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

biomarker of a condition and to understand normal ranges and potential disease cutoff values.

Because protein expressions show multifaceted temporospatial characteristics driven by responses to physical and/or biological stimuli, there are several complexities involved in the process of identifying a novel protein biomarker. In order to confirm the utility of a candidate biomarker for a clinical purpose, several steps must be achieved, including discovery, qualification, verification, optimization, and validation, followed by commercialization and distribution of assays[28] (Fig. 1). Although the discovery of novel proteins is driven by mass spectrometric methods, validation and commercialization more frequently involve traditional antibody-based techniques such as enzyme-linked immunosorbent assay (ELISA), Western blotting, and immunoblotting, with the less common use of MS-based methods in a clinical setting dictated by obstacles in regulatory approval and cross-site/cross-equipment reproducibility.[29] Regardless of the most suitable analytical method, a successful biomarker must be easily measured, low cost, patient friendly, and show high levels of sensitivity and specificity for its purpose (eg, diagnosis, prognosis).[30]

This article highlights the current clinical uses of protein biomarkers in HF (Fig. 2) and discusses the application of targeted and nontargeted proteomic investigations to discover and develop novel biomarkers centered on using a personalized medicine approach for improved prognostic information.

MARKERS OF CARDIAC STRESS

B-Type Natriuretic Peptide/N-Terminal Pro–B-Type Natriuretic Peptide

B-type natriuretic peptide (BNP) is perhaps the most widely used biomarker for cardiac stress. It is a central component in cardiovascular homeostasis and is released from the cardiomyocytes, primarily located in the ventricles, in response to stress and stretch of the cardiac muscle.[31] After binding to specific receptors, BNP is activated and drives a reduction in systemic vascular resistance, antagonizes the actions of the renin-angiotensin-aldosterone system, and promotes vasodilation and natriuresis.[32] BNP has been studied extensively for its role as a diagnostic[33–35] and prognostic[36–38] biomarker in HF, including both chronic patients and acute decompensated admissions. However, an important limitation of BNP for HF diagnosis is that circulating levels may become increased in response to alternative disorders such as renal dysfunction, left ventricular hypertrophy, and right ventricular dysfunction.[39] Furthermore, because factors such as sex, age, and body mass index are also associated with fluctuations in BNP levels, accurate interpretation of circulating concentrations is crucial.[40]

In addition to uses in diagnosis and prognosis, BNP has shown utility for the monitoring of patients treated with diuretics and vasodilators such as angiotensin-converting enzyme inhibitors,[41] angiotensin-II receptor antagonists,[42] and aldosterone inhibitors.[43] Circulating BNP levels

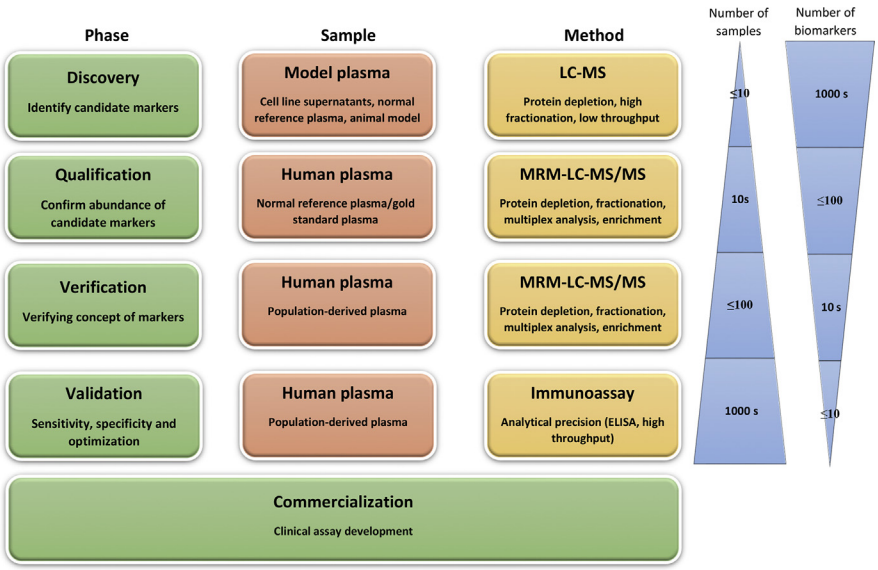

Fig. 1. Protein biomarker discovery pipeline of novel candidate biomarkers. ELISA, enzyme-linked immune-sorbent assay; LC-MS/MS, liquid chromatography tandem mass spectrometry.

