Biomarkers: A Challenging Conundrum in Cardiovascular Disease

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Abstract:

The use of biomarkers has proven utility in cardiovascular medicine and holds great promise for future advances, but their application requires considerable rigor in thinking and methodology. Numerous confounding factors can cloud the clinical and investigative uses of biomarkers. Yet, the thoughtful and critical use of biomarkers can doubtless aid discovery of new pathogenic pathways, identify novel therapeutic targets, and provide a bridge between the laboratory and the clinic. Biomarkers can provide diagnostic and prognostic tools to the practitioner. The careful application of biomarkers can also help design and guide clinical trials required to establish the efficacy of novel interventions to improve patient outcomes. Point of care testing, technological advances such as microfluidic and wearable devices, and the power of “’omics” approaches all promise to elevate the potential contributions of biomarkers to discovery science, translation, clinical trials, and the practice of cardiovascular medicine.
The term “biomarkers” has become common in the conversation regarding cardiovascular disease from both an investigational and clinical perspective. Yet considerable confusion surrounds the definition, uses, validation, and value of biomarkers. Fortunately, an NIH consensus statement published in 1998 provides a set of definitions in the biomarker arena that remain useful currently, albeit often not heeded.¹ (Table 1) A biological marker, or biomarker, is a characteristic that is objectively measured and evaluated as an indicator of a normal biological process, pathogenic process, or pharmacologic response to a therapeutic intervention. Some biomarkers can be qualified as “surrogate endpoints.” A surrogate endpoint is a biomarker that can substitute for a clinical endpoint for clinical or regulatory use. A clinical endpoint is a characteristic or variable that reflects how a patient feels, functions, or survives. Note that our formal definitions of biomarker do not sanction the oft-heard term “surrogate marker.” To accompany a series of articles relating to cardiovascular biomarkers, this prefatory statement puts forth some of the common challenges to the use of biomarkers in investigation and the clinic. While we draw our examples from the cardiovascular arena, the principles apply to many aspects of medicine. The NIH working group definition of biomarkers extends beyond in vitro diagnostics, usually measured in blood or other bodily fluids. Anthropomorphic measurements and commonly measured clinical variables such as blood pressure, oxygen saturation, or heart rate also fall under the
definition of biomarkers. Examples of imaging biomarkers include carotid intima media thickness, coronary calcium, ventricular dimensions, and the like. This review will focus primarily on blood biomarkers.

**Analytical Considerations**

The use of blood biomarkers requires attention to pre-analytical concerns. The mode of blood drawing requires considerable care. For example, markers carried in platelets will vary in blood samples depending on how they are drawn and handled. Performing venipuncture without activating platelets or other cells with granular content, including many leukocytes, necessitates special precautions. The choice of anti-coagulants also requires care. For example, the use of chelating agents such as ethylenediaminetetraacetic acid (EDTA) precludes measurement of metalloenzymes such as the matrix metalloproteinases in an accurate manner. The separation of cellular elements from blood often requires centrifugation, the details of which require consideration to avoid spurious results. Emerging biomarkers such as extracellular vesicles and exosomes, may furnish valuable diagnostic resources in the cardiovascular domain as currently explored in oncology. Sample preparation to harvest this potentially valuable source of relatively unexplored cardiovascular biomarkers requires ultracentrifugation.
The stability of blood analytes can vary considerably. The French physiologist Claude Bernard discovered the glucogenic function of the liver because he noted a difference in the level of glucose assayed at different times after the conclusion of an experiment. The stability of analytes also varies based on storage considerations. Antibodies may remain stable at 4°C whereas other biomarkers require storage below freezing or even at -70°C to maximize stability. To avoid degradation of analytes and improve consistency of results, protocols should minimize freeze/thaw cycles. In designing biobanks, preparing aliquots of frozen samples can forestall the need for repeated freeze/thaw cycles that may degrade analytes.

