Despite great enthusiasm for biomarkers as tools to enhance risk prediction and to lead the way in a transformation towards personalized cardiovascular medicine, progress in the biomarker field has been painstakingly slow, particularly in the area of population screening. Some individual biomarkers such as C-reactive protein (CRP) have demonstrated consistent associations with incident cardiovascular events across multiple studies, but the magnitude of these associations is modest, and only small improvements in discrimination and reclassification are seen. One attractive solution to the limitations of individual biomarkers is to combine nonredundant biomarkers into panels to enhance risk assessment. However, results of studies testing multiple biomarkers for risk prediction in primary prevention populations have not provided a clear picture, with some studies showing qualified promise and others suggesting limited value.

In this issue of *Circulation*, Blankenberg et al report findings from an impressive large-scale effort to screen multiple biomarkers for risk assessment in the general population. The investigators evaluated 30 biomarkers reflecting different components of the pathophysiology of atherosclerotic heart disease in 2 cohorts totaling almost 10,000 individuals. Unfortunately, most of the novel biomarkers tested either did not associate with incident cardiovascular events at all or showed promising results in 1 cohort that did not replicate. Although the novel biomarkers largely failed, consistent, albeit modest, associations with incident events were seen across the 2 cohorts for CRP, N-terminal pro B-type natriuretic peptide (NT-proBNP), and cardiac troponin (cTn) I, 3 well-established cardiovascular biomarkers. A weighted biomarker score was developed in men from one of the cohorts (FINRISK 97) and then validated in a second cohort of men (PRIME Belfast). Adding the biomarker score did improve correct reclassification across the entire PRIME Belfast cohort as well as among those in the intermediate risk category. Findings were similar when data-derived cut points were used for each biomarker.

This study has a number of strengths: It represents one of the largest biomarker projects yet reported in cardiovascular medicine; the investigators developed and validated their biomarker panels in separate data sets with sufficient numbers of cardiac end points; and they used comprehensive and state-of-the-art metrics for evaluating the contributions of the biomarkers to standard risk prediction variables. As would be expected given the “real-world” observational design, the huge numbers of data points, and the complexity of the statistical analyses, there are several potential problems as well. A substantial number of individuals were missing data for at least 1 biomarker, and the authors used multiple imputation techniques to estimate the missing values. This strategy assumes that the biomarkers are “predictable” on the basis of other data collected from the individual. However, this argument has an inherent flaw in that the biomarkers were selected because they offered additional information not contained in the other variables. Reassuringly, the authors do note that results were similar in sensitivity analyses using only individuals with complete biomarker data.

A more important issue to consider is that the reclassification analyses were performed only in the PRIME Belfast cohort, which may provide an overly optimistic impression about the potential clinical value of the biomarkers. The baseline risk factor model did not perform as well in PRIME men compared with FINRISK men and women, almost certainly because PRIME enrolled a much narrower age range of individuals, mitigating the dominant effect of age in the standard risk factor models. Clearly, the PRIME Belfast cohort offered an easier “victory” for the biomarker panel compared with the FINRISK men or women. Indeed, it is notable that in prior large population studies evaluating multiple biomarkers, favorable conclusions have been reported from studies enrolling individuals with a restricted age range, whereas those enrolling a broader age range have typically shown less favorable results. In a recent quantitative review of 79 articles evaluating the incremental performance of a new predictor over the Framingham Risk Score alone, a strong inverse association was observed between the performance of the baseline Framingham Risk Score model and
and the increment in the C statistic reported with the new predictor.\textsuperscript{10}

We believe, however, that a reasonable case can be made for the authors’ approach of evaluating biomarkers in sex-specific cohorts with restricted age ranges. First, this approach helps deal with the dominant effects of age and, to a lesser effect, sex, which marginalize other variables in standard risk factor models. Second, this strategy provides clinically relevant information for the age and sex groups tested. Of course, the results cannot be generalized to other age ranges (or race and sex groups). It would have been instructive to also perform the reclassification analyses in the more heterogeneous FINRISK cohort because this would have likely highlighted the complicated influences of age and sex on the interpretation of results of these studies.