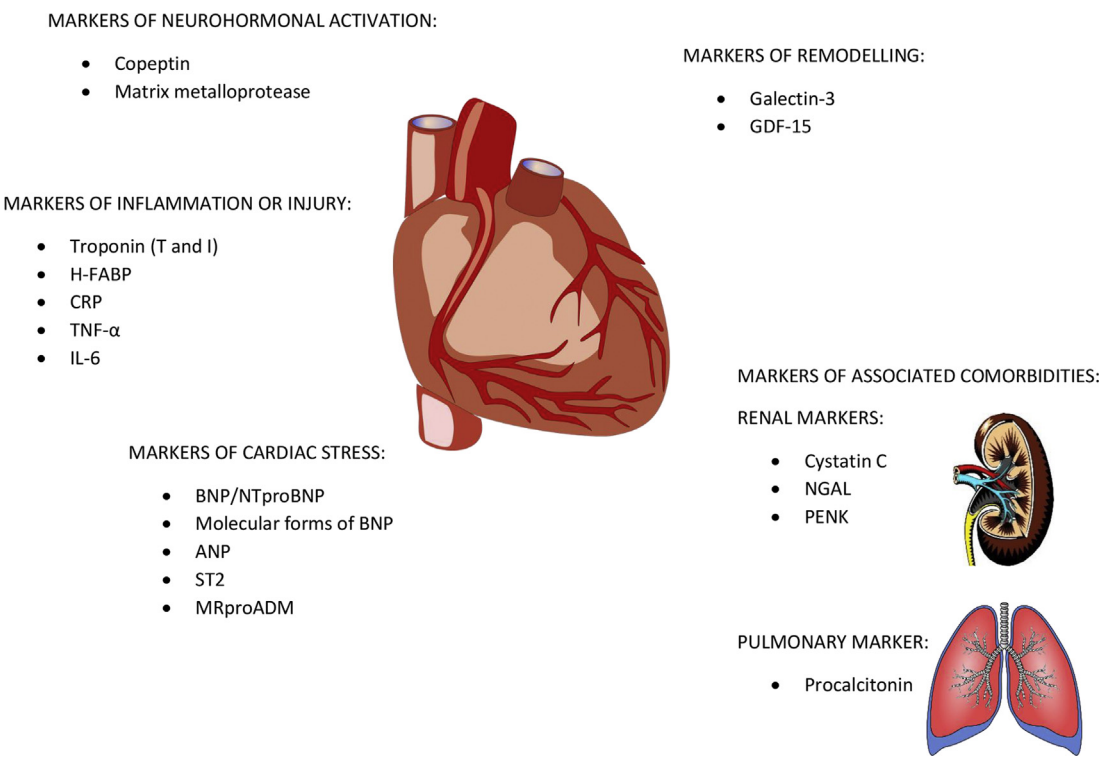

Fig. 2. Protein biomarkers of heart failure and their various pathophysiologic associations. ANP, atrial natriuretic peptide; BNP, B-type natriuretic peptide; CRP, C-reactive protein; GDF-15, growth differentiation factor-15; H-FABP, heart-type fatty acid–binding protein; IL-6, interleukin-6; MRproADM, midregional proadrenomedullin; NGAL, neutrophil gelatinase-associated lipocalin; NTproBNP, N-terminal pro–B-type natriuretic peptide; PENK, proenkephalin; TNF-α, tumor necrosis factor-α.

are known to decrease rapidly following successful treatment strategies, therefore repeat measurements of BNP concentrations provide an observation of responses to medical interventions.

Although widely used in clinical analysis, BNP has a short half-life (approximately 20 minutes) when present in the circulation[44] and, therefore, care must be taken during the sampling and storing of blood samples. N-terminal proBNP (NTproBNP) is released in conjunction with BNP and is considered a more stable alternative because of its longer half-life.[45] NTproBNP is reported to have similar characteristics to BNP as a biomarker for diagnosis,[46–48] prognosis,[42,46,49] and guided treatment[50] in HF.

