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STATE-OF-THE-ART REVIEW

Finding a Needle in a Haystack

Proteomics in Heart Failure

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HIGHLIGHTS

- Proteomics has aided HF biomarker discovery, which allows for greater disease insights.
- Experiment design can be tailored to HF research to discover novel biomarkers.
- Primary methods include MS, protein microarray, aptamer, and PEA-based technologies.
- Proteomics can detect unique low abundance proteins and detect protein modifications.

SUMMARY

Circulating protein biomarkers provide information regarding pathways in heart failure (HF) and can add important value to clinicians. Advancements in proteomics allow researchers to measure a multitude of proteins simultaneously with excellent sensitivity and selectivity to detect low abundance proteins. This helps identify previously unrecognized pathways in HF and discover biomarkers and potential targets for HF therapies. Although several proteomic methods exist, including mass spectrometry, protein microarray, aptamer, and proximity extension assay—based techniques, each have their unique advantages. This paper provides an overview of the various proteomic methods, with examples of how each has contributed to understanding the pathways in HF. (J Am Coll Cardiol Basic Trans Science 2020;5:1043-53) © 2020 The Authors. Published by Elsevier on behalf of the American College of Cardiology Foundation. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

easurement of circulating biomarkers has revealed important insights in the complex pathophysiology of heart failure (HF), identifying unsuspected pathways in diagnosis and providing possible therapeutic targets. Furthermore, in the case of natriuretic peptides, biomarker testing provides clinical value, informing diagnosis, prognosis, and therapy decision-making (1-4). Despite its enormous value in HF, natriuretic peptide testing fails to reflect all pathophysiological aspects, which beyond cardiac myocyte stretching, also results in renal injury, fibrosis, and inflammation (5-7). For these reasons, an approach that reveals a broader spectrum of proteins in HF is necessary.

The pathophysiology of HF may be understood through measurement of its components. From genes to metabolic byproducts, measurement of discrete components of biological pathways is known as "omics" (Central Illustration) (8). The assessment of proteins expressed in a specific state and how these proteins might change over time and with alterations in disease circumstance are notable. Such "proteomics" may reveal previously unrecognized pathophysiological pathways in

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ABBREVIATIONS AND ACRONYMS

2-DE = 2-dimensional gel electrophoresis

HF = heart failure

ICM = ischemic cardiomyopathy

MS = mass spectrometry

PEA = proximity extension assay

HF and solidify understanding of the inter-relatedness of known processes (9). Proteomics allows for testing of thousands of proteins simultaneously, using as little as 1 μ l of a sample (10), which permits rapid and high-throughput discovery of potential biomarkers of HF or identifies druggable targets, thus rapidly translating findings from bench to bedside.

WHAT ARE PROTEOMICS?

Traditionally, to conduct biomarker experimentation, specifically targeted biomarkers would be selected based on the hypotheses of disease pathophysiology involvement. This deductive method was slow because it required initial hypothesis generation and measurement of 1 specific protein, which would have to be repeated for each protein of interest (11). In addition, this methodology ignored the potential for elucidation of unknown pathways and unrecognized inter-relatedness between proteins and pathways. Rather than analyzing on a protein-by-protein basis, the goal of proteomics is to analyze a spectrum of proteins expressed in a system. The introduction of proteomics has allowed for multiple proteins to be separated, identified, and quantified simultaneously on a larger scale. In addition, due to its inductive nature, no hypothesis is generally required because the results drive discovery. Pre-selecting proteins of interest may result in a type I statistical error (12-14).

Proteomics was first explored following the creation of 2-dimensional gel electrophoresis (2-DE) in 1975 by O'Farrell, Klose, and Scheele, and has evolved since then (15). Today, the 4 most common proteomic technologies are mass spectrometry (MS), protein microarray, aptamer, and proximity extension assay (PEA)-based technologies. Advantages and disadvantages for each proteomic strategy are summarized in **Table 1**.





Omics give insight into the state of a system, as affected by a stimulus. In the case of proteomics in heart failure (HF), researchers hope to observe the effect of HF on the types and quantities of proteins produced from the RNA transcripts. Reprinted with permission from Barallobre-Barreiro et al. (8).

