Analytical validation of novel cardiac biomarkers used in clinical trials

Alan H.B. Wu, PhD, DABCC ***San Francisco, CA***

Background Blood-based biomarkers such as cardiac troponin and B-natriuretic peptides are widely used in clinical practice for the diagnosis, rule out, and risk stratification for patients with acute coronary syndromes and heart failure. Because neither these nor any other laboratory test meets all clinical needs, there are many novel biomarkers that are proposed and evaluated each year for possible implementation into clinical practice. Results of clinical trials are used as a means to validate their effectiveness and to obtain regulatory approval.

Methods and results Novel biomarkers are discovered through a targeted approach using knowledge of the pathophysiology disease process and an untargeted approach where proteins from tissues or blood of disease patients are compared against healthy subjects or those with benign conditions. Once a candidate biomarker has been identified, it is important to understand where the protein is located and how it is released into blood. In designing trials, the requirements for Food and Drug Administration clearance and approval should be taken into consideration. There are preanalytical studies that should be considered including the preservative used to collect samples and in vivo and in vitro analyte stability. If the analyte is not stable, a surrogate marker could be used such as stable "pro" molecules (precursor proteins) may be preferred. Assay imprecision and bias, biological variation and criteria for the establishment of a reference range are important analytical attributes. The need for harmonization and commutability and correlation of results to other markers and clinical outcomes are important postanalytical attributes of novel biomarkers.

Conclusions Inadequate adherence to these variables when conducting clinical trials reduces the quality and value of the information contained in literature reports of novel serum/plasma-based biomarkers. (Am Heart J 2015;169:674-83.)

Biomarkers are widely used in routine clinical practice for diagnosis, monitoring, risk stratification, and therapeutic selection for cardiovascular diseases. The "ideal characteristics" of serum-based biomarkers have been reviewed.[1] For acute coronary syndrome, there has been a progression of biomarkers starting with aspartate aminotransferase, creatine kinase and its MB isoenzyme, and now cardiac troponin. For heart failure (HF), the natriuretic peptides (B-type natriuretic peptide [BNP] and N-terminal pro–B-type natriuretic peptide [NT-proBNP]) are widely used. These biomarkers have been successful because they have fulfilled most of the characteristics for an ideal marker. Both troponin and BNP indicate the presence of a pathophysiologic condition, that is, myocardial ischemia and injury, and hemodynamic stress, respectively, and not presence of a specific disease. Therefore, the optimum use of these tests is in conjunction with other data, especially clinical presentation. When present, it raises the pretest probably of myocardial infarction (MI) and HF.

There are several unmet clinical needs with regard to the utilization of existing biomarkers. For acute coronary syndrome, a marker that can diagnose MI early, for example, during the initial presentation of patients with chest pain to the emergency department, would better facilitate triaging. For HF, biomarkers that can be used for long-term disease monitoring and selection of optimum therapy have the promise of reducing the rate of readmission to the hospital for HF exacerbation. For these and other potential clinical applications, there is considerable research conducted in the discovery and clinical validation of novel biomarkers for cardiovascular diseases. This review is intended for researchers, clinicians, laboratorians, regulatory scientists, journal reviewers and editors, and manufacturers of diagnostic assays, who examine literature reports, plan validation studies, consider commercialization of laboratory tests, or implement new clinical testing services. The author is solely responsible for the drafting and editing of the manuscript and its final contents.

Biomarker discovery platforms

There are 2 principal mechanisms to discover new serum-based biomarkers.[1] The "pathophysiologic"

From the and Department of Laboratory Medicine, University of California, San Francisco, CA.
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Reprint requests: Alan H.B. Wu, PhD, DABCC, San Francisco General Hospital, 1001 Potrero Ave, San Francisco, CA 94110.
E-mail: wualan@labmed2.ucsf.edu
0002-8703

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approach involves knowledge of the relevant human physiology and disease processes, an active area of clinical research. Cardiac troponin is an important marker for myocardial ischemia because these proteins participate in muscle contraction and are found in high concentrations within the myocyte. B-type natriuretic peptide is a hormone that participates in the regulation of water and electrolytes, which are essential for the maintenance of the proper blood pressure. Understanding the pathophysiology is important for the development of new pharmacologic agents. It also gives an opportunity for the discovery of novel biomarkers that are produced, released, or cleared during the disease process.

