New Antibody Assays for Cardiovascular Disease: Future Tools for the Clinical Chemist?

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Cardiovascular disease remains the leading cause of death in developed countries, a fact that underscores the importance of effective preventive measures. The accurate identification of those at risk for primary or recurrent events is essential for implementing preventive measures. Over the past several decades, this evaluation has been based on the assessment of classic risk factors, including hypertension, diabetes, hyperlipidemia, and smoking. It has been recognized, however, that a substantial proportion of individuals who develop coronary heart disease have only one or none of the classic cardiovascular risk factors, which has led to interest in identifying new genetic and nongenetic biomarkers to augment risk prediction and illuminate novel disease mechanisms.

Other than measurements of lipids, there are currently no widely accepted biomarkers for cardiovascular screening in clinical practice. In the last decade, however, a number of circulating biomarkers in blood or urine have generated interest because of their success in predicting future cardiovascular events in ambulatory populations and because of their mechanistic involvement in atherosclerosis-associated pathways. These include biomarkers associated with inflammation (high sensitivity C-reactive protein, interleukin-6, lipoprotein-associated phospholipase A2), hemostasis/thrombosis (fibrinogen, plasminogen activator inhibitor 1), neurohormonal activation (renin, B-type natriuretic peptide), insulin resistance (insulin, hemoglobin A1C), and endothelial dysfunction (homocysteine, urinary microalbuminuria). The incremental value of these biomarkers over conventional risk factors remains a source of debate (1). The majority of present biomarkers fall along pathways already known to be associated with atherosclerotic vascular disease, potentially limiting their ability to provide incremental clinical utility or new biological insight. However, technologies are emerging to enable the systematic characterization of variation in genes, RNA, and proteins in individuals with and without disease, permitting the discovery of biomarkers in a manner that is unbiased by current knowledge.

In a study that illustrates the potential utility of a new class of cardiovascular biomarkers, Cleutjens et al. (2) recently described the application of a novel protein-array technique to the identification of antibody biomarkers of ruptured atherosclerotic lesions. The detection of autoantibodies in human serum is currently used for the diagnosis of patients with autoimmune diseases. For example, antibodies directed against the Sm antigens and double-stranded DNA are specific for the diagnosis of systemic lupus erythematosus (SLE). Similarly, anti-Ro and anti-La autoantibodies suggest a diagnosis of Sjogren syndrome, whereas anti-Scl70 antibodies support a diagnosis of diffuse systemic sclerosis (reviewed in (3)). The reason intranuclear autoantigens, protected from the extracellular environment by both the nuclear and cellular membranes, become the target of the humoral immune system in patients with autoimmune diseases is unknown.

Recent studies suggest that autoantibodies may also assist in the diagnosis of patients with early malignancies. Wang et al. (4) reported that potential peptides present in prostate cancer tissue could be used to detect autoantibodies directed against prostate cancer. Similarly, polypeptide panels have been proposed to identify autoantibody signatures associated with colon (5), pancreatic (6), and breast (7) cancers. The rationale for the development of polypeptide panels to detect antibodies is that some tumor antigens may be considered foreign by the immune system and that antibodies directed against these tumor-associated antigens may serve as early, sensitive, and specific markers of malignancy.

In an extension of earlier studies relating to antibody formation in patients with autoimmune disease and cancer, Cleutjens et al. (2) described the identification and implementation of antibody signatures to identify individuals with ruptured, inflammation-rich,

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4 Nonstandard abbreviations: SLE, systemic lupus erythematosus; TnT, troponin T.
Atherosclerotic lesions. The authors generated a phage-display library prepared from mRNA preferentially expressed in ruptured, peripheral, human atherosclerotic plaques. The cDNAs were prepared and expressed as fusion proteins with the minor coat protein pVI of filamentous phage M13. Sera from patients with ruptured atherosclerotic plaques were then used to perform 4 rounds of selection for phage-expressing immunoreactive fusion proteins. Two peptides, E1 and E12, were identified and used to screen serum from larger cohorts of patients with coronary and peripheral artery atherosclerotic lesions, as well as controls. The E1 peptide contained the 16 amino acids present within a potential protein designated 1NFLS. The E12 peptide is a nonsense peptide derived from the untranslated 3’ end of a cDNA encoding protein kinase C η. Antibodies directed against E1 were detected in a majority of patients with ruptured atherosclerotic plaques. Anti-E1 antibodies were not detected in serum from patients with stable plaques or from control individuals. Similarly, antibodies directed against E12 were detected in serum from patients with ruptured plaques compared to serum from subjects with stable plaque or normal controls. The anti-E1 and anti-E12 antibody response appeared to precede the increase in troponin levels in the subjects who presented with acute myocardial infarction. Thus, Cleutjens et al. speculated that the new antibody biomarkers may have applications for early diagnosis of acute coronary syndromes in the emergency room.