When studied in direct comparison, BNP and NTproBNP show comparable utility for diagnosis,[42] prognosis,[51,52] and biomarker-guided therapy[53] in chronic HF, with reductions in all-cause mortality reported with titration of therapies based on repeat measurements. Circulating levels of these natriuretic peptide biomarkers are increased in HF and are strongly associated with disease severity and myocardial stretch.[54] Studies, such as the Valsartan Heart Failure Trial (Val-HeFT), have also shown BNP and NTproBNP to provide superior prognostic information compared with alternative neurohormonal markers of risk.[55]

Molecular Forms of B-Type Natriuretic Peptide

There have been recent research efforts to further understand the degradation pathways of BNP and, with its short half-life in circulation, experiments have also reported the presence of its molecular forms. These molecular forms are truncated BNP peptide chains that are synthesized by the proteolysis of end-chain amino acids and have been identified in the circulation of patients with HF (eg, BNP 3-29, 3-30, 4-29, 5-29), with BNP 3-32, 4-32 and 5-32 reported as the most commonly present.[56] These molecular forms have recently emerged as potential biomarkers for HF, with the major forms previously implicated in ischemic heart disease,[57] which is a major risk factor for development of HF. Furthermore, molecular forms have been reported to associate more closely with clinically measured BNP levels compared with the parent BNP molecule

(BNP 1-32). This finding suggests that the specificity of clinical BNP assays is not unique to BNP 1-32, and that the combination of intact and molecular forms of BNP is a more accurate representation of circulating BNP measurements.[58] More recently, molecular forms of BNP have shown prognostic qualities superior to or comparable with NTproBNP for risk stratification of patients with acute HF. BNP 3-32, 4-32, and notably 5-32 were able to independently predict adverse outcome of patients at 6 months and 1 year, outlining its use as a biomarker to guide outpatient management.[27] However, direct mechanistic actions of these truncated forms, along with the dynamics and kinetics of their degradation pathways, are not currently understood and remain areas of current research.

Atrial Natriuretic Peptide

Atrial natriuretic peptide (ANP) is primarily secreted from the atria and has similar physiological properties to BNP. It is thought to play a role in early HF by preserving the compensated state of left ventricular dysfunction.[59] Although reported to be a prognostic indicator in HF, studies have reported ANP as inferior to BNP primarily because of its decreased stability in circulation and its insensitivity to levels of HF severity.[60,61] Other forms of ANP, such as N-terminal ANP, N-terminal pro-ANP, and midregional pro-ANP (MRproANP), have also been shown to suggest diagnostic and prognostic roles in HF.[61–63] MRproANP has far greater stability in circulation and therefore could prove more suitable for use as a biomarker compared with ANP. An investigation using The Biomarkers in Acute Heart Failure (BACH) cohort reported that MRproANP was a suitable diagnostic and prognostic biomarker in dyspneic patients, with results comparable with those of BNP.[63] Furthermore, additional studies have also indicated that MRproANP provides additive prognostic information to NTproBNP in chronic HF[64] and as a diagnostic marker of acute destabilized HF in patients with dyspnea, again reporting comparable results with the use of both BNP and NTproBNP.[65]

ST2

ST2 is a member of the interleukin (IL)-1 receptor family and has been identified as the target for IL-33, which is expressed under biochemical stress of the heart.[66] ST2 is basally expressed by cardiomyocytes and is detectable in circulation in its soluble form, levels of which are increased in response to mechanical stress of the heart.[67] Its utility has been recognized in HF as an independent predictor of mortality or need for transplant in patients with severe chronic HF (New York Heart Association [NYHA] class III/IV),[67] as well as providing prognostic information for patients with acute HF when combined with natriuretic peptides,[68] suggesting applicability as a biomarker in combination with current clinical testing strategies (eg, BNP and NTproBNP). Furthermore, although NTproBNP showed improved diagnostic accuracy for patients with acute HF, the PRIDE (Pro-Brain Natriuretic Peptide Investigation of Dyspnea in the Emergency Department) study showed that ST2 was a suitable biomarker to predict 1-year mortality in dyspneic patients, irrespective of a positive or negative diagnosis of acute destabilizing HF,[69] suggesting a more generalized biomarker function that can be further refined when applying a multibiomarker approach.

