Biomarkers and Cardiovascular Risk Assessment for Primary Prevention: An Update

Lauren G. Gilstrap1 and Thomas J. Wang1*

BACKGROUND: Interest in cardiovascular biomarkers in primary prevention has increased dramatically in the past decade. This increase has been fueled by an improved understanding of cardiovascular pathophysiology, as well as novel technologies for biomarker identification.

CONTENT: In this review we provide a brief overview of recent concepts in the evaluation of screening biomarkers, because biomarkers may behave differently when used for screening as opposed to diagnosis or disease staging. The following specific biomarker examples are then discussed, with a focus on data from primary prevention studies: high-sensitivity C-reactive protein, B-type natriuretic peptide, lipoprotein-associated phospholipase A2, and high-sensitivity troponin T. The article concludes by addressing novel platforms for biomarker discovery, reviewing recent examples from the field of metabolomics.

SUMMARY: An ongoing challenge is to develop screening strategies that can identify individuals at risk for cardiovascular events well before symptoms appear. For this purpose, the measurement of soluble biomarkers could be an important adjunct to traditional cardiovascular risk assessment. Recent studies highlight both the strengths and limitations of “novel” circulating biomarkers, and suggest that substantial work is still needed to identify biomarkers that are sufficiently accurate and cost-effective for routine use in primary prevention.

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Interest in cardiovascular biomarkers in primary prevention has increased dramatically in the past decade. This increase has been fueled by advances in genetic and molecular research, which have yielded insight about early cardiovascular pathophysiology and simultaneously provided novel platforms for biomarker discovery. Another factor contributing to the increased focus on early screening tests has been the recognition that traditional cardiovascular risk factors (e.g., hypertension, hyperlipidemia, smoking, diabetes) do not fully explain interindividual variation in cardiovascular risk. For instance, a large proportion of individuals who develop cardiovascular disease have few or no risk factors.

The challenge for physicians, researchers, and clinical chemists is to develop screening strategies that safely, accurately, and cost-effectively identify individuals at risk for cardiovascular events well before symptoms appear. Preventive interventions are likely to be most effective in this period, because atherosclerosis can take decades to manifest. The measurement of “novel” biomarkers could be an important component of these strategies. Although a biomarker can be anything that reflects a biological process—from genetic markers to imaging tests—soluble biomarkers are particularly attractive because they are easy to obtain and generally reproducible.

Because biomarkers may behave differently when used for screening as opposed to diagnosis or disease staging, a distinct set of metrics has arisen to assess screening biomarkers. Thus, we begin this review with a brief overview of recent concepts in the evaluation of screening biomarkers. Then, we describe a few specific examples from the recent literature. For the sake of this review, only circulating biomarkers will be discussed.

General Considerations

In 2007, Morrow and de Lemos outlined 3 criteria for evaluating novel biomarkers: (a) ease of measurement; (b) addition of information; and (c) effect on management. The first criterion is typically straightforward to assess. Indeed, one of the advantages of soluble biomarkers is that standardized, reproducible assays exist for most biomarkers of interest. In contrast, addressing the second criterion—the incremental information from measuring a biomarker—can be challenging. In cardiology, a biomarker is useful only when it contrib-
utes information in addition to that provided by traditional risk factors. The best way to evaluate this contribution statistically in low-risk populations has been a source of debate; several approaches have been proposed, which are described in the following section.

Whether a biomarker is used to screen for a disease, diagnose a disease, or inform prognosis, it should affect clinical management. Because few randomized trials have been performed to evaluate biomarkers in the primary prevention setting, little is known about the impact of biomarker-guided strategies on clinical outcomes. The section on high-sensitivity C-reactive protein (CRP)\(^2\) highlights some of the challenges involved in producing the necessary evidence.

A specific consideration in measuring biomarkers for cardiovascular screening is the potential importance of noncardiac sources of variation, given the need to interpret concentration differences that are typically much smaller than those observed in acutely ill patients. Such variation may be particularly relevant for biomarkers originating in extracardiac tissue, such as CRP, which increases with infectious states or weight gain. Nonetheless, it can also be important for cardiac-derived biomarkers such as B-type natriuretic peptide (BNP), which is influenced by noncardiac factors such as sex,\(^5\) sex hormone status,\(^7\) weight\(^9\)–\(^{13}\). An understanding of such effects is essential for implementing biomarker measurements in the ambulatory setting.

