

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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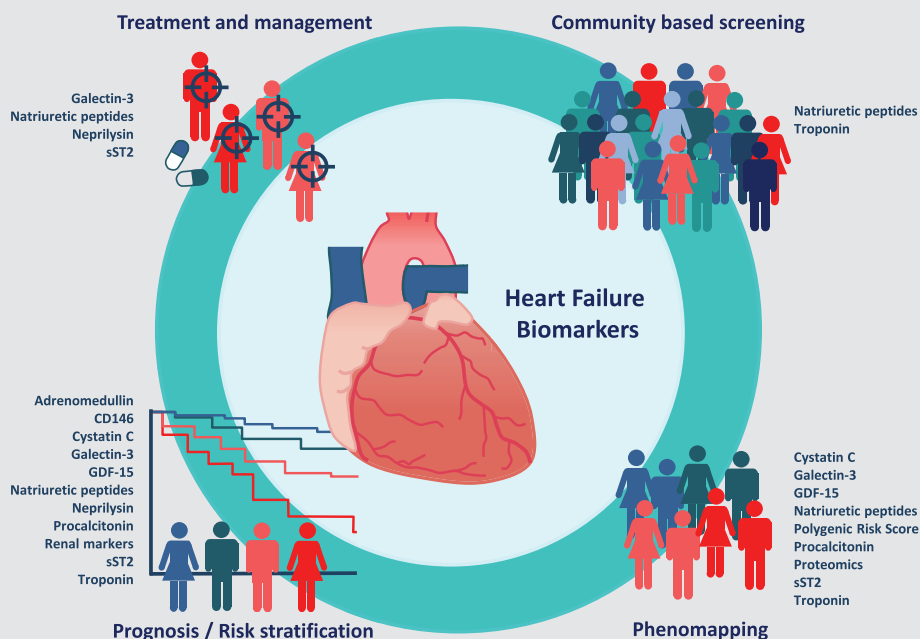
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New biomarkers are being evaluated for their ability to advance the management of patients with heart failure. Despite a large pool of interesting candidate biomarkers, besides natriuretic peptides virtually none have succeeded in being applied into 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 discharge⁶ 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*.

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 MI¹³ (*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

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.

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 variable²¹ and consider consensus definitions¹³ 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 ($\mu\text{g/L}$).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 $\leq 10\%$; (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}

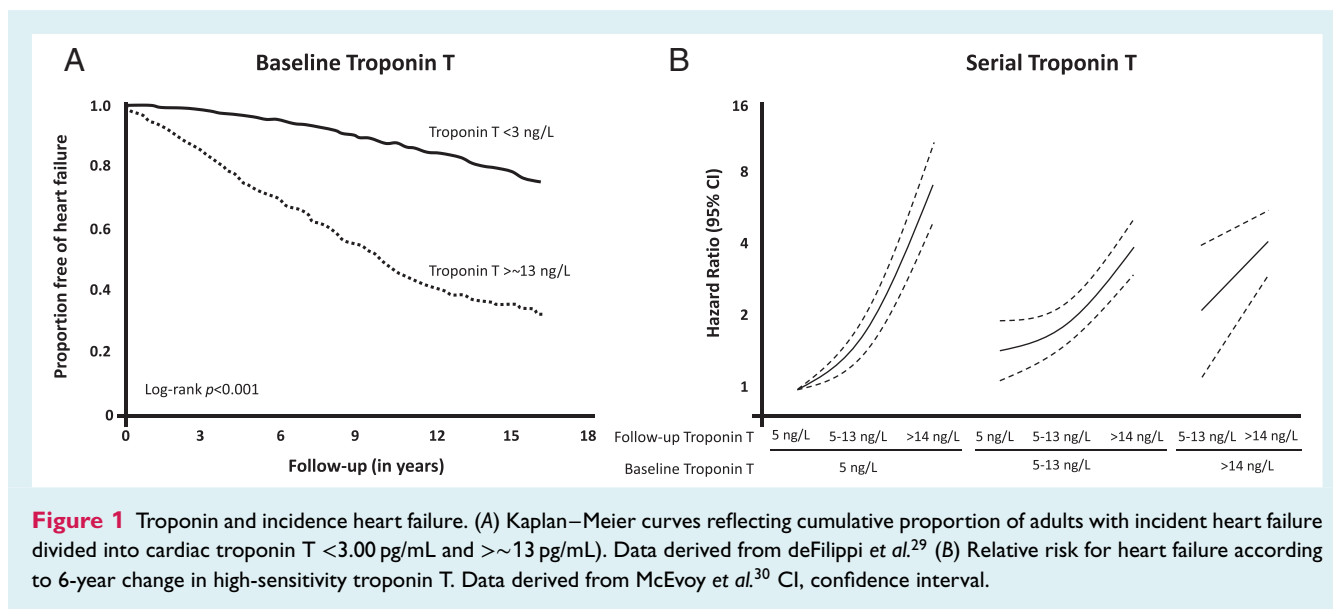
Diagnostic and prognostic studies

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



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) study⁴⁹ 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 ($\sim 25\%$) 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 m².^{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 μ mol/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 μ mol/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 a 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