Midregional Proadrenomedullin

Adrenomedullin (ADM) is a peptide hormone that has natriuretic, vasodilatory, and hypotensive effects on the heart, with plasma concentrations increased in response to these effects, such as in chronic HF.[70] However, because ADM is unstable in circulation, its midregional prohormone fragment midregional proadrenomedullin (MRproADM) is often measured to perform an indirect quantitation.[71] It has reported a varied role in prognosis, providing information for short-term (30 days) and long-term (4 years) prognosis but less success for midrange predictions of outcome (1 year).[72] MRproADM has also been successfully shown as a prognostic biomarker for patients with acute HF presenting with dyspnea.[73] In this study it was reported as a superior biomarker to both BNP and NTproBNP for the short-term prediction of mortality (90 days) as well as for subsequent patient rehospitalization.[73] Data suggest that it has a suitable role for use in HF prognosis; however, further investigations to confirm its superiority to current biomarkers are warranted.

MARKERS OF INFLAMMATION OR INJURY

Troponin

Troponin proteins are found in cardiac and skeletal muscle tissue and are involved in the regulation of actin and myosin interactions during muscle contraction. Cardiac troponin I (cTnI) and cardiac troponin T (cTnT) have unique isoforms that exist only in cardiac muscle, allowing the measurement of these specific isoforms to provide information in cardiovascular disease, notably as diagnostic biomarkers for ACS.[74] However, development of high-sensitivity troponin assays have further

allowed the measurement of increased cTnI/cTnT levels in patients with HF,[75,76] with increased concentrations associated with poor outcome. High-sensitivity assays for cTnT have been applied for prognosis in chronic HF, with circulating concentrations able to predict mortality and hospitalization in stabilized patients.[77] Furthermore, cTnT has been shown as a suitable marker to reflect myocardial damage in severe chronic HF,[78] to risk stratify patients on admission for subsequent mortality and morbidity,[79] and to identify patients at high risk of disease deterioration.[80] For acute decompensated HF in the absence of ACS, cTnT is a prognostic marker for short-term and long-term outcomes.[81]

In addition, cTnI has been shown to reflect increased BNP levels, impaired hemodynamics, and worsening of left ventricular dysfunction.[82] For acute admissions, serial changes in cTnI levels over 90 days were functional in predicting increased likelihood of mortality and rehospitalization.[83] When used in combination with BNP measurements, cTnI measurements on admission were shown to predict in-hospital mortality, and increasing concentrations were associated with risk of death in a large-scale registry cohort (the Acute Decompensated Heart Failure National Registry [ADHERE]).[84,85]

Heart-Type Fatty Acid–Binding Protein

Heart-type fatty acid–binding protein (H-FABP) is a small cytosolic protein involved in transporting long-chain fatty acids in the myocardium and is released in response to myocardial damage,[86] indicating its potential as a sensitive biomarker for acute myocardial infarction (MI).[87] H-FABP concentrations are known to be increased in congestive and chronic HF,[88] and are reported to be more sensitive than troponin to detect myocardial damage and identify patients at high risk.[86] Initial investigations have been performed to understand the role and/or association of H-FABP in HF, but further studies are required to assess its utility in comparison with current clinical measurements.

C-Reactive Protein

C-reactive protein (CRP) is a traditional marker of inflammation and high concentrations are commonly associated with mortality in patients with acute MI. However, studies have also shown increased CRP levels in patients with HF, reflecting myocardial damage[89] and associations with HF severity, mortality and morbidity,[90] and rehospitalization.[91] Data indicate that CRP can be used as a predictor for deterioration of heart function; however, it has also been reported to show no statistical association with left ventricular ejection fraction, providing complications for its suitability and specificity as a biomarker in HF.[92]

Tumor Necrosis Factor-α

Tumor necrosis factor-α (TNF-α) is a cytokine involved in inflammation and has been reported to be at increased levels in chronic HF.[93] TNF-α has also been implicated in patients with newly diagnosed HF, with increased levels associated with abnormal left atrial dysfunction, and advanced left ventricular diastolic and systolic dysfunction.[94] Further associations have been reported with NYHA class and disease severity,[95] along with the use of TNF-α as a predictor of mortality in advanced HF.[96]