What are the implications for the winners and losers from this biomarker competition? First, although the international debate on the utility of biomarkers for population screening has long focused on CRP, it is now time to broaden consideration to include other biomarkers that appear to provide predictive information at least as robust as CRP in this setting. For example, although the incremental value of adding NT-proBNP to CRP was not directly assessed in the present study, as an individual biomarker, NT-proBNP displayed performance characteristics similar to CRP in terms of discrimination and reclassification. In previous population-based studies that directly compared NT-proBNP or BNP with CRP for risk estimation, the natriuretic peptides typically show greater prognostic value.\textsuperscript{5,7,8,11,12} At this point, however, the diagnostic and therapeutic implications of elevated natriuretic peptide levels in the general population have not been clearly defined.

These findings also require that we reconsider long-held notions about where and when cTn measurement may be informative. It is increasingly clear that cTnT and cTnI may be released in response to chronic as well as acute cardiac injury and may be elevated in asymptomatic individuals at increased cardiovascular risk.\textsuperscript{4,5,13} In distinction to the acute care setting, it appears that elevated troponin levels in ambulatory subjects reflect cardiac structural and functional abnormalities more than ischemic risk\textsuperscript{13} and, like NT-proBNP, are particularly informative for death and heart failure events.\textsuperscript{5,14} In this study, cTnI was measured with a contemporary sensitive assay but not an emerging higher-sensitivity assay. Higher-sensitivity assays may allow even greater risk discrimination among apparently healthy individuals.

Although the study represents a qualified victory for multiple biomarker panels that include CRP, NT-proBNP, and cTnI, does it represent the end of the road for the other biomarkers that were tested and failed? More important, does the failure here and in prior studies of the more novel biomarkers suggest that biomarker discovery in this area is likely to be futile? We believe such a conclusion would be premature. First, characteristics of the cohort studied, including size and demographic makeup, can significantly influence biomarker performance. In the present study, for example, differences between subjects enrolled in the 2 cohorts may have contributed to the failure of some potentially informative biomarkers to replicate. Second, biomarker selection may also vary depending on how the standard risk factors are modeled, with subtle differences having potentially important implications.\textsuperscript{10} Third, in our experience with similar data sets, selection and prioritization of biomarkers for inclusion in the multiple biomarker panels are sensitive to several statistical considerations, including degree of collinearity between biomarkers, analysis of biomarkers as continuous versus categorical covariates, and choice of selection and optimization criteria (ie, based on strength of association, indices of model fit, C statistic, or integrated discrimination index). Finally, statistical power is an important issue to consider, particularly when evaluating large numbers of covariates. In the present study, soft end points such as unstable angina and revascularization were included in the composite, and actual numbers of hard coronary end points were modest relative to the numbers of covariates evaluated. A careful review of the data from the present study suggests that each of these factors may have contributed to the exclusion of some biomarkers from further consideration. Although the present study is one of few to report multiple performance characteristics on a host of novel markers, it is clear that much work is needed to define optimal methods for biomarker screening, selection, optimization, and validation.

Progress forward requires movement in several directions. For the established biomarkers, further clinical validation of panels containing CRP, NT-proBNP (or BNP), and a sensitive troponin assay is required in different age, race, and sex groups, given the known influences of these factors on levels of these biomarkers. We also need carefully designed observational and interventional studies to help us understand the full implications of reclassification based on these biomarkers. In particular, it is critical to establish the safety of deferral of preventive therapies for individuals who are reclassified to a lower risk category by biomarkers. With regard to the more novel biomarkers, careful thought is needed with regard to the appropriate target populations for discovery and validation, as well as the criteria used to sort out the contenders from the pretenders.

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References


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Separating the Contenders From the Pretenders: Competitive High-Throughput Biomarker Screening in Large Population-Based Studies

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