Emerging Paradigms, Platforms, and Unifying Themes in Biomarker Science*

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Since the object of pure scientific knowledge cannot be other than it is, the truth obtained by demonstrative knowledge will be necessary.

Aristotle, Analytica Posteriora (1)

The relationship between platelets, leukocytes, and the endothelium highlights a complex balance between normal biology and pathobiology, adaptation and maladaptation, health, and disease. Although it is increasingly apparent that inflammation is a unifying theme for trauma, environmental toxins, and a highly evolved immune system that quickly recognizes and responds to nonself inhabitants and intruders, it is equally apparent that inflammation is in many instances necessary, but not sufficient to explain common clinical phenotypes in cardiovascular disease.

Biomarkers: A Traditional Paradigm

Biomarkers are measurable cells, proteins, and/or metabolic byproducts that represent, either directly or indirectly, one or more processes in a defined biological system or disease state (2). Because biomarkers reflect health and disease according to genetic makeup, gene–gene interactions, gene–environmental interactions, and a dynamic interface between genotype–phenotype relationships (3), one could consider biomarkers in categories of antecedent biomarkers (identifying the inherent or existing risk of developing a disease), screening biomarkers (a screening tool for subclinical disease), diagnostic biomarkers (recognition of existing disease), staging biomarkers (categorizing the severity of disease), and prognostic biomarkers (predicting the natural history of disease, including response to treatment and likelihood of adverse effects related to treatment) (reviewed by Vasan [4]). A category that to date has eluded cardiovascular medicine is target biomarkers (altering the phenotype through specific treatment aimed at the biomarker itself or a functional byproduct).

In this issue of the Journal, Cavusoglu et al. (5) report an association between plasma F11 receptor/junctional adhesion molecule (F11R/JAM-A) levels and human atherosclerosis. In a cohort of 389 male patients undergoing coronary angiography, F11R was shown to correlate with the presence and severity of obstructive disease according to a validated risk score. By multivariate analysis that included high-sensitivity C-reactive protein, plasminogen activator inhibitor-1, matrix metalloproteinase-9, tissue inhibitor of metalloproteinase-1, insulin, and tumor necrosis factor alpha, F11R was the only measured biomarker that was independently associated with coronary artery disease score. The investigators conclude “strategies that block F11R may represent a novel approach to the treatment of human atherosclerosis.”

Biomarkers of Atherosclerotic Plaque and Prevalent Clinical Phenotypes

The relationship between inflammation and atherosclerosis is well established, with a wide variety of molecules being represented in varying stages of disease, including T-lymphocytes, monocyte-derived macrophages, and attendant–mediator proteins such as growth factors, cytokines, chemokines, proteolytic enzymes, and disintegrins (reviewed by Koenig and Khuseyinova [6]). More than 30 cohort and nested case-control studies have been reported for C-reactive protein alone as a prognostic biomarker for future cardiovascular events (reviewed by Armstrong et al. [7]). The experience to date clarifies the need for integrated pathways of investigation.

Applied Vascular Biology

Most cells, including those of the vascular endothelium, communicate with their adjacent neighboring cells via tight junctions. The junction or gap itself is spanned by connecting channels known as connexons that permit water-soluble molecules and inorganic ions to pass directly from the cytoplasm of one cell to the cytoplasm of another, coupling the cells both metabolically and electrically. Under normal circumstances, the functional pore size of a tight junction is 1.5 nm; thus, macromolecules such as proteins, polysaccharides, and nucleic acids would not be shared between cells.

Individual tight junctions are in a state of dynamic flux, opening and closing in response to local cellular conditions and extracellular signals. Based on structural and functional properties, 3 types of integral membrane proteins are recognized. The first type, containing 4 transmembrane domains that are responsible for establishing a structural basis for barrier and gate functions, includes tricellulin, claudin, and occludin (8). The second type, represented by Crumbs...
What is the Likelihood That F11R/JAM-A Inhibition Can Either Prevent or Change the Natural History of Atherothrombotic Vascular Disease?

Cavusoglu et al. (5), based on observations from a cohort of 389 male patients with varying degrees of obstructive coronary artery disease, conclude that “strategies designed to block F11R-mediated adhesion of platelets to endothelial cells may represent a novel approach to the prevention and treatment of human atherosclerosis.” Although identification of a circulating biomarker that is associated with an intermediate phenotype does not establish mechanistic causality, and in turn, an expected benefit derived from its attenuation or complete inhibition, the investigators’ hypothesis is biologically plausible.