Interleukin-6

IL-6 is also a cytokine involved in inflammation, but it has additional cardiovascular properties through regulation of cardiomyocyte hypertrophy and apoptosis.[97] Cardiac IL-6 expression is reported to increase in advanced HF, suggesting a potential role in prognosis.[98] In addition, increased IL-6 levels have been associated with left ventricular dysfunction before HF diagnosis, highlighting its potential utility as a risk marker for the onset and progression of HF.[99] This prognostic ability has been confirmed in acute HF for prediction of short-term and long-term mortality, both as a sole biomarker and in a multimarker approach when combined with NTproBNP.[100] Furthermore, IL-6, along with CRP and IL-4, concentrations have been shown to increase during a coronary event, returning to preevent levels as symptoms of HF subside over time. This observation indicates a potential role for IL-6 in differentiating between the decompensated and compensated states.[101]

MARKERS OF NEUROHORMONAL ACTIVATION

Copeptin

Preprovasopressin is proteolytically cleaved into copeptin, neurophysin II, and vasopressin, with the vasopressin also known as antidiuretic hormone, which has a prominent role in fluid homeostasis and has been shown to be related to the severity of HF.[102] However, vasopressin is known to show instability in circulation and therefore is troublesome for clinical measurements. In contrast, copeptin is considered to have high stability and is released in equimolar concentrations to vasopressin, allowing a more reliable and

reproducible alternative for indirect measurement of vasopressin.[103] Initial research into the clinical role of copeptin as a prognostic biomarker has suggested it offers superiority over natriuretic peptides for prediction of 14-day and 90-day mortality in acute admissions[73,104] and longer-term prediction at 24 months for patients across various stages of disease,[105] as well as for those with advanced HF.[106] Although a contemporary biomarker for HF, data highlight copeptin measurements as a potential clinical tool for risk stratification, particularly in acute cases.

Matrix Metalloproteinases

Matrix metalloproteinases (MMPs) are a family of zinc-dependent protease enzymes required for normal tissue remodeling and mediating collagen metabolism and extracellular matrix (ECM) homeostasis.[107] Four common classes of MMPs have been identified: gelatinases (MMP-2 and MMP-9), collagenases (MMP-1 and MMP-8), stromelysin (MMP-3), and matrilysin (MMP-7), all of which are regulated by tissue inhibitors.[108]

Increased MMP-9 and MMP-2 concentrations have been reported in patients with HF,[105] with the latter associated with mortality.[109] In contrast, MMP-8 has been shown to have decreased concentrations in patients with chronic HF.[110] Although there are variable alterations of MMPs in patients and more extensive research is required to identify their individual suitability for prognostic investigation, data suggest that they offer additional information when included within a multibiomarker panel.[111] These panel risk scores can improve the information available to identify disease process and HF risk in line with changes to ECM collagen homeostasis and activity of enzymes of remodeling.

MARKERS OF REMODELING

Galectin-3

Galectin-3, a member of the lectin family, has been implicated in multiple aspects of HF physiology, including inflammation and ventricular remodeling. It is secreted by activated macrophages that proliferate and cause cardiac fibrosis,[112,113] and has been shown to provide a positive role as a prognostic marker of HF.[114,115] Increased gelactin-3 levels have been associated with an increased risk of HF and mortality,[116] with a 2-fold increase in levels associated with a 2-fold increase in risk of death or rehospitalization over an 18-month period.[114] Furthermore, increased concentrations have also been associated with adverse outcome in patients with HF with preserved ejection fraction (HFpEF).[117] As with many proteomic biomarkers of HF, the role of gelactin-3 requires further research, but initial data suggest potential as a marker to stratify patients for HF with or without remodeling.

Growth Differentiation Factor-15

Growth differentiation factor-15 (GDF-15) is a stress-responsive, transforming growth factor beta-cytokine involved in inflammatory and apoptotic pathways of tissue injury. It is an emerging marker of cardiac dysfunction, and increased levels of GDF-15 have been shown to identify high-risk patients with cardiovascular disease.[118,119] Increased GDF-15 levels have shown mortality prediction in patients with chronic HF, with increased expression of GDF-15 linked to the promotion of protective mechanisms for inhibition of apoptosis, hypertrophy, and adverse remodeling.[120] Increased levels have also been reported to have prognostic utility in patients with both HF with reduced ejection fraction (HFrEF) and HFpEF, adding to current markers such as troponin and NTproBNP.[121,122] For acute decompensated HF, increased GDF-15 concentrations have shown prognostic value for predicting mortality and HF rehospitalization at 1 year, supported by 21 original studies.[123,124]

