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REVIEW

Circulating heart failure biomarkers beyond natriuretic peptides: review from the Biomarker Study Group of the Heart Failure Association (HFA), European Society of Cardiology (ESC)

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New biomarkers are being evaluated for their ability to advance the management of patients with heart failure. Despite a large pool of interesting candidate biomarkers, besides natriuretic peptides virtually none have succeeded in being applied into the clinical setting. In this review, we examine the most promising emerging candidates for clinical assessment and management of patients with heart failure. We discuss high-sensitivity cardiac troponins (Tn), procalcitonin, novel kidney markers, soluble suppression of tumorigenicity 2 (sST2), galectin-3, growth differentiation factor-15 (GDF-15), cluster of differentiation 146 (CD146), neprilysin, adrenomedullin (ADM), and also discuss proteomics and genetic-based risk scores. We focused on guidance and assistance with daily clinical care decision-making. For each biomarker, analytical considerations are discussed, as well as performance regarding diagnosis and prognosis. Furthermore, we discuss potential implementation in clinical algorithms and in ongoing clinical trials.

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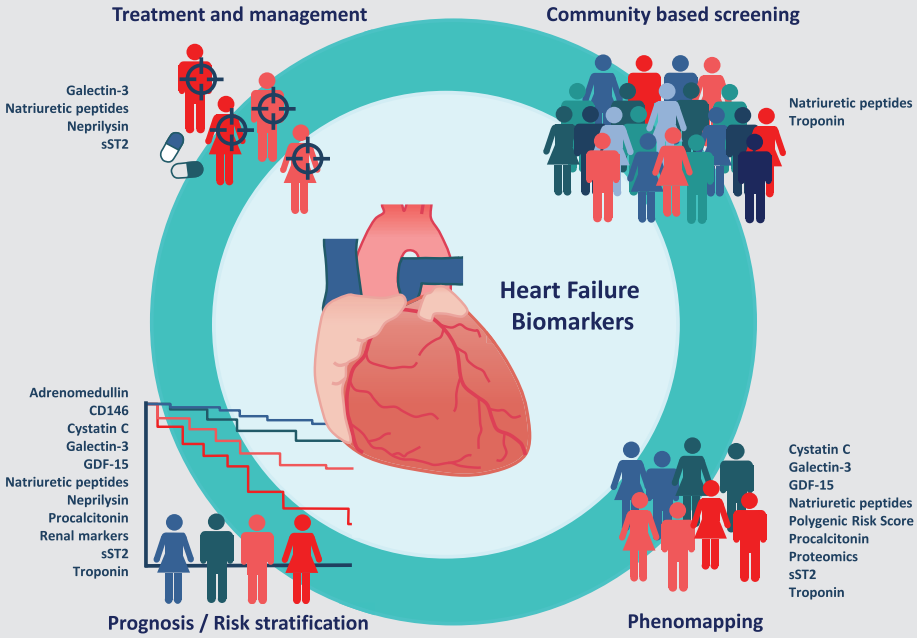
Graphical Abstract



Potential usage of biomarkers in the heart failure spectrum.

Keywords Biomarkers • Heart failure • Cardiac troponin • sST2 • Growth differentiation factor-15 • Galectin-3 • Procalcitonin • Adrenomedullin

Introduction

Physicians routinely use biomarkers for the diagnosis, prognosis and management of patients with heart failure (HF). Established biomarkers such as N-terminal pro-B-type natriuretic peptide (NT-proBNP), B-type natriuretic peptide (BNP), and high-sensitivity cardiac troponin (hs-cTn) are integrated into the current European (European Society of Cardiology, ESC) and American (American College of Cardiology/American Heart Association, ACC/AHA) guidelines.[1,2] In the ESC guidelines, natriuretic peptides are advocated for their utility in diagnosis of HF, with particular value to exclude HF. However, most data regarding biomarkers in HF report on their prognostic utility, while recognizing these markers may not necessarily improve diagnosis or treatment. To improve performance, it has been advocated to use serial measurements or to implement biomarker guided-therapies.[3,4] Factors complicating clinical application of markers include fluctuation in biomarker levels at various time points in clinical decision-making; for example, at the emergency department,[5] prior to discharge[6] or in the outpatient clinic.[7] Further, although biomarkers can both be used to assess high and low risk of adverse events,[8] comorbidities and other patient characteristics including age, sex, coefficient of variation within an individual (biological variation),[9] and kidney function potentially confound the biomarker result and its interpretation. Finally, few prospective trials have been conducted to test if biomarker-based clinical decision-making improves management or outcomes. As a result, the role of biomarkers in ongoing HF treatment decision-making remains limited although it is universally accepted that lower peptide serum concentrations associate with a better prognosis. The role and best clinical use of established markers of renal function such as serum creatinine has recently been summarized elsewhere and is not discussed.[10]

An article in this Journal from 2015 highlighted the most promising biomarkers at that moment in time.[11] The field of biomarkers in HF is very dynamic, with a vast number of candidate biomarkers recently described. We herein provide an updated overview with a focus on biomarkers with clear potential, as only a few have made the difficult translation from initial promise to (pre-)clinical application. In view of numerous previous reviews of natriuretic peptides, including a recent update by this Heart Failure Association (HFA) Biomarker Study Group,[12] in the current review, we do not address natriuretic peptides. We focus on those markers that have passed this first stage including hs-cTn, procalcitonin, novel kidney markers, soluble suppression of tumorigenicity 2 (sST2), galectin-3, growth differentiation factor-15 (GDF-15),

Table 1 Effect of confounders on biomarker level

Biomarker	Male gender	Age	Obesity	Non-White	Renal impairment	Inflammation/ infection
Natriuretic peptide	←→	↑	↓	↓	↑	↑
Cardiac troponin	↑	↑	↑	↑	↑	↑
Procalcitonin			↑		↑	↑
Cystatin C	↑	↑	↑	↓	↑	↑
NGAL	↓	↑			↑	↑
KIM-1					↑	–
sST2	↑	←→	←→	←→	←→	↑
Galectin-3	↓	↑	←→	←→	↑	↑
GDF-15	↑	↑	↑	←→	↑	↑
CD146						↑
Neprilysin			↑			←→
Adrenomedullin						↑

CD146, cluster of differentiation 146; GDF-15, growth differentiation factor-15; KIM-1, kidney injury molecule-1; NGAL; neutrophil gelatinase-associated lipocalin; sST2, soluble suppression of tumorigenicity 2.
↑ Higher; ↓ Lower; ← → Not affected.

cluster of differentiation 146 (CD146), neprilysin, adrenomedullin (ADM), and also discuss proteomics and genetic-based risk scores. We aim to provide guidance in clinical care decision-making. All the biomarkers mentioned in this review are displayed in the *Graphical Abstract*. The biomarkers are depicted next to the clinical setting in which they fit best. We have structured the article so that analytical considerations and biomarker performance in diagnosis and prognosis are presented before discussion on implementation in clinical algorithms and/or current trials (via a search on ClinicalTrials.gov). Imaging biomarkers are upcoming in sight of diagnosis and prognosis in HF but are not part of this review. For all biomarkers mentioned in this article, biological variation indices are displayed in online supplementary *Table S1*. The influence of confounders on biomarker levels can be found in *Table 1*.

Table 2 Interpretation of high-sensitivity cardiac troponin T/I in the setting of acute heart failure

Diagnosis	hs-cTnT/I levels
Type 1 AMI likely	hs-cTnT/I is very high (e.g. >10 times the ULN)
Type 1 AMI likely	Δ hs-cTnT/I within 1 h or 3 h is very high (e.g. >100 ng/L), unless clear alternative cause, as e.g. AF with rapid ventricular conduction or myocarditis
AMI likely	ST-segment elevation and/or depression increases

AF, atrial fibrillation; AMI, acute myocardial infarction; hs-cTnT/I, high-sensitivity cardiac troponin T/I; ULN, upper limit of normal.

Cardiac specific

(High-sensitivity) cardiac troponin

Testing for hs-cTn is commonly performed in patients with HF, particularly in those with acute HF syndromes, where cardiac troponin I (cTnI) or cardiac troponin T (cTnT) measurement is recommended to establish the presence of type 1 myocardial infarction (MI) or acute HF-related injury. In patients with acute HF, substantial elevation [e.g. >10 times the upper limit of normal (ULN)] and/or substantial increases within 1–3 h (e.g. >100 ng/L) suggest the presence of type 1 MI[13] (*Table 2*).

Physicians should be careful when extrapolating data and thresholds from any one assay to another to diagnose type 1 MI. Unless there is evidence of acute myocardial ischaemia, cardiac troponin increases should be considered as myocardial injury.[14,15] Importantly however, concentrations of hs-cTn are frequently elevated in HF patients independent of the presence or absence of myocardial ischaemia due to vascular events. Notably, when elevated – regardless of acute or chronic HF, and regardless of presence or absence of coronary artery obstruction – hs-cTn concentrations prognosticate risk for progressive ventricular remodelling, and predict a heightened risk of death.[16,17]

In a chronic phase, cardiac troponin values better reflect left ventricular end-diastolic pressure, suggesting subendocardial hypoperfusion. The latter can be explained by that transient elevations of left ventricular end-diastolic pressure can lead to cTnI release, apoptosis, and reversible stretch-induced stunning in the absence of ischaemia. Aimo and colleagues recently reported results from a large pooled analysis of patients with chronic HF (n = 9289)[16]; in this analysis, the median concentration of hs-cTnT was 16 ng/L, with >50% of the cohort above the ULN.

The percentage of those with elevated hs-cTn concentrations is even higher amongst those with acute HF syndromes. Myocyte injury might explain cTnI elevations in acute HF.[18,19] Pascual-Figal and colleagues reported 98% of patients with acute HF had

detectable hs-cTnT, and 81% were above the ULN.[17] When measured serially, a rise and/or fall of hs-cTn may be seen in acute HF; and in fact such changes closely mimic cardiac troponin kinetics seen in acute MI.[20] When such changes are noted, clinicians are reminded to interpret cardiac troponin as a quantitative variable[21] and consider consensus definitions[13] to avoid conflating this evidence of myocardial injury, unless of course, other criteria for MI are present.