Limitations of this novel work must be considered, and critical questions arise. Do the antigen targets provide insight into the biology of unstable atherosclerotic lesions? Unfortunately, the anti-E1 and -E12 antibodies described do not appear to be autoantibodies. Autoantibodies by definition are directed against “self” antigens. In SLE patients with anti-Sm antibodies, autoantibodies are directed against protein components of the human spliceosome, including proteins B1, B2, D, and E. In contrast, the anti-E1 and -E12 antibodies identified by Cleutjens et al. appear to be directed against random peptides, not self-antigens. At present, anti-E1 and -E12 antibodies must be considered anti-(random) peptide antibodies, and not true autoantibodies.

Another important question regarding the study of Cleutjens et al. relates to the pathophysiology of antibody formation in patients with cardiovascular disease related to plaque instability. Clearly, antipeptide antibodies do not develop on the day a patient presents with an acute myocardial infarction. The authors speculated that antipeptide antibodies may form during the days or weeks before an acute episode, during periods of “plaque instability.” That is, there may be a substantial delay between plaque rupture and the onset of symptoms. Why do patients with “stable” atherosclerotic plaques not produce antipeptide antibodies? Do they not also have periods of plaque instability? Is there something fundamentally different about the pathophysiology of plaque formation in patients with stable plaques that does not induce antipeptide antibody formation, and might these antibodies be useful in identifying distinct phenotypes among patients with atherosclerosis? These questions remain the subject of future investigation.

What is the potential clinical application of these findings? The biomarkers proposed by Cleutjens et al. are diagnostic biomarkers rather than screening biomarkers, because they identify patients only upon acute presentation. The utility of diagnostic biomarkers is tied to their sensitivity (rate of positive results in individuals with disease) and specificity (rate of negative results in individuals without disease). The sensitivities for the anti-E1 and -E12 antibodies ranged from 88% to 93% in those with acute myocardial infarction. All of these patients had ST segment elevation, however, in which case biomarker elevation is not required to make the clinical diagnosis. The authors also tout that the antibody markers were increased in patients before troponin T (TnT) levels were increased, but they applied an inappropriately high TnT cutpoint, well above the recommended 99th percentile in healthy individuals. It would be helpful to see how the antibody markers performed in comparison to troponin in patients with non–ST-elevation myocardial infarction. Furthermore, sensitivity of the antibody markers was only 33% for detecting unstable angina, a condition also thought to be due to plaque rupture, but without myocardial necrosis (and hence without troponin elevation). Clinically, unstable angina and non–ST-elevation myocardial infarction are often treated the same, and the diagnosis of either often triggers the performance of coronary angiography. Thus, poor sensitivity for detecting some forms of acute coronary syndromes might reduce the utility of these biomarkers.

Cleutjens et al. reported that the antibody markers had 100% specificity for detection of ruptured lesions, but it is notable that nearly two-thirds of rheumatoid arthritis patients also had antibodies directed against one of the antigens. Thus, the antibody markers require assessment in additional control populations, including those with other autoimmune or chronic infectious diseases. Additionally, the persistence of these biomarkers for months after initial presentation reduces their utility for detecting recurrent events.

Studies of cardiovascular biomarkers have typically focused on biomarkers from pathways known to be associated with atherosclerosis and its complications. Although this approach may validate existing hypotheses regarding mechanisms of disease, it is unlikely to lead to the identification of novel biomarkers that...
are substantially better than those already available. Biomarkers from uncorrelated or orthogonal pathways involved in disease pathophysiology are most likely to improve diagnostic or screening performance. New proteomic technologies have great potential for identifying such biomarkers, as shown by the work of Cleutjens et al. Undoubtedly, as panning techniques for autoantibodies improve, new antibody assays are likely to move into the realm of the clinical chemist.

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