Most biomarkers that are in use or being evaluated as markers for cardiovascular diseases were discovered by the pathophysiologic approach. The cardiac troponin complex is a collection of 3 proteins that participates in muscle contraction. B-type natriuretic peptide is a hormone that counters the actions of renin and aldosterone. The discovery is how these markers are used in the diagnosis or prognosis of cardiovascular disease, not the existence of the protein or hormone itself in blood.

The "proteomic" and "metabolomic" approach involves an untargeted search for biomarkers. This science involves the use of techniques such as 2-dimensional electrophoresis and mass spectrometry that enable the simultaneous analysis of hundreds to thousands of proteins and metabolites. These techniques are used to compare the protein or metabolite signature of disease patients compared with healthy individuals. Proteins found in 1 set of samples but not the other may reveal itself as being a potentially useful biomarker. *Metabolites* are defined as low-molecular-weight products of enzymatic reactions. Novel metabolites may be present in blood of MI patients, due to the release of proteolytic enzymes that degrade circulating proteins. Sometimes, the identity of the substance is not known at the time of their discovery. Subsequent analysis is conducted to determine the identity of the protein and its physiologic or pathophysiologic role.

There have only been a few cardiac biomarkers that have been discovered by the proteomic approach. Soluble ST-2 was released into surrounding media from cell cultures of rat myocytes that undergo mechanical strain.[2] The role of ST-2 was delineated after the discovery of interleukin 33, the natural ligand of ST-2.[3] It is likely that more biomarkers will be discovered by untargeted approaches as the mass spectrometry technology for protein identification improves and the analytical sensitivity increases. Although there is some interest in measuring N-terminal and C-terminal peptides degraded from intact troponin, there is no biomarker discovered by metabolomics currently in routine clinical use.

Classification of novel biomarkers

Once biomarkers have been discovered, it is useful to classify the biomarker as an indicator of clinical events (eg, acute myocardial infarction) or a disease (eg, HF). Cardiac troponin is an event marker, whereas C-reactive protein is a disease marker. The natriuretic peptides can be considered as both an event marker (eg, decompensated HF) and a disease marker (eg, chronic HF).

Once the physiologic role of a candidate biomarker is known, it is also useful to classify biomarkers according to pathophysiologic groups, such as myocardial ischemia, necrosis, inflammation, neurohormonal activation, angiogenesis, plaque instability, atherosclerosis, and thrombosis.[4] Markers in each of these categories could provide complementary information using multimarker approaches. Two markers of the same category (eg, BNP and NT-proBNP) do not provide differential information. The selection of members of a multimarker panel must be demonstrated through clinical trials.

Relevance of clinical trials to Food and Drug Administration submission

Extensive validation studies are required before biomarkers can be put into clinical practice. The Food and Drug Administration (FDA) is responsible for the regulatory approval of clinical laboratory tests. Reagents, instruments, and systems are intended for use in diagnosis of disease to cure, mitigate, treat, or prevent disease, or its sequelae are considered medical devices. Their clearance ensures that the devices are safe and the diagnostic claims made by the manufacturer are verified. Table I lists the different FDA classification of biomarkers and approval processes they undergo.

The FDA examines data from analytical and clinical validation studies conducted on tests submitted for approval. Clinical trials are directed to address specific intended claims to be made by manufacturers of the test. The FDA requires a significant fraction of testing to be conducted prospectively. Retrospective biomarker data obtained from pharma-based clinical trials designed to determine efficacy of a therapeutic agent are usually not acceptable as part of an FDA submission for an in vitro diagnostic (IVD) test. The exception is markers designed to be a part of "companion diagnostics." These are tests that are directly linked to specific therapeutics, that is, a requirement for use is based on the result of a clinical laboratory test. For example, the drug trastuzumab is on women with breast cancer who overexpress the her-2/neu receptor.[5] Because of the medical consequences of a false-positive or false-negative result, this and other tests such as those for infectious diseases, oncology, and cardiovascular disease require either a premarket approval submission or a 510(k) with a clinical study to include an adjudication of a patient's discharge diagnosis or outcome.

Clinical trial study design

There are 2 objectives for clinical trials of in vitro diagnostic devices: FDA clearance of a biomarker for which