Other mechanisms have been implicated to explain the rise of hs-cTn in patients with HF; all involving cardiomyocyte injury and apoptosis or necrosis.[22,23] Among these are inflammation, neuro-hormonal activation, ventricular stretch, increased wall tension, supply–demand mismatch, cytotoxicity, cellular necrosis, apoptosis or autophagy, and possibly exocytosis of cytosolic contents.[14]

Analytical considerations

Cardiac troponin I and T are troponin isoforms that are unique to the cardiac myocyte, and measurement of either is sensitive and specific for detecting cardiac injury. Over the years, several generations of troponin assays have been developed and made commercially available for patient care. Despite the fact that the results from these various testing platform systems may yield similar clinical interpretation for diagnosis, i.e. above or below the 99th percentile of the assay with a rise and/or fall of cardiac troponin, there are considerable differences in the numerical cardiac troponin values between assays (from different vendors). This variability may be due to differences in assay calibration, use of different antibodies, differences in assay design, instrument limitations and multiple detection technologies, and must be taken into account when interpreting test results.

Rapid advances in immunoassay technology have allowed manufacturers to develop and calibrate troponin assays with magnificent analytic sensitivity and precision. A contemporary troponin assay detects plasma troponin levels as low as 5–6 ng/L. At the beginning of 2000, the limit of detection fell from 0.5 ng/mL to 6 ng/L, an >100-fold improvement in analytic sensitivity. Due to the sensitivity of the new methods, we express data as pg/mL (ng/L) instead of ng/mL (μg/L).[24]

For a troponin assay to be defined as high-sensitivity, two analytical criteria need to be met according to the 2018 document released by the Task Force on Clinical Applications of Cardiac Bio-Markers: (i) the percent coefficient of variation at the 99th percentile ULN should be ≤10%; (ii) measurable concentrations should be attainable at a concentration at or above the assay limit of detection for >50% of healthy individuals.[25] To provide perspective, the hs-cTn assays can detect troponin concentration in more than 95% of the samples which are of the limit of detection value but this only includes adult men. In women, cardiac troponin can be detected in only 40% to 60% of the samples, especially when women aged <45 years are taken into consideration.[24,26–28]

Diagnostic and prognostic studies

Concentrations of hs-cTn may be of use to predict the onset of HF in apparently healthy individuals, especially when measured serially. hs-TnT measurement was found to be useful in predicting future development of HF in 4221 older, community dwelling adults; in this study hs-cTnT >13 ng/L was associated with a HF incidence rate of 6.4 per 100 person-years (95% confidence interval (CI) 5.8–7.2), and this translated in an adjusted hazard ratio (HR) of 2.48 (95% CI 2.04–3.00).[29] An elevated hs-cTnT was also predictive of future cardiovascular death (incidence rate of 4.8; 95% CI 4.3–5.4), with an adjusted HR of 2.91 (95% CI 2.37–3.58) compared to those with undetectable hsTnT. A repeat hs-cTnT measurement in 2–3 years refined risk even further: >50% change in hs-cTnT was associated with an even higher risk for HF (adjusted HR 1.61; 95% CI 1.32–1.97) and cardiovascular death (adjusted HR 1.65; 95% CI 1.35–2.03) while a decrease was associated with a lower risk. These results were recently confirmed by McEvoy and colleagues, who showed a rising hs-cTnT concentration 6 years after initial measurement predicted substantial risk for incident coronary artery disease (CAD) (HR 1.4), HF (HR 2.0), and death (HR 1.5); in those with a marked rise in hs-cTnT from baseline to 6 years, an even greater risk for incident CAD (HR 4.0) and HF events (HR 8.0) was present.[30] In an even lower risk cohort from the Framingham Heart Study, Wang and colleagues also demonstrated the prognostic importance of hs-cTnI in prediction of death and the onset of HF, even when extensively adjusted for relevant covariates, including other biomarkers.[31] The BiomarCaRE consortium included nearly 49 000 subjects from four population-based cohorts and demonstrated that hs-cTnI predicted new-onset HF over a time period of 6.6 years (HR 1.42; 95% CI 1.31–1.53).[32]

In acute HF, concentrations of cardiac troponin are also substantially prognostic, regardless of the reason for cardiomyocyte injury. For example, in one study, concentrations of hs-cTnT remained a significant and independent predictor of all-cause mortality, even after adjustment for multivariables including natriuretic peptides and sST2 (HR 1.16; 95% CI 1.09–1.24; $P < 0.001$).[33] In a multi-marker strategy, patients with all three biomarkers below their optimal cutoff point (based upon the receiver operating characteristic curves) had the best survival (0% death) at a median follow-up of 739 days, while 53% of those with elevation of all three biomarkers died. Concentrations of cardiac troponin may also be useful to predict in-hospital[20,34] and post-discharge outcomes following hospital-based treatment for acute HF.[35,36] For example, in one cohort of 1074 patients with acute HF undergoing serial measurement of hs-cTnT, higher baseline or peak hs-cTnT and greater peak change predicted adverse outcome, particularly strongest for 180-day cardiovascular mortality (HR per doubling of baseline hs-cTnT 1.36; 95% CI 1.15–1.60).[35] In another cohort of 1653 patients with acute HF,[37] those with low hs-cTnI concentrations had <5% risk for death or rehospitalization within 30 days. Another study demonstrated that if baseline undetected cardiac troponin becomes detectable after 7 days, this is associated with worse outcomes at 60 days.[38]

In chronic HF, cardiac troponin levels are also frequently increased and, as in acute HF, such elevations are prognostic. In a seminal analysis, hs-cTnT concentrations from 4053 stable chronic HF patients in the Val-HeFT (Valsartan Heart Failure Trial) were studied.[39] In this analysis, hs-cTnT was detectable in 92% of the

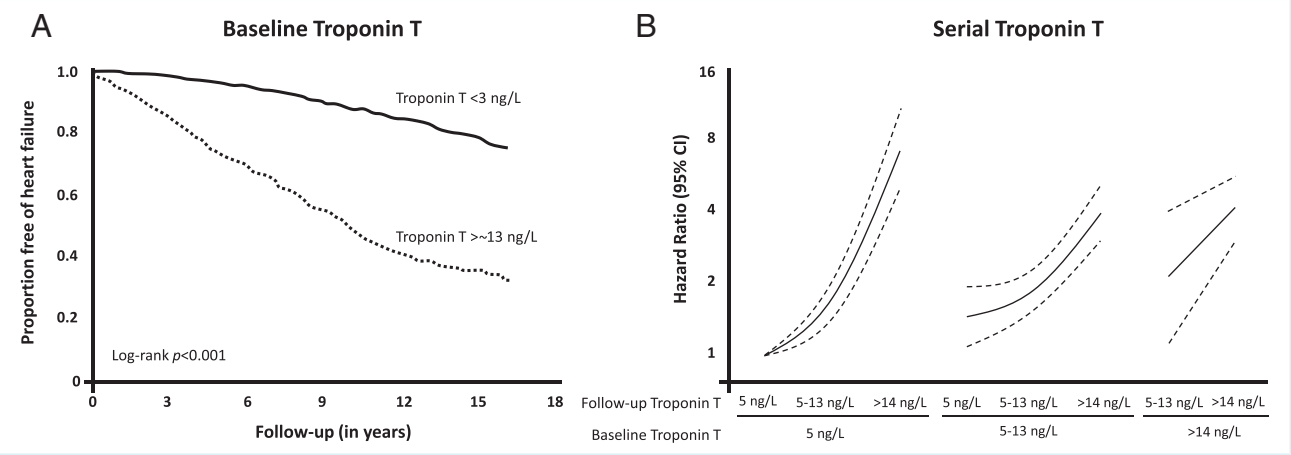


Figure 1 Troponin and incidence heart failure. (*A*) Kaplan–Meier curves reflecting cumulative proportion of adults with incident heart failure divided into cardiac troponin T <3.00 pg/mL and >~13 pg/mL). Data derived from deFilippi *et al.*[29] (*B*) Relative risk for heart failure according to 6-year change in high-sensitivity troponin T. Data derived from McEvoy *et al.*[30] CI, confidence interval.

cohort and predicted future adverse outcomes (HR 1.05; 95% CI 1.04–1.07 for increments of 0.01 ng/mL; $P < 0.001$). Adding hs-cTnT to a baseline model including natriuretic peptides and relevant clinical predictors significantly improved prognostic discrimination. Combining patients from both the Val-HeFT study and the GISSI-HF (Gruppo Italiano per lo Studio della Sopravvivenza nell'Insufficienza Cardiaca-Heart Failure) study, investigators examined serial hs-cTnT measurement in 5284 patients with chronic HF.[40] Increases in hs-cTnT over 3–4 months of follow-up strongly predicted all-cause mortality (HR 1.59; 95% CI 1.39–1.82 and HR 1.88; 95% CI 1.50–2.35, in the Val-HeFT and GISSI-HF study, respectively), after adjustment for traditional risk factors. Improvement in test performance was only modest over a single baseline measurement of hs-cTnT (*Figure 1*).[29,30]

In a subset of TOPCAT (Treatment of Preserved Cardiac Function Heart Failure With an Aldosterone Antagonist Trial), hs-cTnI was measured in 236 out of 1767 patients. hs-cTnI was associated with increased risk for a composite of cardiovascular death or HF hospitalization (HR 1.42; 95% CI 1.20–1.69; $P < 0.001$).[41] Higher hs-cTnI concentrations were associated with male sex, black race, lower estimated glomerular filtration rate (eGFR) and higher NT-proBNP levels.