**Statistical Assessment**

A statistically significant association between a biomarker and cardiovascular disease is necessary but not sufficient to determine if the biomarker is clinically relevant\(^{14}\). Statistical significance, in this case, indicates only that the mean biomarker concentration differs between individuals with and without the disease (or between those who have and don’t have an event). Because the distributions of actual biomarker concentrations between diseased and nondiseased groups may overlap substantially, the value of a biomarker measurement in a given individual may be limited, even if a significant association exists in the overall sample\(^{15}\). Therefore, at least 3 additional statistical criteria have been proposed to assess the utility of new biomarkers: discrimination, calibration, and reclassification (Table 1).

Discrimination is the ability of a biomarker to distinguish those who develop a disease from those who do not\(^{16}\). Discrimination can be characterized by the area under the ROC curve (AUC) or the c-statistic. The c-statistic is a function of the sensitivity and specificity of a test across all diagnostic thresholds. The sensitivity of a test refers to its ability to detect disease when it is present, the so-called true-positive rate. Specificity refers to the ability of a test to exclude disease when it is not present, the true-negative rate. The c-statistic ranges from 0.5 (uninformative test) to 1.0 (perfect discrimination)\(^{17}\). In general, a c-statistic >0.7 is considered good. The c-statistic for coronary heart disease (CHD) with traditional risk factors (e.g., in the form of the Framingham Risk Score) is approximately 0.75\(^{18}\). Thus, the value of a new biomarker can be gauged by determining how much higher the c-statistic becomes with the combination of traditional risk factors and the biomarker test. A test that increases the c-statistic by 0.05 or more is thought to add clinically useful information\(^{19}\). There is no consensus about the importance of lesser changes in the c-statistic (0.01–0.05), which may depend in part on the baseline value. For instance, moving from 0.75 (with the Framingham Risk Score) to 0.77 (with the Framingham Risk Score plus biomarkers) may be more notable than moving from 0.50 to 0.55.

Calibration refers to the concordance between predicted risk and observed risk, assessed by comparing modeled risk estimates with actual event rates\(^{20}\). The most commonly reported calibration statistic is the Hosmer-Lemeshow statistic. Tests or risk models with a Hosmer-Lemeshow P value >0.05 are considered well calibrated because there is no significant difference between predicted and observed event rates. Predicted risk is clinically relevant, because treatment decisions are often based on estimation of future risk\(^{20}\). If a patient’s risk of having a cardiovascular event is quite high, a clinician may be more likely to start early pharmacologic therapy to lower LDL (with statins) or reduce thrombotic tendency (with aspirin). On the other hand, if the patient’s risk is low, the side effects might outweigh the likely benefits of pharmacologic therapy.

Estimates of calibration are sensitive to differing baseline levels of risk. For instance, if a given risk model is derived in a high-risk population but tested in a low-risk population, the predicted risk estimates might be unreliable. Recalibration of the risk model by adjusting the baseline risk estimates to fit the target population may help to mitigate over- or underestimation of risk\(^{21}\). Unfortunately, implementing this method in the clinical setting may not be feasible. Providers typically have access only to published risk scores and do not

\(^2\)Nonstandard abbreviations: CRP, C-reactive protein; BNP, B-type natriuretic peptide; AUC, area under the ROC curve; NRI, net reclassification improvement; CHD, coronary heart disease; Lp-PLA\(_2\), lipoprotein-associated phospholipase a\(_2\); hs, high sensitivity; NT, N-terminal; MS, mass spectrometry.
have the luxury of recalibrating risk scores to reflect their individual patient populations (16).

Reclassification refers to the ability of a test to change an individual’s risk classification. Because reclassification is based on risk categories, it is potentially the most clinically relevant criterion, because treatment decisions are often linked to whether a patient is considered high risk or low risk rather than on their specific predicted event rate. On the other hand, the usefulness of reclassification relies on the existence of well-established risk categories. For cardiovascular disease, the Adult Treatment Panel III is the most commonly used risk stratification algorithm (22). It incorporates the traditional risk factors of the Framingham Risk Score and categorizes individuals as low, intermediate, or high risk, on the basis of their 10-year predicted risk of CHD. Low-risk individuals are those who have a <10% predicted risk of a CHD event over 10 years. Intermediate-risk individuals have a 10%–19% predicted risk, and high-risk individuals have a 20% or greater predicted risk (23). According to the Adult Treatment Panel III, individuals in the high-risk group warrant more aggressive medical intervention, specifically initiation of a statin with an LDL goal of <70 mg/dL (14). If the addition of one or more biomarkers to traditional risk factors changed the risk categorization of a given patient, it could affect their management.