MARKERS OF ASSOCIATED COMORBIDITIES

Cystatin C

Cystatin C is a small protein molecule that is involved in the extracellular inhibition of cathepsins. It is removed from circulation through the kidneys, thus providing biomarker information for renal dysfunction and therefore an interest for cardiovascular disease.[125] Cystatin C has shown prognostic capabilities in patients with chronic HF as well as those with HFrEF.[126–128] These positive relationships with adverse outcome in HF, as well as providing information on dysfunction in the renal system, signify cystatin C as a useful biomarker for a combinatory view of cardiovascular disease and its comorbidities.

Neutrophil Gelatinase-Associated Lipocalin

Neutrophil gelatinase-associated lipocalin (NGAL) is an innate antibacterial factor protein of the lipocalin family, initially found to be expressed in neutrophils and later in kidney tubular cells. In kidney dysfunction, NGAL has been shown to be an early marker of injury in animal models and detectable in blood and urine following acute kidney injury.[129,130]

In patients with chronic HF, NGAL concentrations were found to be increased compared with

healthy subjects.[131,132] However, their applicability as a prognostic marker was proved to be inferior to currently established protocols (eg, NTproBNP).[133] In acute cases, the GALLANT (NGAL Evaluation Along with B-type Natriuretic Peptide in acutely Decompensated Heart Failure) trial indicated that NGAL was a strong short-term (30 days) prognostic predictor of HF-related outcomes.[134]

Procalcitonin

Procalcitonin is a precursor peptide of calcitonin and a diagnostic marker of bacterial infections, such as in pneumonia,[135] with increased levels also measured in patients with HF.[136] Increased procalcitonin concentrations were able to predict the risk of long-term death and rehospitalization in acute admissions, irrespective of bacterial infections,[137] and were observed to be in line with disease severity for chronic patients.[138] In addition, serum procalcitonin concentrations provided diagnostic information for HF with high sensitivity and specificity.[139]

CONTEMPORARY PROTEOMIC BIOMARKERS

Recent research has led to an increased number of protein biomarkers that show promise in diagnosis and prognosis of HF conditions. For example, proenkephalin A (PENK) and chromogranin A (CgA) are proteins measured in the circulation that have shown utility as biomarkers in HF, and efforts to validate these for transition into a clinical setting are underway. PENK is a small endogenous opioid peptide that is cleaved to produce enkephalin. Studies have shown that enkephalins are released from nonneuronal tissues, including the kidneys and heart, in response to ischemia.[140] In chronic and acute HF, PENK is associated with glomerular function but does not offer significantly additive prognostic information in addition to current biomarkers of renal function.[141] However, it has provided useful prognostic information for hospitalization or mortality in patients with stable HF.[142] In addition, PENK concentrations have shown predictive capabilities for in-hospital mortality in patients with acute HF, as well as indicating patients at risk of worsening renal function.[143] CgA is a prohormone produced in various tissues, including the heart. Hyperglycosylations of CgA lead to its impaired conversion to catestatin, an action found to be associated with acute HF outcomes.[144] Mixed prognostic quality has been reported in the literature, with studies showing CgA to be associated with the severity of chronic HF and a prognostic marker for mortality,[145] but providing no additive information for prognosis compared with established protocols and biomarkers.[146] In addition to CgA, chromogranin B, which is colocalized with CgA, has also shown an increase in concentrations to follow the severity and development of HF.[147]

Although PENK and CgA have been taken forward to extended validation studies, discovery-focused proteomics experiments have identified several circulating proteins as potential novel biomarkers for HF. The use of urine sampling as a less-invasive alternative to the traditional blood draw has led to an interest in highlighting urinary proteins for diagnostic testing in HF. Two proteins, insulinlike growth factor–binding protein 2 (IGFBP-2) and orosomucoid 1 (ORM1), have been reported to possess diagnostic potential, providing additive information to current biomarkers such as BNP for IGFBP-2,[148] as well as increasing concentrations in line with the severity of chronic HF and good diagnostic sensitivity (95%) and specificity (85%) for ORM1.[149] These proteins are examples of proteomic biomarkers that have provided initially positive associations, but further validation in extended experiments is required.