Proposal for a clinical algorithm/trial update

While the prognostic ramifications of elevated cardiac troponin in patients with acute HF are clear, the therapeutic steps to follow when such an elevated value is present remain less well defined. Nonetheless, given the great importance of acute MI in the precipitation of acute HF, current position statements recommend the measurement of cardiac troponin in patients with acute symptoms, to primarily diagnose or exclude type 1 MI as the trigger of the current episode.[13]

Though prognostic, how hs-cTn concentrations inform clinical decision-making in chronic HF remains speculative. Higher concentrations of hs-cTn T or I were predictive of future left ventricular remodelling in patients with stable ambulatory HF in parallel with a poor prognosis.[42] Knowledge of continuous elevation in hs-cTn may help in decision-making regarding intensity of follow-up, acquisition of updated cardiac imaging and escalation of therapy. Among patients with HF and reduced ejection fraction (HFrEF), therapy with sacubitril/valsartan was associated with lower subsequent concentrations of hs-cTnT at 4 weeks and 8 months after randomization, when compared to those treated with enalapril.[43] Presently, however, use of hs-cTn as a guide to HF therapy is not defined, although in patients with elevated hs-cTn, reassessment of clinical stability and consideration of optimization of drug therapy is warranted.

Given the current lack of evidence-based clinical strategies for managing elevated hs-cTn in apparently stable patients, firm recommendations cannot be made regarding the approach to management. However, certain concepts are reasonable: control of risk factors linked to hs-cTn concentrations such as hyperglycaemia and hypertension should be reviewed, and the presence of unstable coronary ischaemia might be sought.

Non-cardiac specific

Procalcitonin

Patients at the emergency department who present with shortness of breath require rapid and accurate assessment regarding aetiology. Differentiating between acute HF and pneumonia is of pivotal importance and timely initiation of therapy is a necessity. Misdiagnoses may result in delayed or erroneous treatment, potentially increasing adverse outcomes, mortality, and cost.[29] Procalcitonin has been introduced as a biomarker that might help physicians to screen whether a patient with shortness of breath also has a bacterial infection irrespective of other causes of dyspnoea. It is a protein released directly by endotoxins or indirectly via cytokines [e.g. interleukin (IL)-6]. In other infectious diseases like lower respiratory tract infections, pneumonia and sepsis, procalcitonin was

proven to aid in diagnostic accuracy and may assist with antibiotic therapy stewardship.[44,45] Furthermore, procalcitonin may have additional value for the evaluation and management of the dyspnoeic patient with acute HF.

Analytical considerations

Insights into the analytical capability of procalcitonin are mainly derived from non-HF studies. A biological variation study in haemodialysis patients ($n = 123$) concluded that the relative change in procalcitonin is more important than absolute procalcitonin levels for diagnosing bacterial infection.[46] Furthermore, procalcitonin can also be used to monitor treatment effect. In a prospective study of 36 acute meningitis patients, procalcitonin levels over time reflected an adequate antibiotic treatment regimen.[47] Although very low concentrations of procalcitonin (e.g. <0.10 ng/mL) are useful to exclude the presence of bacterial infection, the mere presence of acute HF tends to cause higher concentrations of procalcitonin irrespective of clinical overt infection.[48] Accordingly, higher thresholds for identifying pneumonia in patients with acute HF might be considered.

Diagnostic and prognostic studies

A post-hoc analysis of the BACH (Biomarkers in Acute Heart Failure) study[49] suggested that procalcitonin levels may improve diagnostic accuracy in identifying pneumonia in patients with acute dyspnoea. The area under the curve to diagnose pneumonia was significantly improved when procalcitonin was added as a diagnostic test independent of chest X-ray, yet still suboptimal. This was further tested with multivariable analyses, which demonstrated an independent additional value of procalcitonin in the diagnosis of pneumonia. These results were confirmed in the PRIDE (ProBNP Investigation of Dyspnea in the Emergency Department) study, where procalcitonin was an independent predictor of pneumonia, substantially reclassified cases (even in the context of clinician uncertainty) and was prognostic for poor outcomes.[48]

In the BACH study, in patients with procalcitonin levels >0.21 ng/mL, the lack of antibiotic therapy resulted in worse survival. Similarly, patients had an elevated risk of mortality when treated – possibly without justification – with antibiotics, if procalcitonin levels were <0.05 ng/mL. More recently, a post-hoc subgroup analysis of a randomized controlled trial suggested that also patients with a past history of HF if presenting with respiratory symptoms might have reduced antibiotic usage and possibly even improved outcomes if procalcitonin-guided treatment is used.[50] This hypothesis was then prospectively tested and unfortunately rejected in two studies specifically enrolling patients with acute HF, i.e. IMPACT-EU (Improve Management of Acute Heart Failure with Procalcitonin in Europe).[51,52] IMPACT-EU was a multicentre, international prospective, randomized, controlled process trial with an open intervention evaluating the effectiveness of procalcitonin-guided antibiotic treatment compared to current treatment practice to reduce 90-day all-cause mortality in a European population of emergency patients with dyspnoea and suspected acute HF. The study was stopped early because of futility – no significant differences between control and procalcitonin-guided groups were observed (log-rank $P = 0.91$). A recent study assessed both the diagnostic accuracy and procalcitonin guidance in prospective trials and compared those with IL-6 and C-reactive protein (CRP) in the diagnosis of pneumonia. Procalcitonin had in fact worse diagnostic accuracy compared to both IL-6 and CRP (area under the curve 0.75 vs. 0.80 vs. 0.82; respectively).[53] Furthermore, the cost of measuring CRP in most countries is <5% of that of procalcitonin. Therefore, based on the aggregate data we cannot recommend the use of procalcitonin in patients admitted with acute HF.

Proposal for a clinical algorithm/trial update

The concept of using a combination of a 'cardiac marker' (e.g. natriuretic peptides) with an 'inflammatory' marker (e.g. IL-6, CRP, or procalcitonin) for the very early discrimination of acute HF vs. pneumonia would in theory be of additional value. Future studies should be conducted in acutely dyspnoeic patients where a true clinical dilemma exists with regard to the underlying cause of dyspnoea, with a substantial proportion that may be explained by respiratory tract infection.

Kidney markers

Acute HF is associated with a high incidence (~25%) of acute kidney injury (AKI), often superimposed on pre-existing chronic kidney disease. From multiple studies we know that nearly 50% of patients with chronic HF have an eGFR <60 mL/min/1.73 m^2.[54,55] Both background kidney dysfunction and acute decrements portend a worse prognosis in acute HF.[54–57] Several studies have shown that congestion is the main driver of adverse outcomes in acute HF, and that it can confound or modulate the prognostic value of changes in serum creatinine.[58–60] AKI, defined as either an increment in serum creatinine of over 26.4 μmol/L within 48 h, or increase in serum creatinine >1.5 times in the last 7 days or an urine volume <0.5 mL/kg/h for 6 h, is associated with doubling of mortality at 1 year and increments in serum creatinine as little as 9 μmol/L are associated with worse prognosis.[56] There is a growing recognition of the great difference between a rise in serum creatinine related to congestion vs. that related to therapeutic effects of diuretics and vasodilators. The latter, when modest, is actually associated with therapeutic success in acute HF, while the former is a marker of high risk, where intensified diuretic therapy is critical. The diagnosis of AKI is currently limited to the measurement of serum creatinine and urine output, which mostly reflects glomerular filtration. Differentiation between AKI and acute tubular necrosis is important and both entities are clearly explained in other reviews.[61,62] Additional markers which reflect tubular injury might complement to diagnose AKI at an earlier stage. Indeed, there is a clear trend to evaluate both glomerular and tubular injury to detect AKI.[63]

Analytical considerations

To date targeting AKI in acute HF with experimental pharmacotherapies has unfortunately failed.[64] It is critical to detect the

development of kidney injury early in order to apply renoprotective measures (avoidance of nephrotoxic drugs, contrast media and hypotension plus protection of renal perfusion pressure), which are known to improve outcome in acute HF.[65] Serum creatinine to monitor renal status is not sufficient.[66] Further, many patients with acute HF with a near-normal serum creatinine on admission have evolving AKI, not apparent in the absence of knowledge of pre-acute HF values. Further, whether AKI in HF from hypoperfusion differs from injury resulting from renal venous congestion is unclear.[67] Early markers of AKI in acute HF are needed. Ideally biomarkers should reflect the causal mechanism, context and duration of the renal insult.[68] Methods have now been developed to define the thresholds for damage biomarkers independent of changes in renal function.[69,70]

Diagnostic and prognostic studies

Kidney cellular damage markers such as kidney injury molecule-1 (KIM-1), neutrophil gelatinase-associated lipocalin (NGAL) and IL-18 might facilitate earlier detection of AKI following ischaemic and nephrotoxic kidney injury.[66,71,72] None has yet entered routine clinical practice.

Urinary or serum NGAL demonstrated an area under the curve of ~0.7 to detect AKI in acute HF.[71–74] However, to add clinical value markers require an area under the curve of 0.75 or more. The large AKINESIS (Acute Kidney Injury N-gal Evaluation of Symptomatic Heart Failure Study) indicated NGAL offered no advantage over serum creatinine.[73,75–77] Data from an intensive care unit-based study in patients with serious acute disease indicate that the cell cycle markers, insulin growth factor binding protein 7 (IGFBP7) and tissue inhibitor of metalloproteinase type 2 (TIMP-2) have an area under the curve for detection of AKI of ~0.8.[78] These markers have not been studied in acute HF patients. Proenkephalin has also recently emerged as a promising candidate marker with less delay in detection of falling kidney function than creatinine.[79]

Another class of biomarkers is emerging from RNA fractions. These novel biomarkers include microRNAs (miRNAs), a class of small (22–28 nucleotides long), non-coding, single-stranded RNAs that negatively regulate gene expression via translational inhibition or mRNA degradation.[80,81] Few studies have reported on circulating levels of miRNAs in AKI.[82] Urinary miR-21 increased 1.2-fold, and miR-155 decreased in AKI.[83] Another study detected that miR-494 increased 60-fold in urine in AKI.[84] The rise in miR-494 preceded increases in serum creatinine, highlighting miR-494 as a candidate early marker for AKI. Lorenzen *et al.*[85] identified 13 miRNAs with altered circulating levels in critically-ill patients with AKI, of which miR-210 was an independent predictor of survival. The diagnostic performance of miRNAs as markers of AKI in the context of acute HF is unknown.