Reclassification can be described by estimating the proportion of individuals in a population who are reclassified, although this metric doesn’t account for whether the reclassification is “correct” or not. Pencina and colleagues have proposed a metric known as the net reclassification improvement (NRI), which summarizes the net proportion of individuals with “correct” reclassification (e.g., those who develop events who were up-classified, and those who don’t develop events who were down-classified) and “incorrect” reclassification (those who develop events who were down-classified, and those who don’t develop events who were up-classified) (24). They have recently extended this concept to include a “category-free” NRI, which does not depend on the existence of fixed risk categories (25).

There are advantages and limitations to each criterion for evaluating biomarkers. The \( c \)-statistic (discrimination) is easily understood, in that it is a function of sensitivity and specificity and does not rely on specific thresholds or categories. Nonetheless, it is often quite challenging for a new biomarker to raise the \( c \)-statistic when existing models, such as the Framingham Risk Score, already discriminate well. Biomarkers

<p>| Table 1. Selected criteria for assessing the incremental predictive value of new biomarkers$^a$ |</p>
<table>
<thead>
<tr>
<th>Metric(s)</th>
<th>Attribute</th>
<th>Description</th>
<th>Advantages</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>c-Statistic, AUC</td>
<td>Discrimination</td>
<td>Captures the sensitivity and specificity of a marker, across a full range of cutpoints</td>
<td>Easily understood</td>
<td>Difficult for new biomarkers to raise the ( c )-statistic when existing variables discriminate well</td>
</tr>
<tr>
<td>No reliance on specific thresholds or categories</td>
<td></td>
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<td></td>
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<tr>
<td>Hosmer-Lemeshow statistic</td>
<td>Calibration</td>
<td>Assesses concordance between predicted and observed event rates in longitudinal data</td>
<td>Assesses accuracy of predicted risks (basis for clinical decisions)</td>
<td>Relatively insensitive to differences between models</td>
</tr>
<tr>
<td>Provides a global measure, even though clinical decisions may be based on predicted risks within a relatively narrow range</td>
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</tr>
<tr>
<td>Net reclassification improvement</td>
<td>Reclassification</td>
<td>Assesses the proportion of individuals reclassified correctly by the addition of new biomarker(s)</td>
<td>Clinically relevant when risk categories are linked to treatment decisions</td>
<td>Sensitive to changes in the number of risk categories and choice of cut points</td>
</tr>
<tr>
<td>Incorporates the accuracy of reclassification</td>
<td>Gives same weight to reclassifications that are unlikely to affect clinical decisions</td>
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</tbody>
</table>

$^a$ Adapted from Wang et al. (16), with permission.
could provide clinically important information despite small increments in the c-statistic. The Hosmer-Lemeshow statistic (calibration) assesses the accuracy of predicted risk estimates, which are the basis for most clinical decisions. However, as noted, recalibration of models may be necessary when applied to different populations. Furthermore, as with discrimination, it may be difficult to measure improvement in calibration when baseline models are already well calibrated (e.g., when the Hosmer-Lemeshow statistic already has a P-value >0.05). Reclassification has clinical relevance because practice guidelines typically refer to predetermined risk categories. The dependence of reclassification metrics on the number of risk categories and the choice of cutoff points has been noted earlier. Furthermore, reclassification metrics generally do not take into account whether the shift in risk classification would lead to a change in clinical management (16). Thus, it has been proposed that studies addressing biomarkers in primary prevention should describe the performance of new biomarkers according each of these complementary criteria (26).

Specific Biomarkers

Over the past decade, multiple new cardiac biomarkers have emerged. Below, we discuss several examples of established biomarkers (CRP, BNP) and newer biomarkers [lipoprotein-associated phospholipase A2 V(Lp-PLA2) and high-sensitivity troponin (hs-troponin)]. We then review findings from one of the newer platforms for biomarker discovery (metabolomics). These examples are meant to be illustrative, rather than providing a comprehensive description of available cardiovascular biomarkers.

