerotic plaque rupture and thrombosis resulting in acute myocardial infarction [1]. This dynamic process involves multiple pathophysiological pathways including oxidative stress, inflammation, platelet activation and coagulation that together are responsible for transitions from an apparently stable state to a life-threatening cardiac event. In practice, an external trigger for this transition is rarely apparent and the period preceding the onset of symptoms is unremarkable. As such, science has sought to translate our understanding of these pathogenic mechanisms into novel tools that could herald imminent events so that one could take action to prevent myocardial infarction.

In this issue of *Nature Cardiovascular Research*, Gustafsson and colleagues combine the latest in proteomic and metabolomic screening techniques to seek new markers of imminent myocardial infarction [2]. The authors performed a nested case-cohort study from six population-based cohorts across Europe who were free from cardiovascular disease at the time of blood sampling. The cohort consisted of 420 individuals who had a myocardial infarction within six months and up to four age, sex, and study center-matched controls without myocardial infarction for every case. They used a proximity extension assay and ultra-high-performance liquid chromatography-tandem mass spectrometry to screen more than 1,800 biomarkers identifying 48 proteins and 43 metabolites associated with imminent myocardial infarction. Only one of these biomarkers remained independently associated with myocardial infarction after adjusting for age and sex, and this was an established marker of cardiac disease, B-type natriuretic peptide (BNP).

This study was the first to prospectively evaluate a very large number of biomarkers of imminent myocardial infarction in the general population. The study design was commendable for including a representative cohort of individuals from nine different European countries. The approach to biomarker discovery was comprehensive including a wide range of proteins and metabolites that may be relevant to cardiovascular disease. Advanced methodological techniques - including machine learning approaches - were applied both for biomarker discovery and the development of risk models. Furthermore, the large sample size facilitated a sex-specific analysis, which demonstrated that their findings were consistent across both sexes. Finally, the authors developed a new clinical risk

prediction tool for imminent myocardial infarction with good discrimination on internal and external validation.

Despite screening more proteins and metabolites than ever before, why was only one biomarker identified that was predictive of imminent myocardial infarction? First, we need to consider the time interval between blood sampling and the onset of myocardial infarction. Atherosclerotic plaque rupture, the most common precursor of myocardial infarction, typically occurs in the hours or days prior to the onset of symptoms [3]. This is in stark contrast to the six-month interval between blood sampling and myocardial infarction evaluated here. It is more likely that any biomarkers identified would reflect the presence of subclinical stable atherosclerosis or the consequences of previous silent myocardial infarction. Indeed, BNP is an established marker of ventricular impairment and chronic heart failure [4] and its association with imminent myocardial infarction is less likely to be an early sign of active coronary artery disease than a consequence of previous events or other underlying pathology associated with atherosclerotic risk.

Second, by assessing biomarkers at a single point in time, the study was not designed to evaluate the dynamic nature of atherosclerosis. To capture changes in dynamic processes preceding a myocardial infarction, such as the impairment of fibrinolytic function or platelet activation, tracking biomarkers over time through repeated measures would be informative. Indeed, recent studies of cardiac troponin as a measure of myocardial injury demonstrate that repeated measurements can help to track and refine estimates of future cardiovascular risk in the general population [5]. Hence, incorporating longitudinal biomarker measurements in the risk estimation for imminent myocardial infarction is of particular interest and likely to provide additional prognostic information.

Third, the choice of the study population may have limited the potential to identify biomarkers of an imminent myocardial infarction. Just 1 in 400 of the 169,053 individuals enrolled across six general population cohorts had a myocardial infarction within 6 months of blood sampling. A population with a higher probability of myocardial infarction, such as those with chronic coronary syndromes, might have improved the statistical power of the study and enabled the deployment of shorter periods from sampling to myocardial infarction.

Finally, the use of stored biological material introduces some uncertainty, as only markers that are stable during long-term storage can be reliably measured. Analytical imprecision and limitations in the sensitivity of proximity extension assays and mass spectrometry may have resulted in type II error where promising biomarkers were incorrectly discarded.

What would the ideal study design look like for the discovery of new biomarkers to predict imminent myocardial infarction, and would this be feasible to deliver? The ideal approach would enable high-frequency, real-time measurements in fresh samples obtained immediately prior to and during an acute plaque rupture event. There are some significant practical challenges in delivering such a study. Based on the event rates reported by Gustafsson and colleagues the chance of an individual in the general population having a myocardial infarction within 7 days of sampling was around 1 in 10,000, and therefore the scale of such a study would be prohibitive unless it were to enroll individuals with a substantially higher pre-test probability of myocardial infarction. The recent BIOMArCS study overcame several of the limitations discussed above [6]. The study enrolled 844 patients immediately following acute myocardial infarction in whom 12,281 blood samples were drawn at an average of 17 per patient during the one-year follow-up period. Forty-five patients had a subsequent event, and longitudinal patterns of both cardiac troponin and growth differentiation factor 15 (GDF-15) were associated with cardiovascular death or recurrent acute coronary syndrome, with GDF-15 levels increasing in the period prior to the event [6]. Additional studies applying the methods of Gustafsson and colleagues with proximity extension assay and ultra-high-performance liquid chromatography mass spectrometry in these high-frequency sampling materials may be insightful.

Alternatively, experimental studies that mimic plaque rupture events during planned coronary intervention with sampling from the coronary sinus could be applied to evaluate these panels of biomarkers. The use of intravascular devices, such as the liquid biopsy system, enable sampling of material from the surface of a ruptured plaque to enrich the sample for biomarkers released from the plaque [7]. Using this system, distinct biomarker gradient profiles were identified in stable and ruptured plaques, suggesting that local measurement of biomarkers could help identify biomarkers of plaque activity. However, any novel biomarkers identified in the coronary circulation during

iatrogenic or spontaneous plaque rupture would require validation to confirm they are quantifiable in the systemic circulation and that they are also present prior to the onset of myocardial infarction. To achieve this, innovative technologies may be required, such as the use of subcutaneous sensors capable of continuous, real-time monitoring of biomarkers - an everyday reality for the monitoring of glucose in patients with diabetes mellitus [8]. Adaptation of these technologies to measure protein biomarkers involved in the pathophysiological processes of atherothrombosis would be a major challenge. Recently, the first high-frequency, real-time monitoring of a protein biomarker, neutrophil gelatinase-associated lipocalin (NGAL), was reported in a modified urinary catheter without the need for sample processing, marking a significant advance in the measurement of protein biomarkers [9].

Whilst further discovery science is needed to identify novel biomarkers of atherothrombosis, there is also a pressing need to harness known predictors and established biomarkers more effectively. For instance, BNP and cardiac troponin have consistently been shown to reflect subclinical cardiac disease and predict adverse cardiovascular events [5, 10], yet their use in population screening and risk estimation systems remains limited. Gustafsson and colleagues have created the first prediction tool for imminent myocardial infarction in the general population. The challenge now is to combine the clinical features identified here with serial measurements of established biomarkers to create a truly dynamic risk estimation system. If realised, the opportunity to track individuals at risk and evaluate responses to preventative therapies could be transformative so that myocardial infarction, which may once have been considered imminent, becomes altogether avoidable.

Contributors

MB, DMK and NLM drafted and revised the manuscript critically for important intellectual content, provided approval of the final version to be published, and are accountable for the work.

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Competing interests

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