The timing of sampling blood biomarkers often requires particular consideration. For example, triglycerides will vary considerably depending on whether they are obtained in a fasted state, at various times after meal, and fluctuate with the fat content of the diet. Other analytes, such as fibrinogen, have diurnal variations. Numbers and activation state of circulating immune cells may also vary during the diurnal cycle. The half-life of an analyte may also affect its utility as a biomarker. Those with short half-lives may prove less reliable in population studies than those with longer dwell times in the circulation.
Measurement of biomarkers requires care in the choice of assay. The standardization, specificity, and scalability of a biomarker as well as the dynamic range of an analyte require consideration. Many proteins pivotal in biological control require processing to obtain biological activity. Whether an assay measures the precursor, the active form, or both needs specification. Examples of analytes with active and inactive forms include angiotensin, interleukin-1-beta, and many proteinases such as the matrix metalloproteinases and caspases. All assays require the requisite quality assurance and quality control.

Interpretation

The proper use of biomarkers requires consideration of the distinction between a causal factor that participates in the pathogenesis of disease and a biomarker that does not engage in a causal pathway. To have utility, a biomarker need not contribute directly to the disease mechanism. Yet, confusion regarding causality to a biomarker can confound the rigorous thinking. The classical schema of Fleming and Demets highlights some of the slips twixt the cup and lip in the use of biomarkers (Figure 1). Many initial studies overestimate the magnitude of the utility of biomarkers. Therefore, the initial results of biomarker studies that do not
employ an independent validation cohort or population merit considerable skepticism.

Use of Biomarkers

The Uses of Biomarkers in Research and Translation

Laboratory investigators often focus on a particular molecule whose interest has emerged from basic science studies. The bench based scientist understandably often wish to test whether the molecule of interest correlates with the presence or absence of a condition (discrimination), prognosis, or outcome. Cholesterol, an example of a biomarker that arose from such a “candidate approach,” emerged from animal investigations performed at the dawn of the 20th century. 8 Multiple studies in the second half of the 20th century validated the utility of blood cholesterol as a biomarker of risk, and subsequently as a causal risk factor in atherosclerosis. 9

The advent of “-omics” technologies has ushered in a new era in the discovery of biomarkers that represents an “unbiased” approach to biomarker discovery. Genomics, proteomics, metabolomics, and lipidomics have all opened new vistas in this regard. While demanding considerable rigor in interpretation and
validation, such exercises can point to new biological pathways and potential therapeutic targets. While the era of analyses of particular single nucleotide polymorphisms (SNPs) shed little light in this regard, contemporary approaches such as genome-wide association scans have yielded some novel pathophysiologic insights. Monogenic traits can also identify novel biomarkers or therapeutic targets. For example, proprotein convertase subtilisin/kexin type 9 (PCSK9), a current therapeutic target, arose from analyses of variations in genes causing autosomal dominant hypercholesterolemia. Mendelian randomization strategies have proven useful in determining whether a biomarker participates causally in the pathogenesis of a disease.

The use of biomarkers can aid clinical investigation in important ways. Biomarkers used as enrollment criteria can serve to enrich a population for risk and hence yield a population with elevated event rates reducing the number of subjects and/or the time of exposure to a putative therapeutic agent to determine its efficacy. This strategy can prove most valuable when the biomarker reflects the biological basis of the contemplated intervention. For example, end-stage renal disease, advanced heart failure, or high coronary calcium score all correlate with increased cardiovascular events. Yet, enriching event rates in a population using such biomarkers may prove futile if the intervention does not attack the cause of
the qualifying biomarker. Using an inappropriate dose commonly confounds clinical trials. The use of a well-chosen biomarker in preliminary or Phase 2 studies could inform the rational selection of an appropriate dose for a clinical endpoint trial.

**The Use of Biomarkers in Clinical Practice**

Diagnostic dilemmas often face the practitioner. Biomarkers can aid a physician to sort individuals into categories of “disease” or “no disease.” This use of biomarkers to discriminate requires validation and refinement. The use of highly sensitive troponin assays to discriminate individuals with acute coronary syndromes who require hospitalization versus those that do not require inpatient observation constitutes one example.