High-Sensitivity CRP

CRP was originally discovered by Tillett and Francis in 1930. It was found in the serum of patients with acute inflammation and was noted to react with the C polysaccharide of pneumococcus, hence its name (27). CRP is an acute-phase reactant, produced primarily by hepatocytes in response to stimulation from interleukin-6 and tumor necrosis factor-α (28). Typically, CRP binds to phosphocholine expressed on the surface of dead or dying cells and activates the complement system via the C1Q complex (29).

CRP is a 224-residue protein with an annular, pentameric disc shape. In general, circulating CRP concentrations can increase up to 50 000-fold in acute inflammation (i.e., infection) within 6 h. CRP typically peaks at 48 h. Because the half-life of CRP is constant, the degree of increase is determined by the severity of the precipitating cause (30). Intraindividual variability of CRP in healthy individuals has been studied by several groups. For instance, Macy et al. found an intrindividua variability (CVI) of 42%, compared with a between-individual variability (CVg) of 93% (31). The index of individuality (CVI/CVg) of 0.46 is comparable to other analytes such as total cholesterol. Another study, involving Framingham Heart Study participants, examined the rank stability of long-term CRP measurements. Between 2 routine examinations (a mean of 16 years apart), approximately half of the more than 2000 individuals studied remained within the same quintile of CRP values (32).

In the past 15 years, more than 20 epidemiological studies have demonstrated a significant association between increased CRP concentrations as measured by use of high-sensitivity assays (hs-CRP) and the risk of a first cardiovascular event among asymptomatic patients (33, 34, 35). A metaanalysis of 22 studies by the US Preventive Services Task Force indicated that hs-CRP concentrations greater than or equal to 3 mg/L were associated with a 60% increased risk of cardiovascular disease (36). The degree to which addition of hs-CRP measurement improves the predictive accuracy of traditional risk stratification models, however, has been controversial. Studies in low-risk populations have generally shown modest increases in the c-statistic with the addition of hs-CRP, from 0.0 to 0.02 (37). The same studies have shown only modest or absent increases in global calibration measures. On the other hand, investigators from the Women’s Health Study reported that the addition of hs-CRP reclassified a significant proportion of individuals, including approximately 20% of those at “intermediate risk.” The majority of the reclassification for intermediate-risk individuals (roughly 75%) were downclassifications into the low risk group (37).

The JUPITER (Justification for the Use of Statins in Primary Prevention: An Intervention Trial Evaluating Rosuvastatin) study was a large, primary prevention trial with a potent statin medication (rosuvastatin) (38). Its use of hs-CRP concentrations as one of the inclusion criteria makes it one of the few major randomized controlled trials in cardiology to incorporate biomarker-directed therapy. JUPITER enrolled nearly 18 000 individuals (men >50 and women >60 years old) with LDL concentrations less that 130 mg/dL, but hs-CRP concentrations >2 mg/L. The study was stopped early due to an approximately 40% reduction in the composite cardiovascular end point with rosuvastatin compared with placebo. Because the study did not randomize individuals with hs-CRP concentration <2 mg/L, it is uncertain whether screening with hs-CRP is necessary to identify older adults who would benefit from statin therapy. In the 2010 American College of Cardiology Foundation/American Heart Association Guideline for Assessment of Cardiovascular
Risk in Asymptomatic Adults, the measurement of hs-CRP in older men and women with LDL <130 mg/dL to aid in the decision to use statins was given a class IIa recommendation (39). Overall, measuring hs-CRP for cardiovascular risk assessment in men >50 years and women >60 years was given a class IIb recommendation.

B-TYPE NATRIURETIC PEPTIDE
BNP is a member of a family of hormones known as the natriuretic peptides. The natriuretic peptides are synthesized primarily in the heart, and upregulated by myocardial wall stress (40). The gene that encodes BNP [natriuretic peptide B (NPPB)] initially produces proBNP. PreproBNP is cleaved to form proBNP, a 108–amino acid peptide precursor. Finally, proBNP is cleaved by the enzyme corin, forming the biologically active C-terminal fragment (mature BNP) as well as the inactive N-terminal fragment (NT-proBNP). Although both BNP and NT-proBNP can be measured in plasma, the latter has a longer half-life (1–2 h, compared with 20 min). In ambulatory populations, more than 90% of individuals have detectable NT-proBNP concentrations, compared with roughly 70% for BNP (41).