Proposal for a clinical algorithm/trial update

We await rigorous assessment of novel candidate markers of AKI including the cell cycle markers, proenkephalin and miRNAs. Pending proof of a reliable marker panel, clinicians must continue to exercise a high index of suspicion of possible AKI and introduce empirical renoprotective measures in those at high risk including the elderly, diabetic, those with known pre-existing renal impairment, arterial disease, hypotension and/or exposure to nephrotoxic pharmaceuticals.

Cystatin C

Cystatin C (encoded by CST3 on 20p11.21) is a cysteine protease inhibitor that functions within the human vascular system. It mostly regulates cathepsins, and serves as a marker of renal function.[86] Multiple studies have compared cystatin C and creatinine based renal function, and in specific patient populations it might even reflect better the renal function, as it is freely filtered by the glomerulus and not influenced by skeletal muscle degradation.[87,88]

Analytical considerations

Besides *in vitro* assays, two widely used assays are developed to measure cystatin C both as an *in vitro* diagnostic testing, as on a clinical chemical analyser.[89] The Gentian Cystatin C immunoassay is a particle-enhanced turbidimetric immunoassay (PETIA). This assay is compatible with a broad range of analysers like ADVIA, ARCHITECT, AU, VITROS, BS and Cobas on the Architect ci8200 platform (Abbott Laboratories, Abbott Park, IL, USA). A second assay is the particle-enhanced nephelometric assay (PENIA) which can be performed on the Siemens analyser. The assay details can be found in online supplementary *Table S1*. Important to note is that cystatin C is not only affected by renal function, but also by age, diabetes, inflammation and proteinuria.[88]

Diagnostic and prognostic studies

Prospective epidemiological studies show a strong association between circulating cystatin C and risk of future cardiovascular disease and HF, independent of renal function.[90–93] In a pooled analysis of several cohorts (n = 76 481), the single nucleotide polymorphism (SNP) rs911119 was investigated. This SNP explains 2.8% of the variation in cystatin C and is associated with a decreased serum cystatin C. Mendelian randomization analysis did not provide evidence for a causal role of cystatin C, with a causal relative risk for cardiovascular disease of 1.00 per doubling cystatin C (95% CI 0.82–1.22; $P = 0.994$).[94] Cystatin C is also explored in patients with HF. In 823 patients with HF who underwent coronary angiography, cystatin C demonstrated to be of prognostic value to predict major adverse cardiovascular events (HR 1.20; 95% CI 1.05–1.36) after adjustment for clinical and biochemical markers. Furthermore, cystatin C and its derived equation to eGFR add significant prognostic value to creatinine (area under the curve 0.529 to 0.581 $P = 0.02$).[95] In patients with acute HF, with a high mortality rate (at least 25% in 1 year), cystatin C was evaluated besides other markers of renal function. Cystatin C above median (1.30 mg/L) was associated with the highest adjusted HR, 3.2 (95% CI 2.0–5.3; $P < 0.0001$). Mortality increased significantly with each tertile of cystatin C. Moreover, in patients with normal plasma creatinine, elevated cystatin C was associated with significantly higher mortality at 12 months (40.4% compared to 12.6%, those with both normal creatinine and cystatin C; $P < 0.0001$).[96]

Proposal for a clinical algorithm/trial update

Currently only trials devoted to evaluate cystatin C and AKI or hypertension are being conducted, and there are no trials with a focus on HF.

Soluble ST2, galectin-3 and growth differentiation factor-15

Soluble ST2, galectin-3 and GDF-15 are markers that are associated with inflammatory disease and fibrosis. Inflammation and fibrosis are hallmarks of the HF syndrome, therefore, these markers may play an important role in the natural history of HF. Plasma concentrations of sST2, galectin-3 and GDF-15 are all prognostic in HF. However, recent studies highlighted that not the heart, but non-cardiac tissues seem to be the dominant source of these proteins in plasma.[97,98] The major source of all these three markers is currently not fully established.

ST2

ST2L is a membrane bound receptor for which IL-33 is the functional ligand. IL-33/ST2L signalling leads to inflammatory gene transcription which results in the production of inflammatory cytokines/chemokines and induction of immune response. Depending on several co-stimulatory factors, IL-33 can either act as a pro- or anti-inflammatory cytokine. In the context of the heart and HF, IL-33 is considered to exert cardioprotective effects. sST2, a soluble truncated form of ST2L, is secreted into the circulation and functions as a decoy receptor for IL-33, inhibiting the effects of IL-33/ST2L signalling. Thus, increased concentrations of sST2 in the circulation attenuate the systemic biologic effects of IL-33.

Though mRNA transcript for ST2 is detected in cardiomyocytes under mechanical strain, no clear trans-cardiac gradient of sST2 has been found. Recent data have implicated the lungs as a relevant source of sST2, specifically related to alveolar epithelial production by type II pneumocytes.[99] This may help to understand how sST2 correlates with presence and severity of pulmonary congestion in HF, but also with severe inflammatory pulmonary conditions such as acute respiratory distress syndrome.[100]

Galectin-3

Galectin-3 is an unique member of chimera type galectins and is involved in a large number of disease processes. It is known to specifically bind β-galactosides. It plays an important role not only in cancer but also in inflammation. In this context, galectin-3 can be viewed as a regulatory protein acting at several stages along the continuum from acute to chronic inflammation and in tissue fibrinogenesis. Indeed, the involvement of galectin-3 in various 'inflammatory/fibrotic' conditions such as arthritis, asthma, pneumonia, atherosclerosis, and kidney disease has been described. Even in the pathophysiology of HF, galectin-3 plays a biological role through inflammation and fibrosis.[101–103]

Growth differentiation factor-15

GDF-15 (also known as serum macrophage inhibitory cytokine-1) is a protein belonging to the transforming growth factor beta (TGF-β) superfamily. It has a role in regulating inflammatory and apoptotic pathways in acute and chronic tissue injury. The expression of GDF-15 is upregulated in many pathologies, including inflammation, cancer, cardiovascular disease, pulmonary disease, diabetes, and renal disease.[104–106] Plasma concentrations of GDF-15 are believed to reflect these stressors and their role in disease progression and prognosis. Notably, the GDF-15 receptor, its downstream signalling pathways and biological actions are poorly defined.

Analytical considerations

In nearly all published studies on HF patients, sST2, galectin-3 and GDF-15 plasma concentrations have been measured with immunoassays.[107–109] Certain assays can be performed fully automated on high throughput analysers in a central laboratory, while for sST2, there is a quantitative point-of-care assay available.[110] Results from different methods are unfortunately not always directly comparable. This is applicable especially for the sST2 assays where a large bias between the methods can be observed. To a lesser extent, this also applies to galectin-3 and GDF-15 assays where apparent plasma concentrations may vary between assays. Another important variable to consider is the variation within a subject over time. Meijers *et al.*[9] have provided an overview of the biological variation indices of all these three markers. GDF-15 exhibited substantial variation over time, whereas ST2 and galectin-3 were more stable.

Diagnostic studies

The three biomarkers sST2, galectin-3, and GDF-15 are not specific for a distinct medical condition but are general markers of disease risk/severity and mortality, as also observed in a recent pre-clinical study.[97] Because increased plasma concentrations of sST2, galectin-3, and GDF-15 are neither specific for a distinct disease entity, nor cardiac specific, the three biomarkers are of no value for diagnostic purposes.[111]

Prognostic studies

There is, however, strong evidence that plasma concentrations of these three analytes provide prognostic information in HF patients independently of, and additive to, other established markers such as cardiac troponins or natriuretic peptides (*Figure 2*).[105,112–119] In contrast to GDF-15, the biomarkers sST2 and galectin-3 have been included in the 2017 ACC/AHA guideline for additive risk stratification in patients with acute and chronic HF.[120] In contrast, the 2016 ESC HF guidelines do not recommend the use of these three biomarkers in clinical practice.[1]

ST2

Soluble ST2 measurements have shown, both in chronic and acute HF, to be of prognostic value. This is further strengthened by two meta-analyses.[112,113] Higher concentrations of sST2 were predictive of new-onset HF in the Framingham Offspring Cohort, and did so even in the presence of BNP and hs-cTnI results.[31] In those with chronic HF, sST2, together with natriuretic peptides and cardiac