TABLE 1 Advantages and Disadvantages of Various Proteomic Methods			
Method	Advantages	Disadvantages	
Mass spectrometry	Produces thousands of results that can be compared with databases available online. Allows for discovery of new protein previously undiscovered biomarkers. Allows for analysis of post-translational modifications,	Methods may result in damage to analyte proteins of interest (acidity conditions, fragmentation by ionization source, etc.) Sensitivity affected by presence of more abundant proteins (e.g., albumin)	
Protein microarray	Allows for quantification of proteins. Allows for studies of interactions with proteins.	Limited to previously known proteins or that can be isolated. Affinity of some proteins low. Conformational changes of protein binding may affect binding strength.	
Aptamer	Sensitive and able to detect low protein concentrations.	Limited to aptamers that have previously been discovered.	
Proximity extension assay	Low use of sample. Sensitive and able to detect low protein concentrations.	Limited to currently developed panels	

TYPES OF PROTEOMIC EXPERIMENTS

MS-BASED EXPERIMENTS. MS proteomic methods provide incredible insight by identifying many proteins and detecting post-translational modifications (16-18). In addition, MS can be readily fine-tuned by changing or combining different methods of separation and analysis (15,19). MS works on the principle that whole molecules can be transitioned to the gas phase, fragmented, and then subjected to an electric or magnetic field to separate fragments based on their mass-to-charge ratio (**Figure 1A**) (10,15).

For protein separation, there are 2 major techniques: liquid chromatography and 2-DE. Liquid chromatography separates proteins by their affinity to either the mobile or stationary phase (usually reversed phase, in which the mobile phase is a polar solvent and the stationary is nonpolar), which affects their ability to pass through a column (20). The mobile and stationary phase polarity can be selected and optimized to increase the resolution of proteins (20). In 2-DE, proteins are separated twice. On a gel with a pH gradient, proteins are first separated by the isoelectric point, which is the point where the acidity of the gel neutralizes any positive or negative charges on the protein and results in a no net charge (16,18). Proteins are then separated on a perpendicular axis by mass using an electrical gradient, which produces spots of individual proteins in 2 dimensions. Like liquid chromatography, 2-DE resolution can be tuned by changing the pH gradient and electrophoresis conditions (16). Although 2-DE has lost popularity due to emerging technologies and its limited ability to separate hydrophobic proteins, it remains a viable technique (21). In either separation technique, it is important to consider the effects of experimental conditions, such as the pH of the gel or the composition of the mobile phase in liquid chromatography. The experimental environment may inadvertently damage proteins or be unable to separate all proteins.

Several attempts may be necessary to optimize separation and ensure proteins are isolated by performing multiple separations under different conditions.

Once separated, proteins are ionized for MS analysis, usually by electrospray ionization or matrixassisted laser desorption ionization. In electrospray ionization, the analyte is dissolved and sprayed through a narrow, electrically charged capillary, which creates small droplets. The charged droplets travel and the solvent evaporates, leaving only charged analyte ions (22). The advantage of electrospray ionization is that it results in little fragmentation; however, the analyte may have high charges that could affect analysis of the mass-to-charge ratio (23). In matrix-assisted desorption ionization, the analyte is suspended in a gel matrix and a laser is used to heat the gel-analyte mixture to evaporate and charge the analyte. Matrix-assisted desorption ionization is useful for large proteins; however, the laser may fragment the analyte (23).

The final consideration is ion analysis, usually through either quadrupole or time-of-flight. In quadrupole analyzers, there are 4 rods with varying electric fields between rods that oscillate. Ions with the correct mass-to-charge ratio will pass through the analyzer and be detected, and other ions will collide with the rods (15,19,20,24). Time of flight uses an electric field to accelerate all ions to the same kinetic energy, which will have different velocities. Heavier ions will take longer to arrive to the detector. The time it takes for a given ion to travel the length of the time of flight analyzer (a set distance) allows for calculation of the analyte's mass, and therefore, its mass-to-charge ratio (15,23).

Once fragment masses are characterized, the results can be searched in online databases to identify the protein, allowing for untargeted elucidation of thousands of proteins (19). Although all these methods have their advantages and disadvantages (Table 2), it is possible to combine approaches to



Although there are several methods to profile the proteome, the most common means are (**A**) mass spectrometry, (**B**) protein microarray chips, (**C**) Aptamers, and (**D**) proximity extension assays. Mass spectrometry fragments proteins and measures the mass-to-charge ratios to identify and quantify proteins. The other methods involve highly specific binding of detectors (either antibodies [**B and D**] or oligonucleotides [**C**]), to proteins which are measured through fluorescent markers (**B and C**), or quantitative polymerase chain reaction.

improve separation and analysis. For example, in many cases, liquid chromatography-MS/MS is used; liquid chromatography first separates the proteins and then MS is performed twice, once to separate the parent ions, and then again to further analyze the fragments (23).