Both BNP and NT-proBNP are markedly increased in individuals with acute heart failure (42), which makes them valuable biomarkers in the setting of suspected heart failure. Interestingly, within the “normal” reference interval of values, there is significant variation in BNP among apparently healthy individuals, and this variation is prognostically significant (43). For example, in the Framingham Offspring Study, BNP concentrations >20 pg/mL were associated with a 60%–200% increased risk of cardiovascular events, stroke, heart failure, and all-cause mortality during a mean follow-up of 5.2 years (45). A recent metaanalysis of 40 prospective studies, approximately half of which were performed in primary prevention populations, found consistently strong associations between BNP concentrations and cardiovascular risk (Fig. 1) (46). Subtle increases in BNP could reflect increased ventricular wall stress resulting from subclinical ischemia, high afterload, or increased neurohormonal activation. Interestingly, the association between BNP and future events persists even after adjustment for standard echocardiographic measures (45).

There are fewer data on whether BNP measurements improve discrimination, calibration, or reclassification for predicting cardiovascular events, on top of traditional risk factors. In the Malmo Diet and Cancer cohort, NT-proBNP was evaluated along with CRP, lipoprotein-associated phospholipase 2, midregional proadrenomedullin, and cystatin C (41). During more than 12 years of follow up, NT-proBNP predicted cardiovascular and coronary events, even after adjusting for CRP and other biomarkers. In the whole sample, there was only a modest increase in c-statistic, and a nonsignificant NRI. Results were stronger when analyses were restricted to intermediate-risk individuals, in whom significant NRIs were noted (ranging from 5% to 15%). Most of the correct reclassification consisted of “down-classification” to lower risk categories.

LIPOPROTEIN-ASSOCIATED PHOSPHOLIPASE A2
Lp-PLA2 is a 441–amino acid protein, encoded by the phospholipase A2, group VII (platelet-activating factor acetylhydrolase, plasma) (PLA2G7) gene, which is produced by inflammatory cells. It circulates mainly with LDL (<20% is associated with HDL or remnant lipoproteins), and it is responsible for hydrolyzing oxidized phospholipids in LDL. Specifically, it catalyzes the degradation (hydrolysis) of platelet activating factor to inactive products (47). It is highly upregulated in atherosclerotic plaques, and may be directly involved in development of atherosclerosis (48) and plaque rupture (49). Because it is produced by macrophages and foam cells in the vascular intima, Lp-PLA2 is thought to be potentially more specific for vascular inflammation than other inflammatory markers, such as CRP (50).

Early in vivo studies showed that both rabbits and humans with increased concentrations of circulating Lp-PLA2 have a higher plaque burden in the coronary arteries than those with normal concentrations (51). Furthermore, it has been shown experimentally that inhibiting Lp-PLA2 leads to a reduction in atherosclerotic lesions in hyperlipidemic rabbits (51). Given its low biological variation, specificity for the vasculature, and association with atherosclerotic plaque burden, Lp-PLA2 has been proposed as a potential biomarker for identifying individuals at increased risk of cardiovascular disease.

There have been more than 10 studies assessing the role of Lp-PLA2 in primary prevention. For instance, in the West of Scotland Coronary Prevention Study study, both hs-CRP and Lp-PLA2 were significantly associated with increased cardiovascular risk (52). Furthermore, after adjustment for traditional risk factors, Lp-PLA2 maintained its association whereas hs-CRP did not (52). In the Atherosclerosis Risk in Communities study, the weighted mean of both Lp-PLA2 and hs-CRP were higher in those who went on to develop coronary events than in those who did not (53). As with BNP, there are fewer data regarding the impact of Lp-

3 Human genes: NPPB, natriuretic peptide B; PLA2G7, phospholipase A2, group VII (platelet-activating factor acetylhydrolase, plasma).
PLA2 measurements on discrimination, calibration, or reclassification. A recent nested case-control study from the Nurses Health Study examined discrimination with Lp-PLA2 in women (54). The additional of Lp-PLA2 raised the $c$-statistic from 0.72 to 0.733, and improved net reclassification. In contrast, a report from the Malmo Diet and Cancer Study suggested nonsignificant changes in discrimination and reclassification (41).

Fig. 1. Relative risks for cardiovascular disease (CVD) in individuals in the top vs bottom third of baseline BNP or NT-proBNP concentration, according to different study characteristics. Reproduced with permission from Di Angelantonio et al. (46).
HIGH-SENSITIVITY TROPNONIN

In 1963, Ebashi and colleagues discovered that a new myofibrillar protein, troponin, was integral to both skeletal and cardiac muscle contraction (55). Troponin has 3 subunits: troponin C, I, and T. Troponin C is responsible for sensing and responding to the presence of calcium. Troponin T binds tropomyosin, forming the troponin–tropomyosin complex. Troponin I binds to actin in thin myofilaments, and holds the troponin–tropomyosin complex in place (56).