The use of biomarkers to determine risk of an event or prognosis as a continuous variable demands careful consideration. Various risk scores in the cardiovascular arena have generated considerable controversy. Some risk scores use categorical biomarkers with arbitrary assignment of weighting (e.g. the CHADS2 Score for Thrombotic Risk). Others use continuous variables and weighting of components of the score based computational algorithms (e.g. the
Reynolds Risk Score for Women). A number of recent reviews consider the statistical test used to determine the ability of various biomarkers or risk scores to discriminate, calibrate, and prove clinically useful in practice.

As used in clinical trials, biomarkers can help to target interventions in clinical practice. For example, drug treatment of hypertension or diabetes depends on achieving certain cutpoints of biomarkers such as systolic blood pressure or glucose or hemoglobin A1c. While various guideline-mandated cutpoints can vary considerably, and some have engendered controversy, the concept that biomarkers can guide therapy governs a great deal of contemporary clinical practice. Yet, large-scale clinical trials have validated such clinical cutpoints surprisingly seldom. In JUPITER, allocating statin therapy on the basis of the degree of inflammation as reflected by high sensitivity C-reactive protein (hsCRP) not only yielded a primary prevention population with enhanced risk of a cardiovascular event, but identified individuals who benefitted particularly from statin therapy.

Widespread clinical adoption of a biomarker should await the validation of the ability of the biomarker to inform therapy that improves patient outcomes. In particular, imaging biomarkers such as coronary calcium score, while indubitably enriching cardiovascular risk, lack clinical trial support as a guide to therapy.
When considering variables to include in the generation of an a priori (as opposed to an unbiased derivation) risk score, selecting biomarkers that report on orthogonal aspects of pathogenesis makes sense. For example, a biomarker of lipid risk such as LDL, of myocardial stress such as a natriuretic peptide, of myocardial injury such as troponin, of inflammation such as hsCRP, and glycemia such as hemoglobin A1c, each report on different biological pathways. Including biomarkers that lie in a common pathway (e.g. LDL cholesterol, apolipoprotein B, and non-HDL cholesterol) would not be expected to add as much information to a risk assessment instrument as biomarkers that reflect independent, orthogonal pathogenic pathways. (Figure 2)

Biomarkers can also inform regulatory decisions. For example, the U.S. Federal Food and Drug Administration (FDA) has approved drugs for marketing based on their ability to effect such biomarkers as LDL cholesterol, hemoglobin A1c, or systolic blood pressure. The approval of PCSK9 inhibitors provides a good example of a regulatory action based on biomarker changes before completion of large scale clinical outcome studies. Yet, counter examples have raised the regulatory bar for biomarker qualification as surrogate endpoints. Large-scale clinical trials have not always borne out that effects on biomarkers (e.g. hemoglobin A1c lowering or HDL raising) correlate with an improvement in
clinical outcome. Recent guidance from the US FDA mandates assessment of cardiovascular safety beyond affecting a biomarker, notably in the diabetes therapeutic area.\textsuperscript{24} Regulatory authorities have established criteria for the qualification of biomarkers and have variable levels of acceptance of biomarkers for registration of novel therapeutics.\textsuperscript{25,26}

**Future Perspectives Regarding Cardiovascular Biomarkers**

A number of trends may transform the use of biomarkers in cardiovascular research and practice. For example, enormous technological innovations in areas of miniaturization, system integration, and usability are rendering point of care testing more feasible and reliable.\textsuperscript{27,28,29} Point of care testing in the home or in institutions spanning primary to quaternary care settings promise to render clinical decision making and delivery of care much more efficient and efficacious. Point of care testing in the field could enhance global health by expanding access to biomarker analyses in rural areas and developing regions. The adoption of point of care technologies could also “democratize” clinical trials by permitting more widespread inclusion of participants, reaching beyond traditional hospital-based research facilities.
The “big data” approach to biomarkers may also revolutionize medicine and provide new avenues for expanding medical knowledge beyond the traditional carefully conducted cohort studies or clinical trials. For example, the growing number of commercially available wearable devices that report longitudinally on parameters such as motion or heart rate will furnish large data sets to enable correlation of derived biomarkers with clinical outcomes and reveal hidden relationships between cardiovascular outcomes and, for example, physical activity. Obvious confounding regarding access and assumptions regarding continuity of use of such wearable technologies pertain, yet the enormity of the data sets could counterbalance these concerns. Continuous monitoring of biomarkers in acute care and ambulatory settings enabled by microfluidics, wearable technologies, and associated analytical techniques may also transform research and practice in cardiovascular medicine.30