PROTEIN MICROARRAY CHIP-BASED EXPERIMENT. Protein microarray chips identify and quantify proteins in a sample proteome. The technology is based on DNA microarray analysis but has now been extended to capture and characterize proteins (25). There are 2. primary methods of microarray proteomics: analytical and functional. In analytical microarrays, various capture antibodies are attached to a chip platform. Detection of protein binding takes place either through analyte labeling (the proteins of interest have a fluorescent or radioactive marker) or through sandwich detection (the protein binds to the capture antibody then to a reporter antibody containing a marker) (Figure 1B). Of the 2 methods, sandwich detection has more specificity because it requires a second binding process (26). However, in certain cases, the presence of a marker may alter protein structure. To avoid this, more sophisticated analytical methods can be used, such as atomic force microscopy or optical measurements. In atomic force microscopy, a sensitive probe can detect structural changes as it moves across the surface, almost like the needle on a turntable, which are translated into an electrical signal to provide an

image of the protein surface (27). Optical measurements, using methods such as optical ellipsometry or reflectometric interference spectroscopy, measure the optical dielectric response, which is the physical and chemical change in the surface upon the protein of interest binding (25,26). Another format is functional microarray proteomics. In this method, previously selected proteins are attached to the plate and exposed to a variety of conditions, such as certain drugs, nucleic acids, and lipids, to test the biochemical functions of a proteins (25,26).

An important consideration in microarrays is plate design. Plates are usually glass, but the scaffold in which the antibodies or proteins are attached can be a variety of materials. In the past, plates were coated in materials like polyvinylidene difluoride or nitrocellulose. Polyvinylidene difluoride was a popular material; however, its soft surface allowed proteins to scaffold laterally into other plate positions. Nitrocellulose also experienced a similar problem and produced a low signal-to-noise ratio. Three-dimensional scaffolds (usually polyacrylamide or agarose gel) are currently the preferred choice because they allow the protein to maintain its structure and avoid lateral scaffolding, which allows for more positions per plate (25). Despite these advantages, 3-dimensional scaffolds do not bind proteins as tightly and are prone to being washed away (26).

TABLE 2 Advantages and Disadvantages of Various Mass Spectrometry Separation, Ionization, and Analysis Methods			
Method	Advantage	Disadvantage	
Separation			
Liquid chromatography	Great degree of tunability by using different conditions to separate by polarity.	Polar conditions may damage proteins. Limited resolution if proteins have similar polarity.	
2-dimensional gel electrophoresis	Separates on basis of pH and mass.	Acidic/basic conditions may damage proteins. Poor resolution of hydrophobic proteins.	
Ionization			
Electrospray	Little fragmentation	May result in highly charged analyte species.	
Matrix-assisted laser desorption ionization	Useful for large analyte molecules.	Analyte may disintegrate and fragment in laser desorption process.	
Analysis			
Quadrupole	Fast analysis Low cost	Limited mass to charge range and resolution	
Time of flight	High mass-to-charge ratio Fast analysis	Low sensitivity and resolution	

Protein microarrays allow for many proteins in the proteome to be detected at a given time. In addition, once proteins are identified, a better understanding of their function and post-translational modifications can also be found through microarray technology (25,26). Despite these advantages, there are still limitations in specificity, as well as potential bias introduced through its targeted nature (25-27). Protein microarrays also have analytical limitations, such as lower sensitivity and smaller protein libraries.

APTAMER-BASED EXPERIMENTS. Like microarray proteomics, aptamer-based scanning involves proteins binding in solution to receptors. However, instead of using antibodies, short, tightly wrapped oligonucleotide strands called aptamers bind to the proteins (11,28). The benefit of aptamers is that they have a stronger binding affinity compared with antibodies, even compared with sandwich detection (11).