Cardiac troponins have become the standard biomarker in the diagnosis of acute myocardial infarction. On the basis of consensus guidelines, a troponin concentration above the 99th percentile (of a healthy population) is considered diagnostic of an acute myocardial infarction. With conventional troponin assays, troponin concentrations are undetectable in the vast majority of healthy individuals. However, newer generations of cardiac troponin I and T assays, the so-called high-sensitivity assays, permit detection of concentrations significantly lower than the 99th percentile of the normal reference population (57). The high-sensitivity assay, which can detect femtomolar concentrations of troponin, has increased the sensitivity of acute myocardial infarction diagnosis and introduced other potential clinical applications (58).

There have been 3 recent cohort studies examining the clinical utility of hs-troponins. A recent study by de Lemos et al. measured troponin T concentrations using both standard and highly sensitive assays in over 3500 adults in the Dallas Heart Study (59). The prevalence of detectable troponin T was 25% with the high-sensitivity assay, compared with only 0.7% for the conventional assay. The investigators found that hs-troponin T concentrations were associated with baseline structural heart disease (as determined based on MRI) and all-cause and cardiovascular mortality over 6 years of follow up (Fig. 2). This association was substantially attenuated with the use of conventional troponin T. The addition of hs-troponin T to a multivariable-adjusted model for mortality improved the c-statistic (+0.02, P = 0.001) and integrated discrimination index (0.04, P<0.001).

Fig. 2. Association of cardiac troponin T (cTnT) detected with a highly sensitive assay and all-cause and cardiovascular mortality.

The blue segment of the vertical axis indicates the range from 0% to 20%. Reproduced with permission from de Lemos et al. (59).
deFilippi et al. studied the prognostic value of hs-troponin T concentrations in more than 4200 older adults (age >65 years) (60). Increased hs-troponin T concentrations were associated with the development of heart failure and cardiovascular death. The addition of hs-troponin T to traditional risk factors raised the c-statistics for heart failure and cardiovascular death by approximately 0.01. Similarly, investigators from the Atherosclerosis Risk in Communities study found that hs-troponin T concentrations were associated with incident CHD, mortality, and heart failure in nearly 10000 individuals (44). Furthermore, the addition of hs-troponin T to traditional risk factors modestly improved discrimination and reclassification.

Comparisons of Biomarkers

A growing number of studies have provided data on the comparative performance of cardiovascular biomarkers. For instance, several cohort studies suggest that NT-proBNP concentrations are more strongly related to future vascular risk than hs-CRP concentrations (61–63). Similarly, recent studies indicate hs-troponin measurements have predictive value comparable to NT-proBNP and greater than hs-CRP (44, 59). Not surprisingly, panels of multiple biomarkers appear superior to individual biomarkers (61, 63, 64), although the optimal composition of multimarker panels remains to be established.

Furthermore, biomarkers may differ in their ability to predict specific types of cardiovascular events. This variation often reflects the physiology underlying the biomarkers. For instance, BNP and NT-proBNP strongly predict incident heart failure risk (45, 65), which is not surprising in view of their association with cardiac wall stress. hs-CRP and Lp-PLA2 are very good markers of vascular risk, probably reflecting the importance of vascular inflammation in atherogenesis (66). On the other hand, “myocardial” biomarkers such as NT-proBNP and hs-troponin are also surprisingly good predictors of future vascular events, for reasons that are not entirely understood (44, 67).

New Directions

Newer technologies permit the systematic study of proteins (proteomics) and small molecules (metabolomics) in biospecimens, including plasma or serum. Proteomics and metabolomics have similarities to genomics, in that they provide an unbiased approach to biomarker identification (1). In contrast to genetic profiles, however, proteomic or metabolomic profiles can respond to environmental influences and provide a “snapshot” of current physiologic states (Fig. 3).