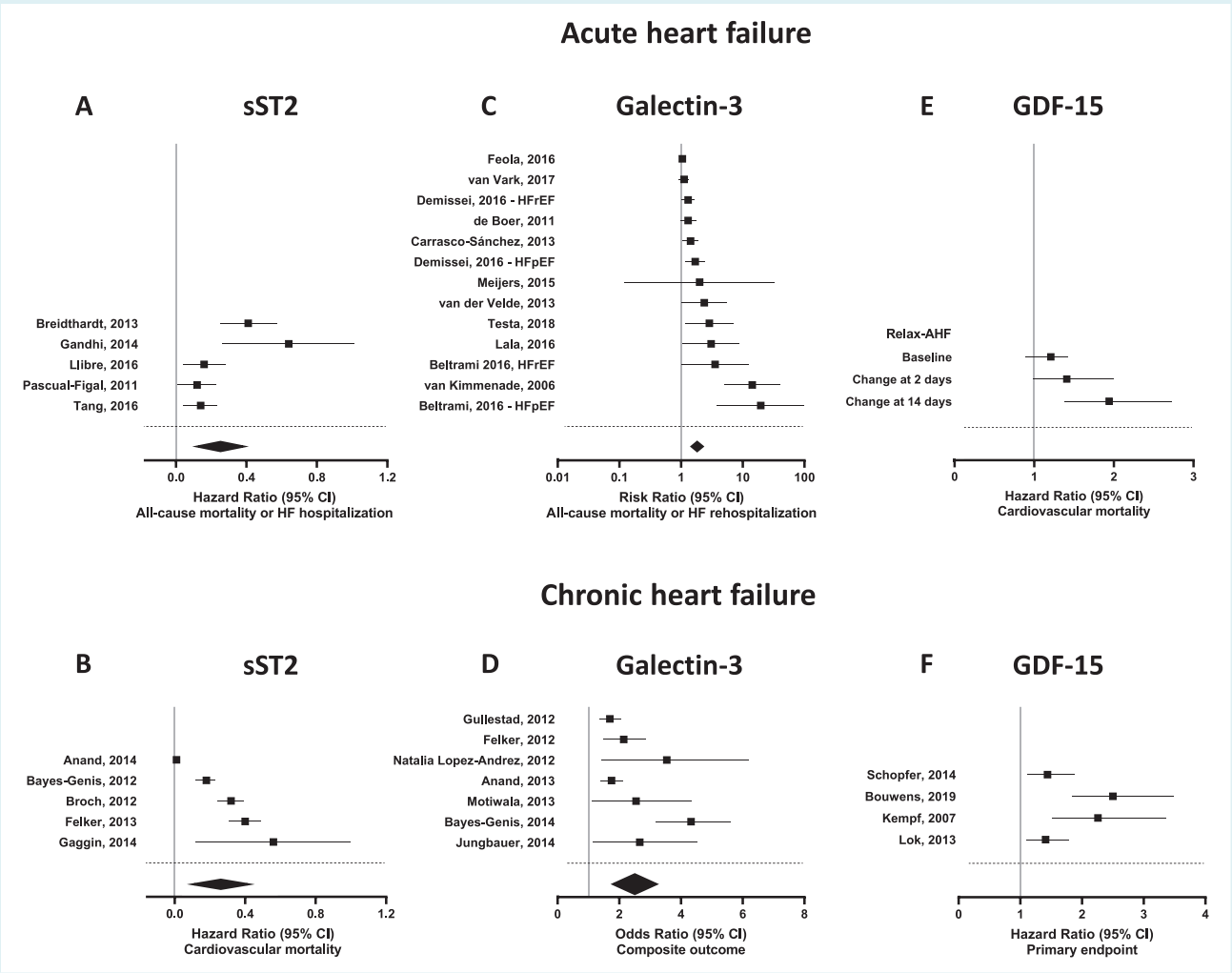


Figure 2 Performance of soluble suppression of tumorigenicity 2 (sST2), galectin-3 and growth differentiation factor-15 (GDF-15) in both acute and chronic heart failure (HF). (*A*) Meta-analysis of sST2 and prognosis in acute HF.[112] (*B*) Meta-analysis of sST2 and prognosis in chronic HF.[113] (*C*) Meta-analysis of galectin-3 and outcome in acute HF.[114] (*D*) Meta-analysis of galectin-3 and outcome in chronic HF.[115] (*E*) GDF-15 performance regarding 180-day cardiovascular mortality in acute HF.[116] (*F*) GDF-15 and outcome in chronic HF.[105,117–119] CI, confidence interval; HFpEF, heart failure with preserved ejection fraction; HFrEF, heart failure with reduced ejection fraction.

troponin, constitute the triad of biomarkers with predictive value in the Barcelona Bio-HF calculator (www.BCNbioHFcalculator.org), a risk score that calculates the risk of all-cause death and/or HF hospitalization at the individual level.[121] Furthermore, sST2 demonstrates predictive value in elderly patients with HF with preserved ejection fraction (HFpEF).[122] Another strength may lie in repeated measurements as shown in the TRIUMPH (Translational Initiative on Unique and novel strategies for Management of Patients with Heart failure) trial, wherein serial sST2 values during follow-up predicted the composite endpoint of all-cause mortality or readmission for HF.[123] Further, sST2 predicted sudden death in a nested case-control study of ambulatory patients with HFrEF.[124]

The relevance of sST2 in clinical decision-making remains limited. In patients with post-infarction ventricular dysfunction, a post-hoc analysis from the EPHESUS (Effects of Eplerenone on Left Ventricular Remodelling Following Heart Attack) trial showed that patients with low sST2 had less adverse left ventricular remodelling, regardless of the treatment arm.[125] In patients with chronic HF, sST2 plasma concentrations dropped for each upward titration of the beta-blocker dose.[126] Lastly, benefit of mineralocorticoid receptor antagonism appeared particularly significant among those with elevated sST2 in HF and those post-MI.[127] In the recent STADE-HF (sST2 As a help for management of Diagnosis, Evaluation and management of HF) trial, use of sST2 to guide therapy at day 4 after admission did not reduce readmissions.[128]

Galectin-3

The prognostic utility of galectin-3 measurements in HF has been demonstrated in several cohorts.[129,130] In the Framingham Offspring Cohort and PREVEND (Prevention of Renal and Vascular End-Stage Disease) study, galectin-3 was associated with an

increased risk for new-onset HF and all-cause mortality after adjustment for natriuretic peptides and several other clinical variables.[131,132] This was further strengthened by a meta-analysis in over 30 000 subjects demonstrating that galectin-3 added prognostic value for new-onset HF.[133] A study with 180 ambulatory patients with HF examined serial galectin-3 measurements and concluded that, in multivariable models adjusted for natriuretic peptides, cardiac troponin, and clinical variables, serial galectin-3 did not reclassify patients into higher risk groups.[134] However, another much larger study [CORONA (Controlled Rosuvastatin Multinational Trial in Heart Failure), $n = 1329$] with external validation [COACH (Coordinating Study Evaluating Outcomes of Advising and Counseling Failure), $n = 324$] demonstrated that in both acute and chronic HF, galectin-3 increases >15% over time were prognostic.[135] Furthermore, a pooled analysis of three trials in acute HF demonstrated robust prognostic value for near-term rehospitalization if galectin-3 was >17.8 ng/mL, with a large number of patients correctly reclassified.[130] Galectin-3 appears valuable in prognosticating patients with HFpEF; indeed, galectin-3 was found to be the most accurate risk predictor of adverse events within 5 years in patients with HFpEF.[136] In addition, galectin-3 is able to identify HF patients at low risk for adverse events.[8] No interactions between HF therapies and galectin-3 have yet been identified.[137,138] The HOMAGE (Heart OMics in AGing), revealed that spironolactone did not decrease procollagen type III N-terminal propeptide (PIIINP), nor was its effect on PIIINP modified by galectin-3.[139] However, pathological myocardial fibrosis is characterized by accumulation of Type-I collagen, which are large-diameter fibres with a high propensity for cross-linking that make a substantial contribution to myocardial stiffness compared with the finer type-III collagen fibres.[140] Spironolactone reduced procollagen type III N-terminal peptide (PINP) and increased collagen type-1 C-terminal telopeptide (CITP), suggesting reduced deposition and increased degradation of type-1 collagen.[141]

Growth differentiation factor-15

GDF-15 plasma concentrations in HF are independently associated with all-cause-mortality and composite endpoints of death and HF events in both acute, including cardiogenic shock, and chronic HF as demonstrated in the RELAX-AHF (RELAXin in Acute Heart Failure), HF-ACTION (Heart Failure: A Controlled Trial Investigating Outcomes of Exercise Training) and PARADIGM-HF (Prospective Comparison of ARNI with ACEi to Determine the Impact on Global Mortality and Morbidity in Heart Failure) trials.[108,142–145] Although pharmacological treatments reducing circulating GDF-15 concentrations in HF remain elusive, left ventricular assist device implantation can lead to a significant decrease of circulating GDF-15 in patients with advanced HF,[146,147] showing that even large increases in GDF-15 are to some extent reversible by potentially life-saving therapeutic intervention. GDF-15 has also been explored in patients with right-sided HF. In acute pulmonary embolism for example, GDF-15, but not natriuretic peptides, enhanced the prognostic information provided by an echocardiographic assessment of right ventricular function.[148] Further, in patients with idiopathic pulmonary arterial hypertension, GDF-15 added prognostic information to haemodynamic variables and natriuretic peptides regarding the long-term risk of death or lung transplantation.[149]

Proposal for a clinical algorithm/trial update

ST2. The potential role of sST2 in clinical practice lies with the ability to change with beta-blocker and mineralocorticoid receptor antagonist therapy. An interesting ongoing study investigates the effect of ramipril in suppressing ST2 expression in rheumatic mitral stenosis patients (NCT03991910). Another study focusses on the prognostic value of sST2 in cardiothoracic surgery patients. This study aims to investigate the release of sST2 and its association with cardiovascular events (mortality and HF). The underlying aetiology of the disease is not pre-defined (NCT04460131).

Galectin-3. Currently, multiple studies investigate the modality of using anti-galectin-3 therapy. These studies are currently conducted in other fibrotic diseases, but might be of interest in the future to cardiovascular patients.

Growth differentiation factor-15. The STRONG-HF (Safety, Tolerability and efficacy of Rapid Optimization, helped by NT-proBNP and GDF-15, of Heart Failure therapies) trial is a multicentre, randomized, parallel group trial designed to evaluate the efficacy and safety of up-titration of standard of care medical therapy including beta-blockers, angiotensin-converting enzyme inhibitors, angiotensin receptor blocker or angiotensin receptor–neprilysin inhibitor, and mineralocorticoid receptor antagonist, on morbidity and mortality when initiated and up-titrated early during hospitalization for acute HF.[150] In the treatment arm of this study, repeated assessments of clinical routine data as well as biomarkers including natriuretic peptides and GDF-15 will foster, encourage and ensure the safety of the optimization of oral HF therapies. This will further expedite the clinical utility of biomarkers.

Cluster of differentiation 146, soluble neprilysin and adrenomedullin

Soluble CD146

CD146 (also named MCAM, MUC18) is a component of endothelial junction primarily expressed in endothelial cells and involved in the control of cell and tissue architecture, in cell signalling and more recently described in angiogenesis.