Despite the similar theory of a protein-binding substrate, aptamer-based technologies consist of a series of binding and separation steps, with intermediate washes to remove excess and unbound reagents. In the first stage, a protein binds to an aptamer containing a biotin and fluorescent tag. This complex then binds to a streptavidin bead, and unbound reagents are washed before the protein is also tagged with biotin. Next, the samples are subjected to ultraviolet light, which cleaves the aptamer-bead bond. Dextran sulfate is used to remove any proteins that bind to aptamer with weak affinity (noncognate complexes). The aptamer-protein complex binds to a new streptavidin bead via the biotin tag of the protein, and free aptamers are removed. Base is added, removing the aptamers. These aptamers then undergo DNA microarray analysis, in which they bind to complementary DNA strands on the array, and the aptamers fluoresce to be detected (Figure 1C) (28).

Aptamer proteomics has several advantages. First, it has strong affinity for target proteins even at low concentrations, which is accomplished through aptamer design, multiple washings, and removal of non-cognate complexes (11,28,29). With an ever-growing aptamer library, a large number of proteins in the proteome can also be detected (29). However, this method also has disadvantages. Aside from involving multiple steps that may introduce human error, this technology is limited by the aptamers in libraries, challenges with detecting proteins with post-translational modifications, and the inability to generate absolute concentrations of detected proteins (29).

PEA. Much like aptamer proteomics, PEAs provide a high degree of sensitivity and specificity (30,31). PEAs work like protein microarrays, in that a tagged antibody will bind to a protein at a specific site. However, rather than containing a fluorescent or radioactive probe, the antibody has a short single-stranded oligonucleotide sequence attached to its F_c region. Subsequently, another antibody with a complimentary single-stranded oligonucleotide also binds to the protein. If both bind to the correct protein, the 2 separate oligonucleotide Fc strands will hybridize with each other and serve as a template for DNA extension (Figure 1D). These newly hybridized DNA strands are amplified and measured using quantitative polymerase chain reaction (30,31). An advantage of PEA is its minimal cross reactivity, which is accomplished by the proximity of oligonucleotide strands (30). PEA is advantageous in sample conservation because experiments require as little as 1 µl for experiments (10,31). However, like with aptamer proteomics, results may be affected by conformational changes as a result of binding and may have limited ability to detect post-translational



modifications, as well as limitations in oligonucleotide libraries (32).

LIMITATIONS OF MODERN PROTEOMICS. Despite the increasingly realized power of proteomics, several problems exist for each of the previously mentioned methods.

In MS, the cost of equipment is a determinant, and separation and ionization techniques may affect findings. Protein microarrays require instrumentation to detect florescence or more sensitive equipment (such as atomic force microscopy or optical ellipsometry) when markers may affect protein structure. Although aptamer and PEA platforms are commercially available, both are expensive and not without their shortcomings. For example, commercially available aptamers have limited dynamic range and ability to detect proteins generated from single nucleotide polymorphisms (33,34). Current PEA assays have inconsistent relative error in measurement that depends on the analyte, rather than protein abundance (35). Although all platforms provide insights into relative protein regulation, absolute quantification is only possible in PEA or MS, but these assays require the use of labeled standards that may not be feasible in untargeted experiments (35-38). As a result, proteomics and standard immunoassay measurements are unharmonized, and the detected

protein concentrations for these experiments must be viewed in isolation. To overcome this, proteomics can be used to discover novel proteins, and immunoassays are used to quantitate larger sample sets even in platforms capable of absolute quantification (39).

It is also important to consider the sample source when designing experiments. Although peripheral blood sera samples are readily collected, the blood proteome may be influenced by a large number of systemic issues that may or may not be related to the system of interest. Proteomics conducted on tissue samples, although more difficult to obtain, may better reflect the proteome of the system of interest.

ANALYSIS OF PROTEOMICS DATA. A challenge of proteomics is the requirement for high-level data analysis of multidimensional datasets. In the data analysis, the first step is determining the proteins of interest. Traditionally, principal components analysis is used to extract features or groups of proteins with relatedness, using rotations of values in either an orthogonal or oblique manner. Although orthogonal rotations have been widely used in HF proteomics due to their ease, oblique rotations of <90-degree angles allow for proteins to retain a one-to-many relationship, meaning a protein can be involved in multiple pathways, as is often the case physiologically. If orthogonal rotations are used, these one-toomany relationships may not be observed, which ultimately affects interpretation (40). After extracting features, matrixes are used to select individual proteins within components that have been found to be statistically significantly regulated (41).