F11R/JAM-A, a transmembrane protein expressed at tight junctions of endothelial cells and on the surface of platelets and leukocytes, is known to participate in leukocyte diapedesis (reviewed by Nourshargh et al. [10]). In addition, tumor necrosis factor alpha, a proinflammatory cytokine, provokes changes in endothelial cell morphology and permeability through reorganizing of tight junction proteins, including JAM-A and occludin (11). The role of JAM-A in leukocyte transmigration in vivo has been observed directly by intravital microscopy using JAM-A neutralizing monoclonal antibody (BV11) and in JAM-A deficient (KO) mice (12). Although leukocyte transmigration was reduced in both wild-type mice treated with BV11 and in JAM-KO mice after stimulation with interleukin-1 beta or ischemia-reperfusion injury, neither had an effect on responses elicited by LTβ4 or platelet activating factor. In vivo data generated with blocking antibodies or through inactivation of the JAM-A gene suggest that its contribution to leukocyte recruitment is highly dependent on local conditions (reviewed by Nourshargh et al. [10]). Soluble forms of JAM-A protein within atherosclerotic plaques derived from patients with aortic peripheral vascular disease (19). The findings were reproduced in atherosclerosis-prone apolipoprotein E⁻/⁻ mice (19). Finally, they developed an F11R/JAM-A enzyme-linked immunosorbent assay, and herein report, for the first time, the detection, quantification, and characterization of F11R/JAM-A protein within atherosclerotic plaques derived from patients with aortic peripheral vascular disease (19). The platforms for biomarkers in cardiovascular disease.

Platforms for Biomarkers in Cardiovascular Disease

Conceptually, biomarkers of interest should be based on the biology of health and disease, with an established link to the phenotype under investigation and tested in several relevant model systems. The investigative group responsible for the current work (5) followed a highly laudable and exemplary path. They identified, sequenced, and cloned the human gene for F11R/JAM-A (18), and subsequently demonstrated high levels of F11R/JAM-A mRNA and F11R/JAM-A protein within atherosclerotic plaques derived from patients with aortic peripheral vascular disease (19). The investigators have established a platform for future studies that will provide a basis for answering several important questions such as: What is the site(s) of origin for soluble F11R protein, and how does this influence functionality? Does the lower soluble F11R concentration among individuals with myocardial infarction represent a change in protein conformation and/or condition-modified molecules with altered biological activity? Last, is the relationship
between coronary artery occlusive disease and F11R similar or different among women compared with men?

U.S. Food and Drug Administration Position on Biomarkers

The U.S. Food and Drug Administration has a long-standing interest in biomarkers. Several recent examples in which advanced biomarkers might have been particularly useful for detecting early signals of vascular toxicity and/or drug-induced prothrombotic effects include cyclooxygenase-2 inhibitors (20), torcetrapib (21), and rosiglitazone (22).

National Institutes of Health Position on Biomarkers

The National Institutes of Health, in multiple forums (4), has made their position on biomarkers clear. They view genomics, proteomics, metabolomics, and combinatorial chemistry (as well as high-throughput technologies) as representing a means to relieve an existing bottleneck in drug discovery, improve the efficiency of clinical trials, speed the translation of basic science to the bedside, and address emerging public health issues.

Biomarkers: A Contemporary Paradigm

A contemporary view of biomarkers builds on a strong foundation provided by early observations and introduces 2 fundamental, translatable constructs: (1) platforms for scientific discovery and integration of large complex datasets through bioinformatics, and (2) technology-based and systems-based approaches for probing mechanisms of disease and establishing overarching themes for advanced study.

Accordingly, the scientific community must embark on a contemporary approach, using emerging molecular, cellular, protein-chemistry, metabolic, and tissue imaging platforms (23) (Fig. 1).

Testing a Hypothesis

The 5 phases of biomarker development outlined by Pepe et al. (24), with recent modifications by Vasan (4), warrants consideration and is relevant to the potential use of F11R/JAM-A as a marker of atherothrombotic coronary artery disease. The journey to phase 5 requires commitment, vision, and resources, particularly when a biomarker is ultimately selected as a target for intervention.

The cardiology community must acknowledge that it is 20 years behind our oncology colleagues who have, several times over, connected the operative dots of genotype–phenotype treatment, tying them together with a golden thread of molecular and protein biomarkers to distinguish the presence of disease, establish a prognosis, apply specific therapy, and predict treatment response. There have been advances within cardiology (25,26) and translatable road maps for success (27,28), but progress remains wanting.

Cavusoglu et al. (5) have introduced a new piece to the puzzle that defines atherothrombotic coronary artery disease. One must applaud their attention to scientific rigor and commitment to the tenet of translatability. Will F11R/JAM-A emerge as a biomarker worthy of favored status, and with it, an opportunity to be fully characterized for its merit, or will it be sentenced to a life of obscurity and isolation along with scores of other biomarkers? The answer to the question lies in the hands, minds, and imagination of those who have grown weary of hearing year after year “cardiovascular disease is the leading cause of death among men and women in the United States and developing world.”

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REFERENCES


Figure 1 Systems Biology Creates a Human Biosignature

An ability to understand human health and disease, cell-based and advanced biomarkers, and both safe and effective pharmacotherapies requires an integrated approach to investigation. DNA = deoxyribonucleic acid; mRNA = messenger ribonucleic acid.