Various studies indicate that whereas natriuretic peptides are released from the heart in acute HF, sCD146 is released from congested vessels. Indeed, no differences were seen between levels of sCD146 in peripheral venous blood and in blood from the coronary sinus obtained simultaneously, suggesting a predominant extracardiac source of sCD146 in HF patients. This was confirmed by an experimental model of venous congestion (increased venous pressure in an arm via tourniquet) in HF patients showing that sCD146 was rapidly and markedly increased in the congested arm compared to the other arm.[151] Other studies showed that circulating sCD146 was positively correlated with the size of inferior vena cava in acute dyspnoeic patients[152] and to overhydration in dialyzed patients.[153]

Various studies indicate that whereas natriuretic peptides are released from the heart in acute HF, sCD146 is released from congested vessels for example by the stretch of pulmonary veins.

Neprilysin, a key path in heart failure

Neprilysin is responsible for the degradation of natriuretic peptides and other substances (e.g. angiotensin I, angiotensin II, bradykinin, endothelin-1, substance P).[154] Neprilysin recently became topical with the demonstration of the superiority of sacubitril/valsartan, a combination of a neprilysin inhibitor sacubitril and the angiotensin receptor blocker valsartan, to enalapril with respect to survival in PARADIGM-HF.[155] Benefits of sacubitril/valsartan have been demonstrated in HF patients with reduced left ventricular ejection fraction with mild-to-moderate symptoms. Furthermore, the PIONEER-HF (Comparison of Sacubitril-Valsartan versus Enalapril on Effect on NT-proBNP in Patients Stabilized from an Acute Heart Failure Episode) study demonstrated that the initiation of sacubitril/valsartan after haemodynamic stabilization in an acute HF hospitalization setting led to a greater reduction in NT-proBNP concentration than enalapril therapy; evident within a week of initiating therapy.[156] Reduction of NT-proBNP with introduction of sacubitril/valsartan was also observed in ambulatory patients.[157] With respect to mechanisms, in the EVALUATE-HF (Effects of Sacubitril/Valsartan vs. Enalapril on Aortic Stiffness in Patients With Mild to Moderate HF With Reduced Ejection Fraction) trial, sacubitril/valsartan did not result in a reduced central aortic stiffness, when compared to enalapril.[158] In patients with preserved left ventricular ejection fraction participating in the PARAGON-HF (Prospective Comparison of ARNI with ARB Global Outcomes in HF with Preserved Ejection Fraction) study, there was a modest, although statistically non-significant, lower rate of hospitalizations for HF with sacubitril/valsartan than with valsartan and no significant difference in the risk of death from cardiovascular causes.[159] Extracellular matrix haemostasis is altered by sacubitril/valsartan as depicted in *Figure 3*.[160]

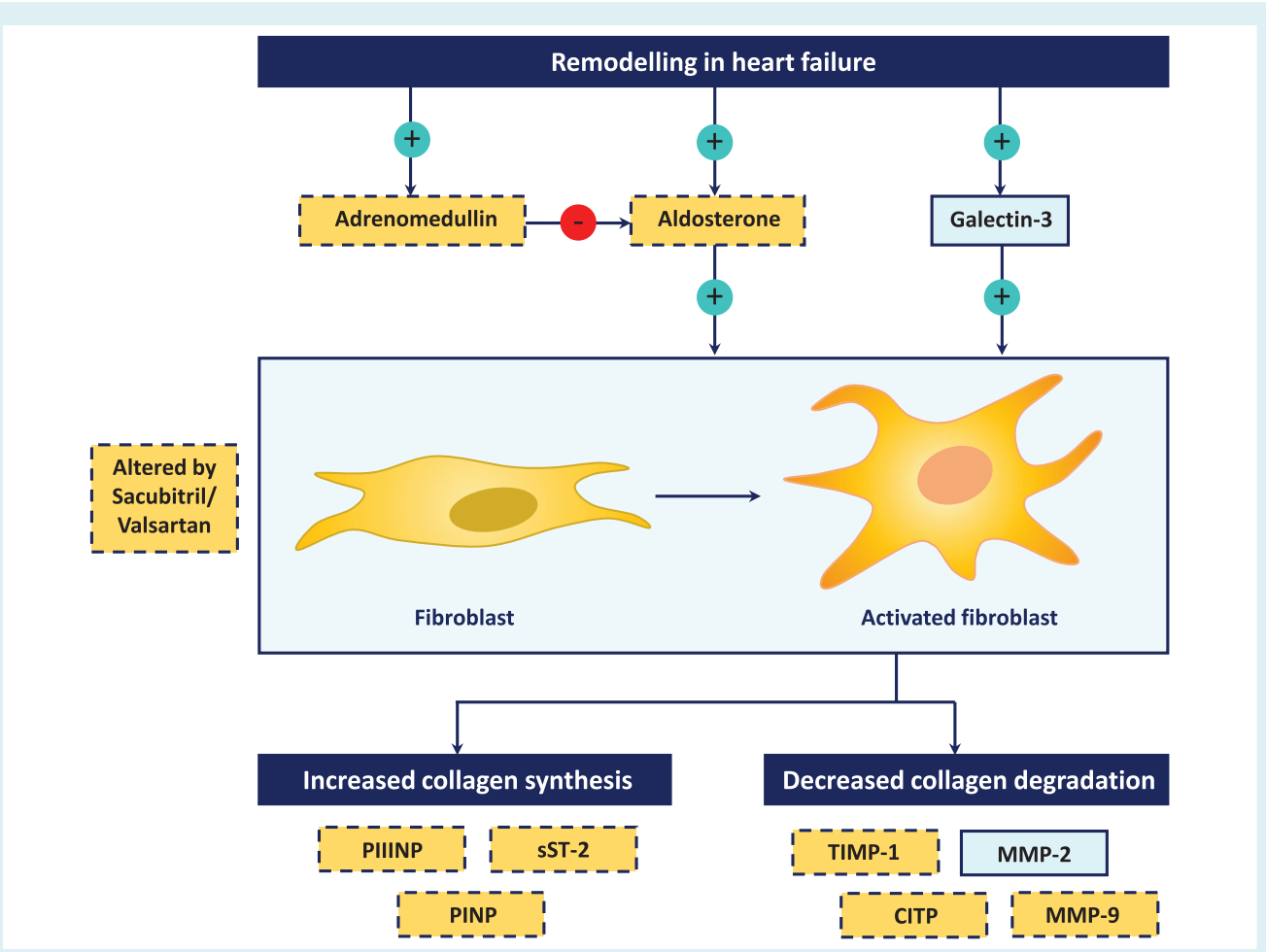


Figure 3 Effect of sacubitril/valsartan on fibrotic markers in heart failure. Changes in sacubitril/valsartan-treated patients are indicated by yellow dashed boxes. Adapated from Zile *et al.*[160] and Cunninghame *et al.*[161] CITP, collagen type-1 C-terminal telopeptide; MMP, matrix metalloproteinase; PINP, procollagen type I N-terminal propeptide; PIIINP, procollagen type III N-terminal propeptide; sST-2, soluble suppression of tumorigenicity 2; TIMP-1, tissue inhibitor of metalloproteinase type 1

Adrenomedullin

Adrenomedullin is a hormone first discovered in pheochromocytoma, hence the name. It is synthesized in many cells and tissues, especially in vascular smooth muscle and endothelial cells and is metabolized by neprilysin (*Figure 3*). The main function of ADM is vasodilatation, resulting in decreased blood pressure and increased vascular flow. A second mechanism of ADM is preserving the integrity of the endothelial layer. Lack of ADM results in vascular leakage and systemic and pulmonary oedema.[162,163] The latter mechanism is important in sepsis. A prospective observational study in severe sepsis and septic shock patients demonstrated the relationship between bioactive ADM (bio-ADM) and disease severity scores, such as the Sequential Organ Failure Assessment (SOFA) score.[164] ADM levels were able to predict sepsis-related organ dysfunction 24 h before onset. This predictive role can be considered as being equivalent to a diagnostic marker for sepsis.[165] Bio-ADM has been identified in several studies as a prognostic marker for the prediction of mortality in sepsis and septic shock patients.[164,166,167]

Bio-ADM can also be considered as a target for therapy. Exogenous administration of ADM appears to be protective in animal models of acute respiratory distress syndrome.[168] In contrast, inhibition of ADM results in attenuation of sepsis-induced multi-organ failure in several murine models.[169–171] Below we will discuss adrecizumab, a humanized monoclonal anti-ADM antibody which is currently investigated in a clinical trial.

Analytical considerations

The definitive assessment of neprilysin as a marker is complicated by the lack of a fully validated assay.[154] Few studies further showed that elevated soluble neprilysin predicts increased risk of recurrent all-cause, cardiovascular, and acute HF admissions in ambulatory patients with HF.[172] In a recent study in dialysis patients, circulating neprilysin activity (but not concentration) and GDF-15 provided incremental diagnostic information over clinical covariates and NT-proBNP in diagnosing HF.[173]

Not many studies in HF with sCD146 have been reported. However, those published describe a Biocytex ELISA with a detection limit of 10 μg/L and coefficients of variation for both repeatability and reproducibility ≤20%. The only assay to measure bio-ADM is a sandwich immunoassay developed by Sphingotec GmbH (Hennigsdorf, Germany). The interassay precision is described as 20% or lower. The limit of quantification was 11 pg/mL. The detection limit of the assay was determined at 3 pg/mL.[174] In 2020, a point-of-care test for bio-ADM was launched by the Sphingotec GmbH. Information about this assay is scarce, but the company brochure describes the following: time to result 20 min; measuring range 45–500 pg/mL, limit of detection 45 pg/mL and an interassay coefficient of variation <16.8%.