After determining the proteins of interest, researchers can learn more about their function using enrichment analysis to determine their roles in a biological process. Resources such as gene ontology terms list the functions a protein is involved in (e.g., inflammation). Pathway analysis, using databases (e.g., Kyoto Encyclopedia of Genes and Genomes), helps relate individual proteins in cellular processes (41,42). In addition, understanding individual protein-protein interactions using resources like Search Tool for the Retrieval of Interacting Genes/ Proteins may help elucidate the function of proteins in various pathways (41,43). Finally, motif analysis is useful for understanding proteins at the molecular level, including protein sequences, folding, and posttranslational modifications that may provide insights into proteins in a disease state (41). As technology, such as machine learning and artificial intelligence, as well as current database catalogs improve, researchers will be able to interpret and understand proteomic experiment findings more readily.



(A) In patients with incident heart failure (HF), 16 proteins were differential; when analyzing these same proteins in the HF reversal group after transplantation, only 12 of the 16 were regulated to reflect levels of patients without HF. For both analyses, there was limited network associations with other proteins. (B) In patients with manifest HF, 421 proteins were significantly regulated relative to control subjects; after transplantation, 138 proteins were regulated towards normal levels. After transplantation, many proteins were no longer associated with manifest HF and no longer maintained associations with other proteins. All data provided by Egerstedt et al. (48), and Figures produced with STRING Protein-Protein Interaction Networks v11.0 (53).

CURRENT USE OF PROTEOMICS IN HF STUDIES

We use the remainder of this review to provide examples of how each proteomic method can be applied, as well as the strengths and future directions of this research.

LIQUID CHROMATOGRAPHY-MS/MS PROTEOMICS.

Several studies have examined MS/MS proteomics to evaluate the disease state of HF (9,44-47). In a study of ischemic cardiomyopathy (ICM), Yi et al. (21) aimed to profile the proteome of patients with ICM. They hypothesized the presence of proteins in patients with ICM would provide future diagnostic, prognostic, and therapeutic usefulness. The Yi et al. (21) group aimed to build on previous ICM 2-DE proteomic analyses, which was limited by poor separation of strongly hydrophobic proteins. The investigators collected samples from infarcted left ventricular myocardium in patients who underwent cardiac transplantation (n = 6). Seven control left ventricular tissue samples were collected from healthy deceased donor hearts. After tissue digestion and protein extraction, proteins were separated using liquid chromatography, ionized using electrospray, and analyzed with time-of-flight in tandem MS/MS.

Using these methods, the investigators identified 1,723 proteins. Of these proteins, 104 were found to be upregulated and 63 downregulated. Upregulated processes included wound healing, inflammation, response to stimulus, complement system activation,



and protein maturation. Downregulated processes included energy use and metabolism, muscle processes, and muscle contraction. These differential regulation patterns in the left ventricle resulted in overall changes in the extracellular matrix, immune response, metabolism, muscle contraction, and signal transduction (Figure 2).

In addition to these findings, Yi et al. (21) reported that an inhibitor to complement C1, SerpinG1, was upregulated in the left ventricle (21). This was noteworthy because a more contemporary liquid chromatography tandem MS/MS study of patients with cardiogenic shock by Rueda et al. (46) identified SerpinG1 as a predictor of short-term mortality in shock, along with fatty acid binding protein, beta-2-microglobulin, and fructose-bisphosphate aldolase B. Taken together with clinical variables, this biomarker panel had high discrimination (C-statistic 0.84; p < 0.001) for predicting death (46). **PROTEIN MICROARRAY PROTEOMICS TO IDENTIFY A POSSIBLE DIAGNOSTIC AND PROGNOSTIC BIOMARKER**. To expand the field of possible biomarkers of value for evaluation of HF, Jiang et al. (39) used protein microarrays to measure 507 proteins of various biological functions. For this initial analysis, there were 3 groups of 3 patients: those with HF with preserved ejection fraction (defined by this study as patients with HF with an ejection fraction of >40%);

patients with hypertension; and healthy control subjects. In their analysis, 11 proteins were up or downregulated by the HF group >5-fold relative to the control group. When the HF and hypertensive groups were compared, 17 proteins were up- or downregulated in significantly greater amounts by the patients with HF. Between these 2 analyses, the protein most upregulated was angiogenin, a 123 amino-acid peptide involved in angiogenesis (Supplemental Table 1). To validate their finding, the investigators performed a traditional angiogenin immunoassay to measure protein levels in healthy control subjects (n = 16), in patients with traditional HF with a preserved ejection fraction (ejection fraction \geq 50%; n = 9), and in patients with midrange HF with a preserved ejection fraction (ejection fraction 41% to 49%; n = 7). As with the proteomic analysis, angiogenin levels were significantly increased in patients with HF, and after traditional HF risk factors were accounted for, angiogenin was found to be a potential marker of HF (39).

