Proteomic Biomarkers of Heart Failure

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KEYWORDS

• Biomarkers • Heart failure • Prognosis • Diagnosis • Proteomics

KEY POINTS

- Heart failure is associated with significant morbidity and mortality.
- Biomarkers are commonly used for diagnostic and prognostic purposes.
- Protein-based biomarkers have been identified to aid clinicians in the early diagnosis of heart failure and provide added information for prognosis.
- Proteomics is an ever-expanding field that uses techniques to measure a wide range of proteins and peptides in the search to identify potential protein biomarkers.

INTRODUCTION

It is estimated that in excess of 20,000 protein-coding genes are responsible for the presence of more than 1 million proteins found in biological matrices.[1] The measurement of these proteins, commonly in plasma, serum, urine, saliva, and tissue samples,[2] has provided critical advancements in medical science through the development of diagnostic and prognostic assays for patients presenting with, or at risk of, a multitude of diseases.[3] The use of protein measurements has been particularly beneficial for the assessment of cardiovascular disease, with the notable inclusion of natriuretic peptides and troponin isoforms in clinical decision making for heart failure (HF)[4] and acute coronary syndromes (ACS),[5] respectively. Clinical measurements of endogenous biological substances, such as proteins, lipids, and metabolites, are commonly referred to as biomarkers and provide pathophysiologic information through an associative or direct mechanistic interaction with the diseased system, organ, or tissue.[6] The relationships of protein biomarkers with disease allow physicians to assess the presence, severity, and/or prognosis of a condition with improved precision and accuracy.[7]

The progression in medical diagnosis and treatment of HF has been heavily influenced by the inclusion of protein biomarker analyses, with measurement of natriuretic peptides commonly used in hospitals worldwide.[8] HF is a major worldwide epidemic associated with high morbidity, mortality, and health care costs affecting more than 23 million people, especially those aged 65 years or older[9]; therefore, any improvements in diagnosis, prognosis, and therapeutic monitoring using protein measurements provide direct improvements in patient care and outcome, as well as economic burden. Difficulties in HF diagnoses exist because of the multifactorial pathophysiology (eg, cardiac stress and injury, neurohormonal activation, and endothelial congestion), and because the signs and symptoms may not arise during early stages of the disease.[10,11] Current guidelines suggest that patients presenting with suspected HF should

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be referred for measurement of circulating natriuretic peptides to aid in diagnosis of the condition.[12]

The development and pathophysiology of HF is associated with changes in the expressions of an array of metabolic, signaling, and structural proteins.[13] Although there are several protein-based assays currently used in clinical laboratories, extensive research is being performed to isolate and identify novel protein biomarkers associated with HF in a bid to improve sensitivity and/or specificity of biomarker information. Leading these discovery-led investigations are mass spectrometry (MS)–based assays, which involve a nontargeted approach to protein measurement and come under the remit of proteomics. These assays measure all detectable proteins that are expressed by a cell, tissue, or organism, known as the proteome, and reflect levels present at the time of sample collection.[14,15]

PROTEOMIC BIOMARKER DISCOVERY

For discovery-led proteomics investigations, the initial phase involves methods using either a widespan-targeted or nontargeted approach in order to measure a large number of proteins and/or peptides from various biological sample types. This method generates a list of numerous proteins that are identified as associated with the condition being investigated and, therefore, are selected as candidate proteins for subsequent verification experiments. Although many candidate protein biomarkers may be identified through these experimental workflows, very few survive the rigorous validation processes leading to the development of high-throughput assays for measurement.[16] MS is the most widely used instrumentation for nontargeted discovery and identification of potential protein biomarkers. It allows quantitative and qualitative analysis, and peptide sequencing and identification, with great accuracy and sensitivity.[17] Proteomic workflows vary greatly across investigations, including sample preparation, chromatographic gradients, and inclusion of complementary analytical techniques such as ion mobility spectrometry. Furthermore, differences across studies in data processing and statistical testing can lead to misidentification or masking of candidate biomarkers. These widely varied approaches provide limitations in that biomarker identification may not be reproducible across multiple methods, complicating the validation process for novel protein biomarkers. Typically, MS method workflows include fractionation to crudely separate proteins in the sample, removal of highly abundant proteins such as albumin in plasma samples, further separation of each fraction using liquid chromatography, and MS using electrospray ionization (ESI) in positive ion mode coupled to accurate mass analyzers such as time of flight (ToF) and orbitrap.[18] Alternatively, gel-based approaches are initially used to separate proteins by their isoelectric points and then by mass using polyacrylamide gel (sodium dodecyl sulfate polyacrylamide gel electrophoresis [SDS-PAGE]), followed by staining, excising, digesting using trypsin, and analysis by MS.[19] Following identification of candidate biomarkers, mass spectral data are cross-referenced with large-scale databases to confirm protein identification. Errors in protein quantitation in global discovery techniques can be associated throughout the analytical work flow from sample preparation to analysis. To assist in reducing these errors, isotopic labeling of internal protein standards can allow the relative quantitation of multiple proteins. Examples of these include metabolic labeling (^{15}N) and isotope-coded affinity tags; however, they lack accuracy and precision and more reliable approaches for sample-wide quantitation are required.[20]

Traditional nontargeted MS–based methods are important in candidate biomarker identification; however, complex sample preparation and analysis steps create a time-consuming process that limits the throughput required for larger-scale validation studies. Once a list of candidate biomarkers is produced, a shift toward targeted MS approaches allows improved specificity, reproducibility, and quantitation of candidates, and also drastically reduces the analytical run time. A commonly used approach for targeted MS is to develop assays using selective reaction monitoring (SRM) or multiple reaction monitoring (MRM), in which a single ion (SRM) or up to 5 fragment ions (MRM) are monitored in association with a specific product ion, typically using a triple quadrupole MS system, which is able to provide enhanced discriminating power, leading to increased sensitivity, absolute quantitation,[21,22] and improved cross-compatibility between instrumentation.[23] Aside from ESI-MS, matrix-assisted laser desorption ionization (MALDI) ToF–based MS is used for targeted MS, in which proteins of interest can be isolated using immunoprecipitation or liquid chromatography before spotting onto a target plate for analysis. Several targeted protein analyses using MALDI have been reported,[24,25] including an application in clinical studies.[26,27] Before commercialization, targeted protein experiments must replicate the results observed from the nontargeted investigations, as well as expanding to larger sample cohorts including diseased and nondiseased populations to validate as a