Diagnostic and prognostic studies

Plasma CD146 was found to have similar diagnostic properties to natriuretic peptides for discriminating acute HF from non-cardiac causes among patients presenting with acute dyspnoea.[175] In acute HF patients, the greater the degree of mitral regurgitation, the greater the level of sCD146.[176] Furthermore, in patients with mitral stenosis, sCD146 is markedly elevated and drops within few hours of mitral angioplasty, although natriuretic peptides remained unchanged.[177] This might imply sCD146 may be a biomarker of congestion, released by a stretched venous endothelium. However, far more data are needed before this assertion may be confirmed. Bio-ADM is another endothelial marker of congestion.[178]

The heart is likely the source of soluble neprilysin in HF patients.[179] Indeed, it was recently described that the removal of failing ventricles and the implantation of a total artificial heart led to a marked decrease in circulating natriuretic peptides and in soluble neprilysin concentration and activity, both partially recovering afterward.[179] These data, in conjunction with a positive trans-cardiac gradient and a neutral trans-pulmonary gradient observed in patients with impaired cardiac systolic function, indicate the heart as a source of soluble neprilysin in HF.

Bio-ADM was measured in 246 patients with acute HF admitted to the emergency department. Within 30 days of admission, 34.6% of the patients met the primary composite endpoint, which consisted of all-cause death, cardiac arrest with resuscitation, respiratory failure, emergency dialysis, acute coronary syndrome, hospitalization >5 days, and repeat emergency department visit or hospitalization. Patients who experienced the primary outcome showed higher plasma bio-ADM levels compared to patients who did not. Additionally, bio-ADM demonstrated predictive value for the composite endpoint, even after multivariable adjustment (odds ratio 2.68; 95% CI 1.60–4.51).[180] A recent analysis in patients with new-onset HF and worsening HF ($n = 2179$) strengthened the observation that bio-ADM is closely linked to congestion, and demonstrated that it was independently associated with an increased risk of all-cause mortality and HF hospitalization (HR 1.16; 95% CI 1.06–1.27; $P = 0.002$). These data were validated in a separate cohort of 1703 subjects.[178] Another study investigating 927 patients who developed HF symptoms after acute coronary syndrome demonstrated elevated bio-ADM levels compared to those who did not develop HF, which underpins the association of bio-ADM with signs of congestion.[181]

Importance of pre-discharge measurements of bio-ADM was recently investigated in a post-hoc analysis of the PROTECT (Placebo-Controlled Randomized Study of the Selective A1 Adenosine Receptor Antagonist Rolofylline for Patients Hospitalized With Acute Decompensated Heart Failure and Volume Overload to Assess Treatment Effect on Congestion and Renal Function) trial ($n = 1236$). Patients with higher bio-ADM levels at discharge were hospitalized longer, had higher natriuretic peptide levels, and poorer diuretic response (all $P < 0.001$). High discharge bio-ADM levels combined with higher use of loop diuretics were independently associated with a greater risk of 60-day HF rehospitalization (HR 4.02; 95% CI 2.23–7.26; $P < 0.001$). Assessment of bio-ADM levels at discharge may be a readily applicable marker to identify patients with residual congestion and higher risk of early hospital readmission.[182]

Proposal for a clinical algorithm/trial update

No data exist regarding clinical use of sCD146 and studies evaluating its role for treatment decision-making are lacking.

There are several ongoing studies of neprilysin inhibition but most do not evaluate how soluble neprilysin might inform treatment decision-making with sacubitril/valsartan. PROVIDE-HF provided information regarding sacubitril/valsartan and the change from baseline to 12 weeks in the Kansas City Cardiomyopathy Questionnaire (KCCQ), a self-administered, 23-item questionnaire that quantifies physical limitations, symptoms, self-efficacy, social interference and quality of life in patients with HF.[183] The mean KCCQ change at 12 weeks was not statistically significant following adjustment, but sacubitril/valsartan initiation was associated with early improvements in QOL. The CNEPi (Circulating NEP and NEP Inhibition Study in Heart Failure With Preserved Ejection Fraction, NCT03506412) trial will evaluate effect of therapy in those with either 'high' or 'low' neprilysin concentrations.

An open, standard therapy controlled clinical trial using a single intravenous infusion of adrecizumab, an anti-ADM antibody, is being conducted in patients hospitalized for acute HF (NCT04252937). This study will serve as a safety trial for adrecizumab in acute HF, using a dose escalating design. This phase 2 safety and proof-of-concept study aims to enhance the plasma concentration of bio-ADM in the circulation to restore and stabilize vascular integrity and function in patients with acute HF after initial stabilization with the current standard of care.

Metabolic markers

In this review, we address several biomarkers that might be implemented in clinical algorithms in the foreseen future. However, several biomarker niches like iron deficiency, thyroid (dys)function and thiamin deficiency have not been addressed.

Briefly, iron deficiency, a common nutritional disorder accounts for approximately one-half of anaemia cases.[184] The diagnosis of iron deficiency and anaemia is confirmed by the findings of low iron stores (ferritin <100 ng/mL or ferritin 100–300 ng/mL and transferrin saturation <20%) and a haemoglobin level two standard deviations below normal (based per gender).[185] In patients with HF, iron deficiency (~50%) and anaemia (~35%) are highly prevalent. Iron deficiency is associated with poor outcome in HF patients.[186] Another review in this Journal describes the practical guidance regarding screening, diagnosis and treatment of iron deficiency in patients with HF based upon the ESC HF guidelines.[187]

Physicians encounter thyroid dysfunction frequently. Hypothyroidism is a known risk factor for CAD, and on the other hand hyperthyroidism can cause atrial fibrillation (AF). Cardiac medication, including amiodarone, may precipitate a variety of thyroid disorders, and severe heart disease, such as left ventricular failure or acute MI.[188] Elderly subjects with a high-risk cardiovascular profile with either low or very high thyroid-stimulating hormone along with normal free T4 appear at increased risk of incident HF.[189] Furthermore, in HF patients subclinical hypothyroidism and isolated low T3 levels are associated with poor prognosis.[190] A recent review summarizes our current knowledge on thyroid and cardiac interaction.[191]

Thiamin deficiency has been reported to have a high incidence in HF patients. There is growing evidence that patients with HF have micronutrient deficiencies. Thiamin deficiency has been found to be more prevalent in HF patients than in the general population, with a prevalence reported to vary from 3% to 91% in different studies.[192,193] Supplementation has been argued as a possible treatment strategy. A recently published prospective randomized trial investigated thiamin supplements (200 mg vs. placebo) in eligible ambulatory patients with HFrEF for 6 months. Left ventricular ejection fraction, quality of life, or exercise capacity did not improve, despite increases in thiamin concentrations. These findings did not support routine thiamin supplementation in the treatment of HFrEF.[194]

Omics

Proteomics

Given the complexity of the HF syndrome, gaining a better understanding of the systemic and local responses to injury is a necessity. Omics phenotyping might provide a broad overview whereas a single biomarker can never fully encompass the complex pathophysiology of the HF syndrome.[195] Accordingly, proteomic approaches (measuring a spectrum of markers and analysing their connectivity through a more systems-biology approach) may add substantially greater information. Omics approaches potentially bridge the gap between function at the cell, tissue and plasma level. However, these techniques will not be implemented in daily clinical care, but will be used more often in the near future to discover new possible mechanisms and targets. Furthermore, it will help to determine unrecognized phenotypes within the HF syndrome.

Analytical considerations

Mass spectrometry (MS) is the gold standard for experimental proteomics but newer techniques utilizing apatmers and proximity extension assays have brought proteomics to a much wider user environment.[196] Recent developments in proteomic platforms allow the reliable detection of less abundant plasma proteins, which could lead to the discovery of new biomarker profiles in HF. There is a large body of literature underscoring the very important pre-analytical and analytical considerations in proteomic assays. An important aspect is analytical incompleteness, leading to multiple runs needed to get a full list of present proteins.[197–199] Innovation and advancement in this area are occurring in instrument sensitivity, increasing signal/noise and quantifying post-translational modifications on peptides. We believe to optimize and implement proteomic data, data storage, information integration, data robustness and standardization and unifying data repositories need to be improved.

Diagnostic and prognostic studies

As described above, the performance of proteomic profiling heavily relies on the methodology. For instance, a multiple reaction monitoring (MRM) MS assay for determination of cardiovascular

markers has been developed for 67 markers associated with cardiovascular disease with accuracy and sensitivity.[200] This demonstrates that MS might be a feasible tool to determine multiple markers reflecting varied pathological pathways (for example, cardiac stretch, inflammation, fibrosis) at once. Another study, using the MRM assay, validated 123 peptides corresponding to 73 proteins and identified 10 proteins with differential expression levels in the serum of patients with HFrEF compared to those with HFpEF.[201] MS in murine HF specimen detected 101 peptides (relating to 81 proteins) in plasma and 227 peptides (for 159 proteins) in cardiac tissue.[202]

A recent large genome-wide association study (GWAS) meta-analysis of HF comprising over 45 000 cases and nearly a million controls demonstrated that 12 independent variants at 11 genomic loci are associated with HF.[203] Shared genetic aetiology is hypothesized because these loci were associated with CAD, AF, or reduced left ventricular function. This was further validated by Mendelian randomization, which supported a causal role. This study is an example how this approach can extend our knowledge of the pathways underlying HF and may inform new therapeutic strategies.

Clearly, in patients it is easier to obtain blood samples compared to cardiac tissue samples, and as a result most biomarker studies relied upon serum or plasma for discovery and validation studies. It is important to realize that many differential regulated proteins will be from non-cardiac sources, such as kidney, vessels and liver. Another useful source for proteomics is urine. Two studies evaluated MS analysis on urine samples of HFrEF patients and identified 103 and 107 peptides, respectively, that were differentially excreted compared to urine samples of control subjects, independent of renal function.[204,205] A retrospective study in which the prognostic value of a defined urinary peptide panel was evaluated over a follow-up period of more than 2 years, demonstrated accurate prediction from asymptomatic diastolic dysfunction to clinical HF.[204]

The strength of proteomics clearly lies in discovery, and multiple discovery studies demonstrated this strength in identifying new proteins and peptides. For example, low levels of serum amyloid P measured in samples derived from the coronary sinus, were associated with progressive left ventricular diastolic dysfunction.[206] The same study also identified leucine-rich alpha-2-glycoprotein 1 (LRG1) as a biomarker that could better identify HF subjects compared with BNP. LRG1 seemed to be involved with fibrogenesis and angiogenesis by modulating TGF-β signalling.[207] As well, proteomics approaches may provide unique insights for understanding different severities of HF and how HF therapies such as left ventricular assist device placement or sacubitril/valsartan therapy affect the proteome after treatment.[208] This may therefore allow for understanding of new therapeutic targets for care of HF.