Metabolomics focuses on small molecules such as lipids, sugars, nucleotides, and amino acids. Current metabolomic platforms rely on 2 principal technologies: nuclear magnetic resonance and mass spectrometry (MS) (68). The latter includes GC-MS and LC-MS. There are several important distinctions between metabolomics and proteomics that are relevant from an analytic standpoint. The number of circulating metabolites is unknown, but is estimated to be in the thousands, several orders of magnitude lower than the number of proteins. In addition, circulating metabolites span a much smaller range of concentrations than proteins (69). Metabolites typically reflect activity that is further downstream of gene expression and poten-
tially more closely related to cellular function than do proteins. Thus, circulating metabolites have the potential advantage of providing immediate information about an organism’s physiologic condition, but the potential disadvantage of being affected by multiple sources of variability including diet, activity, and medication use.

An early study by Brindle and colleagues demonstrated the promise and potential pitfalls of metabolomic approaches to cardiovascular screening (70). They analyzed (1) H-NMR sera in individuals with angiography-proven coronary artery disease, and compared the profile with those in healthy controls. They developed a profile that identified the cases with >90% accuracy. However, a subsequent report showed that common variables, including sex and statin use, were important confounders of the nuclear magnetic resonance spectroscopy profile (71).

Several groups have used targeted LC-MS approaches to reduce potential susceptibility to confounding inherent in more global screens. For instance, Shah and colleagues used targeted LC-MS to profile 69 metabolites in individuals with and without coronary artery disease referred to an academic cardiac catheterization laboratory (72). Two principal components that were analysis-derived factors, one comprising branched-chain amino acids and another comprising urea cycle metabolites, were associated with the presence of coronary artery disease. Similarly, we recently employed targeted LC-MS to investigate predictors of future diabetes in the Framingham Offspring Study (73). Five branched-chain and aromatic amino acids were strongly associated with incident diabetes over 12 years of follow-up (P = 0.001 to <0.0001). The findings were replicated in an independent Swedish cohort.

Another recent study demonstrated the potential for metabolomic approaches to elucidate etiologic factors in cardiovascular disease. Wang and colleagues used nontargeted LC-MS to profile specimens from individuals with and without incident myocardial infarction (74). They identified 3 metabolites derived from phosphatidylcholine—choline, betaine, and trimethylamine-N-oxide—that were associated with incident events. Dietary supplementation of these metabolites promoted atherosclerosis in animal models, which was inhibited by suppression of gut microflora involved in the metabolism of phosphatidylcholine.

Gene expression profiling represents another relatively unbiased technology for biomarker discovery. Rosenberg and colleagues recently derived and tested a 23-gene expression profile from whole blood for detecting coronary artery disease in individuals without diabetes (75). At a score threshold corresponding to a 20% likelihood of coronary artery disease, the sensitivity and specificity were 85% and 43%. More studies are warranted to validate this approach, and to investigate whether profiles from specific cell populations might enable more accurate detection of occult cardiovascular disease.

Conclusion

Biomarkers hold the promise of earlier and more accurate cardiovascular risk stratification. Their role in acute cardiovascular diseases, such as myocardial infarction and heart failure, has been well studied. An increasing number of studies have investigated the role of biomarkers in primary prevention. To date, no biomarker has emerged as the best screening marker for cardiovascular disease, and it is likely that no single biomarker will be sufficiently sensitive or specific to be used on its own.

Future strategies will likely involve larger biomarker panels and more specific target populations. Larger panels require new biomarkers that provide nonoverlapping information to already-available biomarkers or risk factors (16). Newer, “unbiased” approaches, such as proteomics or metabolomics, show some promise in this regard. Further studies are also needed to better define the target populations for biomarker screening, because a very broad approach may be unnecessary as many individuals are adequately risk stratified by traditional risk factors.

Author Contributions: All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.

Authors’ Disclosures or Potential Conflicts of Interest: Upon manuscript submission, all authors completed the Disclosures of Potential Conflict of Interest form. Potential conflicts of interest:

Employment or Leadership: None declared.
Consultant or Advisory Role: T.J. Wang, Diasorin, Inc.
Stock Ownership: None declared.
Honoraria: T.J. Wang, Roche Diagnostics and Quest Diagnostics.
Research Funding: T.J. Wang, NIH R01-HL086875, R01-HL102780, R01-DK081572, R01-HL088283, and R01-HL083197; Diasorin; Brahms; Critical Diagnostics; Singulex; Siemens Healthcare Diagnostics; and Roche Diagnostics.
Expert Testimony: None declared.
Other: T.J. Wang, coinventor on patent applications relating to the use of metabolomic and neurohormonal biomarkers in risk prediction.
Biomarkers in Primary Prevention

References


Ebashi S. Third component participating in the...