Biomarkers may also serve increasingly as “companion diagnostics.” As we move towards greater personalization in an era of “precision medicine” the use of biomarkers to indicate responsiveness to drugs and other interventions or to inform dosage decisions will likely assume increasing importance. The current use of genetic markers to choose targeted therapies in oncology exemplifies this trend.
Increased research may enable similar advances in companion diagnostics involving biomarkers in cardiovascular disease as well.

**Conclusions: Biomarkers Friend and Foe**

While biomarkers have already proven their utility in cardiovascular medicine and afford great promise for future advances, their use requires considerable rigor in thinking and methodology. New biomarkers need validation. Studies reporting the use of biomarkers require rigorous and critical interpretation. Numerous confounding factors can cloud the clinical use of biomarkers as well as their research applications. Yet, the thoughtful and critical use of biomarkers can certainly aid discovery of new pathogenic pathways and therapeutic targets. Biomarker technology should speed the translation of advances in laboratory science to the clinic. Biomarkers can provide diagnostic and prognostic tools to the practitioner. The careful application of biomarkers can help design and guide clinical trials required to establish the efficacy of novel interventions to improve patient outcomes. Thus, the promise of harnessing biomarkers in cardiovascular applications by far outweighs their perils, if judiciously applied.

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References:


Table 1
(Modified from Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. Clinical pharmacology and therapeutics. 2001;69:89-95.)

Figure 1
“Failure modes” of biomarkers. A, Depicts the situation that provides the greatest potential for a biomarker to serve as a surrogate endpoint. B, Depicts a case in which the surrogate does not lie in the causal pathway of the disease pathogenesis. C, Depicts a case in which of several causal pathways of disease, the intervention affects only the pathway mediated through the biomarker. D, Depicts a case in which the biomarker does not report on the pathway of the intervention’s effect or does not reflect its effect. E, Depicts a case in which the intervention has mechanisms of action not involved in the disease process. The dotted lines represent possible mechanisms of action. (Modified from Fleming TR, DeMets DL: Surrogate end points in clinical trials: Are we being misled? Ann Intern Med 125:605, 1996.)

Figure 2
Orthogonality of biomarkers that report on distinct pathogenic pathways.

BNP = brain natriuretic peptide; TG = triglyceride; LDL = low-density lipoprotein; HDL = high-density lipoprotein; hsCRP = high-sensitivity C-reactive protein.

**Table 1**

National Institutes of Health Biomarkers Definition Working Group (1998)

<table>
<thead>
<tr>
<th><strong>Biologic Marker (Biomarker)</strong></th>
<th>A characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention.</th>
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<tbody>
<tr>
<td><strong>Surrogate Endpoint</strong></td>
<td>A biomarker intended to substitute for a clinical endpoint. A surrogate endpoint is expected to predict clinical benefit (or harm or lack of benefit or harm) based on epidemiologic, therapeutic, pathophysiologic, or other scientific evidence.</td>
</tr>
<tr>
<td><strong>Clinical endpoint</strong></td>
<td>A characteristic or variable that reflects how a patient feels, functions, or survives.</td>
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Figure 1

A: Disease → Surrogate endpoint → True clinical outcome
B: Disease → Surrogate endpoint → True clinical outcome
C: Disease → Surrogate endpoint → True clinical outcome
D: Disease → Surrogate endpoint → True clinical outcome
E: Disease → Surrogate endpoint → True clinical outcome
Figure 2

Biomarkers

- Lipids: LDL, HDL, TG
- Cardiac chamber stress: BNP
- Renal function: Cystatin C
- Myocardial injury: Cardiac troponins
- Inflammation: hsCRP

Figure 2