APTAMER PROTEOMICS TO IDENTIFY BIOMARKERS

FOR DIFFERENT STAGES OF HF. Egerstedt et al. (48) used aptamer-based proteomics to detect the concentrations of 1,305 proteins in 3 patient groups: 1) a group of 768 healthy control subjects, 185 of whom would later develop HF; 2) a group of 84 patients who would eventually be admitted for acute decompensation; and 3) a group of 30 patients with end-stage HF who had samples collected at the time of cardiac transplantation and 6 months after. Among those who ultimately developed HF, 354 proteins were upregulated, and greater activation of the complement system and coagulation-related proteins were observed. After a Bonferroni-adjusted significance threshold was applied, a total of 16 proteins was associated with those who developed incident HF; 12 proteins were secreted, whereas 11 proteins were tissue-enriched. In the patients with established HF, several classical markers linked to HF, such as N-terminal pro-B-type natriuretic peptide, C-reactive protein, ST2, galectin-3, troponin T, tumor necrosis factor-α, renin, matrix metalloproteinases, and tissue inhibitors of metalloproteinases, were found. Following aptamer proteomics and after Bonferroni adjustment, the investigators identified a total of 421 proteins associated with prevalent HF. In the reversal group, compared with the patients with incident HF, 12 of the 16 proteins changed their regulation to mirror the levels that approached the patients without HF. Likewise, 138 of the 421 proteins significant to patients with manifest HF changed significantly toward normal levels. These results suggested that after rapid improvement in cardiac function, such as after transplantation, the proteome could adjust and return to normal expression levels (Figure 3) (48).

PEA PROTEOMICS IN HF. In an effort to identify signatures predictive of incident HF in older adults, Stenemo et al. (49) used PEA to measure 92 proteins, 80 of which were selected based on previous studies of HF risk proteins to validate their usefulness. After analysis, 9 proteins were associated with HF incidence when adjusted for traditional HF risk factors (growth differentiation factor 15, T-cell immunoglobulin and mucin domain 1, tumor necrosis factor-related, apoptosisinducing ligand receptor 2, spondin-1, matrix metalloproteinase-12, follistatin, urokinase-type plasminogen activator surface receptor, osteoprotegerin, and suppression of tumorigenicity 2) (Supplemental Table 2). When N-terminal pro-Btype natriuretic peptide was added to the model, metalloproteinase-12, osteoprotegerin, urokinasetype plasminogen activator surface receptor, and T-cell immunoglobulin and mucin domain 1 still predicted incident HF. It is believed that these proteins are involved in HF functions, such as inflammation, immune response, and changes to the extracellular matrix (49).

Other PEA HF studies have yielded important insights on the proteomes of patients with ischemic versus nonischemic HF (50), those with and without atrial fibrillation (51), and between those with HF with preserved and reduced ejection fractions (52) (**Figure 4**, Supplemental Table 3). In each case, careful network analyses revealed previously known pathways, along with numerous plausible but novel protein-protein relationships.

CONCLUSIONS

Thanks to emerging proteomic technologies, understanding of HF biology is poised to rapidly advance, allowing for detection of novel protein pathways and creating obvious opportunities for development of focused diagnostic biomarker panels and targeted therapeutic interventions. Proteomics can detect thousands of proteins, even in low concentrations, allowing researchers to discover proteins in systems previously unrecorded. In addition, investigators can quickly identify protein presence, concentration, and even post-translational modifications with high throughput and minimal sample loss. As technology advances, proteomics research will become a standard part of studies of HF diagnosis, prognosis, and therapy.

AUTHOR RELATIONSHIP WITH INDUSTRY

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APPENDIX For supplemental tables, please see the online version of this paper.