Proposal for a clinical algorithm/trial update

We used ClinicalTrials.gov to track the latest ongoing trials in the field of HF and used proteomics as a secondary search term. Two studies are of particular interest (NCT02141607 and NCT01677494). The first is a recently completed study that investigated molecular triggers of acute HF induced during shock, and it is anticipated the proteins starting the downward spiral in shock might be associated with a specific proteomic signature.

Proteomics could shed light on the pathophysiological mechanism behind the transition from shock to HF. An interesting aspect in the study design is the serial assessment of proteomics 3 months after the initial event. The researchers will determine which proteins are still influenced by the event and which have returned to baseline levels, all compared to control subjects. Another study investigated HFpEF patients with the aim to characterize markers of myocardial fibrosis. The work-up consisted of echocardiography (speckle tracking), magnetic resonance imaging (late-enhancement imaging), biology (markers of collagen turnover) and proteomics; the study has been terminated due to lack of recruitment.

The potential value of proteomics approaches in HF mainly lies in the identification of dysregulated proteins and protein networks. With ever improving platforms, undiscovered proteins and more subtle mechanisms and regulators in HF (development) might be uncovered. Further, better profiling of patients with HF will be a goal for the next decade. Proteomics is a promising approach to add clinical value beyond established biomarkers. Although complex statistical methodology is needed to derive accurate results, this biomarker/technique will likely not be implemented as a bedside tool in the near future.

Polygenic risk score

A polygenic risk score (PRS) is based on the variation in multiple genetic loci and their associated weights. Multiple GWAS have focused on SNP–phenotype associations, but with PRS the associations are multi-fold and the overall associations are much stronger. Therefore, PRS hold the promise of superior predictive performance compared to individual genome-wide significant hits, which underscores the fact that the trait (HF) examined is affected by many more genetic variants than only one.

Analytical considerations

First, the density of the array used in the GWAS, and the heterogeneity of the trait or disease being studied will affect the results. But most importantly, the statistical power to detect associations between variants in the DNA and a trait strongly depends on the sample size, as the millions of genetic variants will necessitate strict corrections for multiple testing.

Diagnostic and prognostic studies

A recent study in a population cohort aimed to identify those at risk for several diseases, including CAD and AF. A constructed PRS in this study identified 8.0% and 6.1% of the population at greater risk for CAD and AF, respectively. With respect to CAD, the PRS-associated prevalence was 20-fold higher than the explained prevalence due to rare monogenic mutations.[209] Another study focused on AF in patients with end-stage renal failure. The PRS, incorporating 13 SNPs, was significantly higher in those with AF compared to control subjects. Moreover, the association of this

PRS with AF was independent of classical risk factors. The disease model comprising three variables (i.e. PRS, age, and MI) covered nearly 10% of the phenotypic variability, and a post-hoc analysis demonstrated that it allowed for a proper classification of 75% of the cases.[210]

A recent study in over 300 000 subjects of the UK Biobank assessed the association of PRS in combination with lifestyle for the incidence of CAD, AF, stroke, hypertension, and type 2 diabetes. Subjects with a high PRS exhibited an increased risk of new-onset cardiovascular and diabetic events, independent of lifestyle. Within and across different PRS risk groups, adherence to poor lifestyle was associated with increased risk of cardiovascular disease and diabetes. This study demonstrates that genetic composition and lifestyle have an additive effect on the risk of developing disease and that the relative effects of poor lifestyle are comparable between PRS-based risk groups.[211]

Proposal for a clinical algorithm/trial update

We used ClinicalTrials.gov to determine which trials are currently being conducted in the field of HF with a special focus on GWAS and/or PRS, which resulted in four hits, of which two are currently recruiting patients. The first study, called HF-EXPRESS (Predictors of Heart Failure After ST-segment Elevation Myocardial Infarction, NCT02650934) investigates post-infarct cardiac repair processes on the basis of cellular, molecular and imaging techniques. The investigators will use GWAS to determine common varying genetic loci in order to anticipate whether these findings and its related pathways would be predictors of adverse remodelling after MI. Another study (NCT02347540) investigates the risk of HF in women with a history of pre-eclampsia. GWAS will be used to associate novel biomarkers of endothelial function and cardiac diastolic function and identify those at risk prior to the development of HF. These studies do not specifically mention PRS but given that PRS is preferred over simple GWAS analyses, PRS analyses likely will be performed.

PRS is a new promising method with superior predictive value compared to single GWAS hits. Implementation in daily practice might be readily feasible with lower prices to build a 'genetic passport', and will help to much better risk stratify patients into low-medium-high risk categories based upon their genetic upmake.

Conclusion/discussion

Several biomarkers have been developed and their diagnostic and prognostic performances reported. The gold standard markers are the natriuretic peptides and hs-cTn. Before widespread use of newer biomarkers in clinical care, either as diagnostic or prognostic markers, further research questions should be answered. It is currently not fully established how and why HF therapies and common confounding factors, such as age, sex, renal function, influence these markers, and what the optimal time of sampling is. In fact, the kinetics of even the best studied newer markers over time have not been properly defined in HF patients. Thus, to our opinion, there is a broad field for further studies to demonstrate that these markers reflect specific changes in disease severity or comorbidity, and such data will likely be essential for algorithms for

Table 3 Origin and pathway of heart failure-related biomarkers

Biomarker	Source of production	Pathophysiological pathway
Cardiac specific		
Natriuretic peptides	Cardiac myocytes	Volume overload, stretch
Cardiac troponin	Cardiac myocytes	Cardiomyocyte injury
Non-cardiac specific		
Procalcitonin	Parafollicular cells of the thyroid neuroendocrine cells of the lung and intestine	Infection
NGAL	Neutrophils, kidney, liver and epithelial cells	Acute kidney injury
KIM-1	Proximal tubular cells in kidneys	Tubular injury
Cystatin C	Eukaryote	Renal function
sST2	Mostly non-cardiac, suspected cardiac and lung cells	Fibrosis/inflammation
Galectin-3	Mostly non-cardiac, macrophages, fibroblasts	Fibrosis
GDF-15	Mostly non-cardiac, unknown	Inflammation
CD146	Endothelial cells	Vascular remodelling
Neprilysin	Kidney	Degradation of vaso-active peptides (natriuretic peptides, angiotensin II, bradykinin)
Adrenomedullin	All tissues and cell types	Vasodilatation, endothelial integrity
Omics		
Proteomics	–	Unbiased
Polygenic risk score	–	Cluster of unbiased results

CD146, cluster of differentiation 146; GDF-15, growth differentiation factor-15; KIM-1, kidney injury molecule-1; NGAL; neutrophil gelatinase-associated lipocalin; sST2, soluble suppression of tumorigenicity 2.

biomarker-guided therapy, beyond the routine biomarkers, such as cardiac troponins and natriuretic peptides.

The GUIDE-IT (Guiding Evidence Based Therapy Using Biomarker Intensified Treatment in Heart Failure) trial[212] is a great example of a well designed biomarker-guided study. However, the biomarker-guided approach should probably be changed towards a pre-set period of time in which the biomarker target level has to be reached well before the end of the study. For any future biomarker-guided study, information is necessary for what is an expected response in biomarker levels after a medication change, so as to be able to preview the difficulty of attaining the targets on time. The question for future trials is whether attaining a specific target or perhaps an expected target is more realistic.

Clearly, it is ultimately required that patients should benefit from biomarker measurements. To answer the question whether the measurement of biomarkers contributes to an improved outcome in HF, randomized studies with treatment arms should be performed, with treatment allocation made by physicians that are unaware (control) or aware of the biomarker result. If these studies prove that the addition of biomarker measurements improve patients' outcomes, the concept of using biomarkers for routine use in clinical practice will be strengthened.

Another task will be to investigate whether biomarker signalling pathways can be used for therapeutic approaches in humans with HF. However, because of the non-specificity of several pathways, such as 'inflammation' or 'metabolism', drug development and development of clinical scenarios will be very challenging based on such results. Also because HF is a layered complex disease, a single-marker strategy might not provide enough additional value, while a multiple biomarker approach likely will be needed to adequately reflect the full spectrum of the disease. It needs to be studied whether such a set of markers has a therapeutic implication, and whether it may be used in an easily deployed, cost-effective manner. As already stated, it is important to realize that many differential regulated proteins will be from non-cardiac sources. Furthermore, because the origin of these markers differ (*Table 3*), multiple imbalances need to be corrected.

Another hurdle to overcome and to better implement a new biomarker into clinical care is that the methodology of the assay should be optimized. Full automated routine clinical available assays are a necessity. Only for a limited number of the new generation HF biomarkers there is some kind of fully automated protocol (sST2, galectin-3, GDF-15) but some of those new generation HF biomarkers do not even have a Food and Drug Administration approval or a CE label. Although smaller point-of-care devices for in- and out-of-hospital use are interesting, unfortunately attempts within this area are not explored widely.

Lastly, consensus regarding a protocol on how to report biomarker studies across the different HF areas of interest should be defined. This consensus strategy may prevent bias, incomparable results, and will prompt more transparency, helping the field forward. For example, currently a wide variety exists of (regression) models to adjust the performance of a certain biomarker. Clearly, such guidelines may be implemented for diagnostic, prognostic, interventional, and monitoring studies. When implemented, these approaches will help the field of biomarkers to come of age.

Systematic reviews have been published for certain biomarkers that are included in this narrative review.[16,113,133,213] We believe the community is best served if the same occurs for emerging biomarkers that we have addressed in this review.

Supplementary Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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