Perspective

A staggering number of people are exposed to excess cardiovascular risk over their lifetimes, ranging from a single risk factor such as high blood pressure to a typical cluster of risks. Such reality has urgent and growing implications for personal and public health. All too commonly, coexistent risk factors lead to arterial damage, precipitate ischemic injury, and ultimately yield reduced ventricular function and heart failure.

To gain better insights into heart failure and produce new value for communities and clinics, it is necessary to study patients from various disease groups in a stratified manner: those at risk, with early occult disease, with early clinical disease, with progressive disease, or at the precipice of heart failure. It will be necessary to tabulate drug therapies and other interventions for heart failure in a more quantifiable and rigorous way.

Heart failure, the leading cause of human morbidity and mortality worldwide, can result from various structural or functional cardiac changes imposed by ischemia, infection, immunity, metabolic derangements, genetic and epigenetic factors, and other incursions. Although myocardial diseases may be due to different individual or combinations of initiators, the pathway can be marked from injury to immune responses, inflammation, reparation, and variable recovery. Many of the diseases noted above operate by the same fundamental mechanisms to cause failure. Certain biologic processes are the nuggets of uniqueness for certain cardiomyopathic risks and disease, and a contemporary focus is to identify and sort the unifying from the unique markers at different stages of disease. As such, there is an emergent demand and much anticipated potential for novel biomarkers of risk and disease that could supplement and fortify classical phenotypes of wellness and illness. Which biomarkers can be used to assess the likelihood of patients developing diseases that are known to lead to organ failure? Do any biomarkers exist that can serve as risk factors or indicators of early disease or progression toward organ failure—regardless of the underlying disease—or as a specific consequence of a particular disease? Which biomarkers are robust; how are they derived, discovered, validated, and qualified; how relevant are they to current therapies; and in which way are they connected to target identification and development? These are central questions for patients on the verge of heart failure. Integrative development with strong validation and qualification steps for preventive, diagnostic, prognostic, and therapeutic biomarkers will help us answer many questions for heart failure patients that will yield improved social and economic well-being. What does this process of biomarker development look like?

Biomarker Discovery Strategy

Biomarkers are indicators used to objectively measure and evaluate normal biologic processes, pathologic processes, or pharmacologic responses to therapeutic interventions. In medical practice, biomarkers may facilitate prediction, diagnosis, and prognosis of disease and allow monitoring of clinical response to therapy. Currently available biomarkers, such as blood tests for cardiac troponins in the diagnosis of myocardial infarction, have been identified during targeted studies of physiologic processes. The aim of biomarker research is to more actively identify previously unrecognized substances that are specific and sensitive to disease state and stage from protein, mRNA, and metabolite candidates in biologic tissues, cells, or fluids. Advancements in genomics, proteomics, and metabolomics have revolutionized the potential for novel biomarker discovery with new tools underpinning high-performance strategies. Although closely related to molecular signatures from studies of gene–environment interactions, the nature of the gene sequence variation, common nucleotide variants, and modifications of DNA such as methylation are not typically defined as biomarkers.

The parallel use of multiple platforms for initial identification and quantitation of potential molecular bio-
markers offers more possibilities for discovery. By using well-standardized and technologically validated platforms to assess mRNA expression, thousands of genes can be studied and compared between normal subjects and those with specific medical risks or conditions. The emergence of micro-RNAs as regulators of gene expression has deepened the level of discovery possible, and recent results have shown that these tiny RNA strands play big roles in some disease phenotypes. Proteomic approaches can provide additional regulatory and functional information, including that arising from posttranslational modifications. Analysis of peptidic and nonpeptidic metabolites provides molecular phenotypes of relevance to signaling, ionic regulation, drug metabolism, and other processes. Many metabolomic signatures are highly specific reflections of the state of a biologic system. As such, metabolomics serves as a great complement to other platforms, allowing the biomarker discovery process to reach a more holistic level of inquiry.

Three phases are usually defined for the biomarker development process. First is the discovery phase, when multiple platforms are used to find candidate biomarkers in a patient population from a small geographic area, usually a single institution. This phase involves careful selection of patients with clear clinical phenotypes and multiple time-point samples for parallel “omics” analysis. The data within a single platform can be analyzed using statistical methods to identify differentially expressed genes, proteins, and/or metabolites in healthy subjects versus patient cohorts or from baseline status for patients through the course of illness. These analyses include those for single time points and those derived serially over time. Data-mining techniques also can be used to find candidate biomarkers. In addition, bioinformatical tools are used in complement to one another to deduce clusters, groupings, associations, and biologic functions or pathways of potential biomarkers. The latter approaches help to provide a better understanding of the markers’ roles in the actual biologic system in health and disease. After the candidate biomarkers within each platform are identified, combinatorial analysis methods can be applied to assess different data types for their additive or geometric influence on the separation of one patient group from another or one patient’s clinical state from another over time. Importantly, the clinical phenotypes are used in this combinatorial approach to avoid losing the discriminative value of those phenotypes alone, be they physiologic or anatomic. The result of the tiered analysis is a number of plausible predictive, diagnostic, or prognostic markers of potential utility in a biomarker panel that may embrace clinical, genomic, proteomic, and/or metabolomic markers numbering from a few to more than a hundred.

The second phase of the biomarker development is internal validation, whereby the same type of platforms and statistical/informatical analyses are carried out on a second patient cohort from the same institution. The purpose of this phase is to evaluate the sensitivity, specificity, and predictive values of the candidate biomarkers. Consensus analysis is then performed between the discovery and internal validation phase for a modified biomarker panel. Subsequently, an external validation (qualification) phase is carried out to evaluate the biomarker panel in patient cohorts from different institutions.

**Biomarker Validation Strategy**

Only the strongest candidate biomarkers identified in the discovery phase may be selected for validation. The decision to carry candidate biomarkers forward should not be based solely on statistical significance but also on the potential to contribute cost-effectively to disease management and prevention.

Biomarker validation can be generally defined as a multifaceted process that includes methodologic validation to determine assay performance characteristics and clinical validation (qualification) to demonstrate the evidentiary link of the biomarker to clinical processes or end points. The fundamental parameters on which method validation is based include accuracy, precision, selectivity, sensitivity, reproduicibility, and stability. Ideally, assay and analytic specificity and sensitivity should be established and validated early in development. This ensures that the clinical phases of validation or qualification are performed using proven analytically robust methods. However, biomarker validation is often a continually evolving process that integrates method and clinical validation. Platforms of utility in the discovery phase of biomarker development will usually be supplanted by simpler, more universally pertinent laboratory methods for the phase of advanced validation.

Settling on specific and detailed universal validation guidelines has been difficult because regulatory guidance is not uniform and biomarker research is quite diverse. Whether a biomarker candidate is a gene, protein, metabolite, or a panel of various types of biomarkers, the consensus is that validation efforts must ensure that the assayed biomarker is reliable for its intended use. This principle is now commonly referred to as the “fit-for-purpose” validation.

For fit-for-purpose validation, the objectives, processes, and types of validation will most likely differ depending on the intended purpose of a biomarker. The risk and consequence involved in using a biomarker for one purpose may necessitate increased validation stringency when the same biomarker is used for a different purpose. For example, a biomarker intended as a population screen to identify individuals with high risk of cardiovascular disease must be very sensitive and specific. The biomarkers currently used in clinics for such purposes still need improvement in these regards.

In summary, the phases of validation include internal validation, external validation, clinical trials, and continued surveillance. All phases may rely on various