Other novel protein discoveries have been supported with initial mechanistic and/or clinical investigations and therefore are following the required pathway for translation into a clinical setting. Leucine-rich α2-glycoprotein (LRG) was reported to have an exaggerated expression in patients with a measured BNP of greater than or equal to 100 pg/mL and provided similar diagnostic statistics to BNP.[150] In addition to this, the investigators showed cardiac myocytes to be the origin of LRG release, and more recently it was observed that LRG was active in suppressing adverse remodeling post-MI,[151] and that LRG release in HF may be in response to pressure overload. Calcium-binding proteins A8/9 (S100A8/9) have also been reported to have an upregulated expression as a protective mechanism in HF development, with an observed contribution to the anti-HF effect of hypertrophic preconditioning.[152] In addition to their mechanistic interactions, S100A8/9 have also been shown to provide predictive qualities for mortality in elder patients with severe HF.[153] Similarly, levels of circulating heat shock protein 70 (HSP70) have been shown to increase concurrently with cardiac expression[154] and HF severity,[155] and HSP70 has been implicated as a potential biomarker for early diagnosis of HF.[155]

Although the prior examples of novel biomarkers have undergone initial stages of mechanistic and clinical validation, more recent experiments have indicated further potential biomarkers that remain at the discovery phase. For example, quiescin

Q6 (QSOX1) has shown promise as a biomarker that is specific to acute decompensated HF with dyspnea, with reduced and comparable levels measured in patients with chronic HF and healthy volunteers.[156] QSOX1 has been shown to increase in the left ventricle in an animal HF model[157] and provides diagnostic qualities that are equal to those of BNP and NTproBNP,[156] with increased specificity compared with natriuretic peptides for diagnosis of acute decompensated HF, irrespective of the presence of previous stable HF.

Many candidate proteomic biomarkers for HF have been discovered and are still in the research phase to determine their roles in HF. Limited information regarding their additive role as biomarkers in HF is available and further research for their capacity is required. Extending from single biomarker analyses, proteome-wide investigations have provided insight into multibiomarker models to predict future disease developments. A notable example was presented by Hollander and colleagues,[158] who identified a list of 17 candidate protein biomarkers that, when combined with BNP measurements, were able to provide 97% sensitivity and 100% specificity for classifying patients on recovery from cardiac transplants. This finding provides an opportunity to provide outpatient screening to monitor response to HF treatments but requires extensive additional testing to validate its applicability for everyday clinical use.

SUMMARY

A wide range of protein-based cardiac biomarkers have shown success in diagnostic and prognostic applications, with several already established as routine measurements in clinical laboratories. Extensive research efforts are currently underway to enhance the current knowledge of protein–cardiovascular disease interactions, led by proteomic-based organizations such as Human Proteome Organization (HUPO). The flagship venture has been The Human Proteome Project (HPP), which is working to map the entire human proteome to further understanding of the localized and systems biology of proteins and protein-protein interactions for diagnostic, prognostic, and therapeutic roles in disease.[2] In particular, HUPO has emphasized the need to develop open-access databases that allow the sharing of proteomics research data across equipment and institutions to detail the human proteome library.[159]

The advancement of technologies, such as MS, that complement traditional enzyme-based assays has allowed the development of highly sensitive and selective methods with the ability to measure multiple relevant biomarkers in a high-throughput manner. Although at a stage of infancy for translation to functional clinical laboratories, these methods offer the potential for future advancements in the breadth and depth of clinically relevant biomarkers. In addition, the use of multiple omics-based investigatory pathways, including proteomics, metabolomics, lipidomics, and genomics, may lead to the discovery and validation of novel biomarkers that provide improved clinical information to patients in cardiovascular disease and beyond. An example of this approach includes the focus of implementing the combination of protein and metabolite biomarkers to improve risk stratification, with recent demonstration of enhanced prognostic capabilities in chronic and acute HF when combining BNP/NTproBNP with trimethylamine *N*-oxide, a metabolite biomarker linked to gut microbial breakdown of dietary molecules.[160,161] Continued efforts for biomarker discovery and validation promise to unearth novel and contemporary molecules for application in personalized and precision medicine, with the potential to lead to improved prognosis, treatment, and early diagnosis of conditions at the center of public health concerns.

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