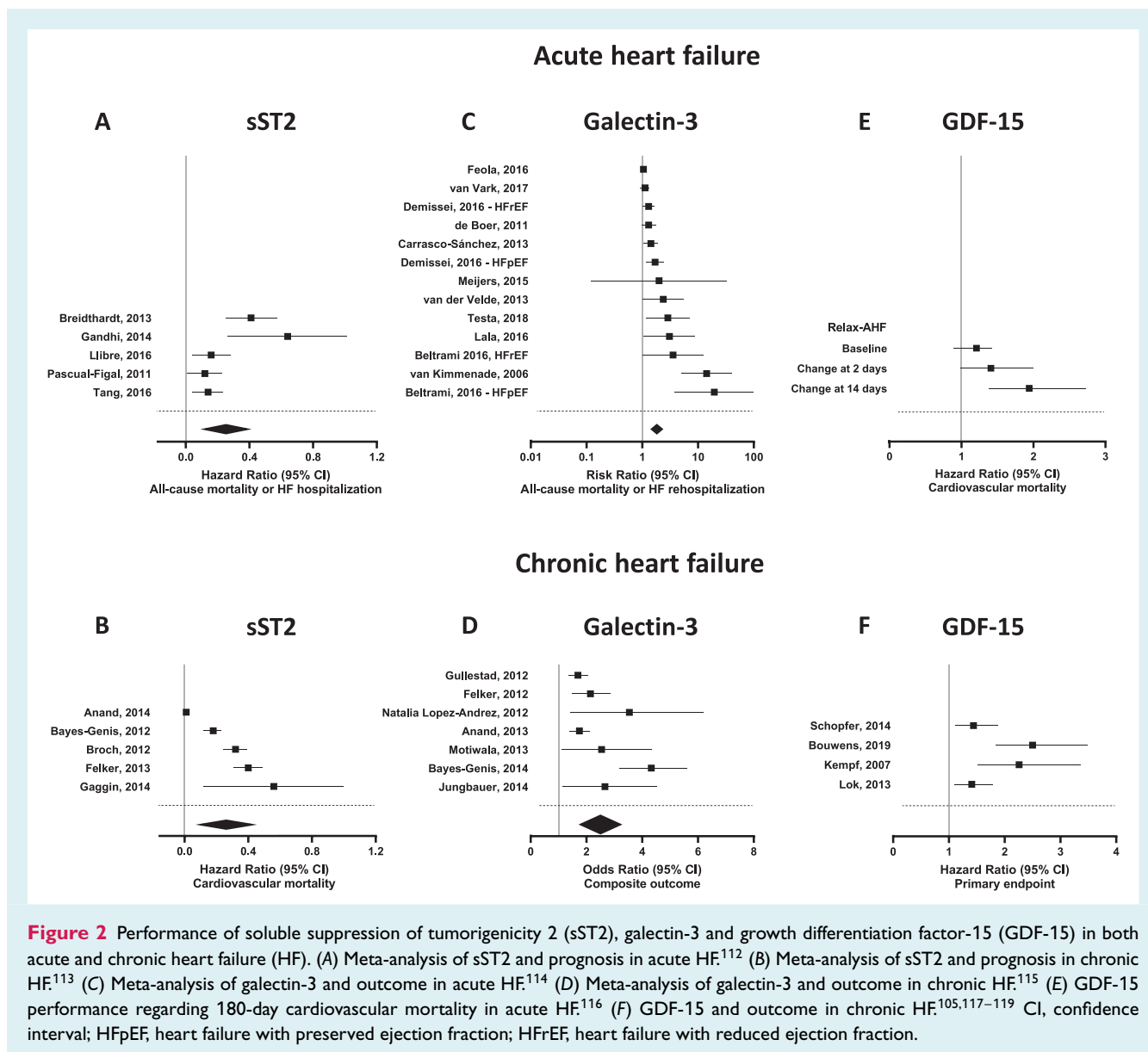
The three biomarkers sST2, galectin-3, and GDF-15 are not specific for a distinct medical condition but are general markers of disease risk/severity and mortality, as also observed in a recent pre-clinical study.⁹⁷ Because increased plasma concentrations of sST2, galectin-3, and GDF-15 are neither specific 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



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 EPHEBUS (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 PREVENTD (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 dyspnoic patients¹⁵² 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

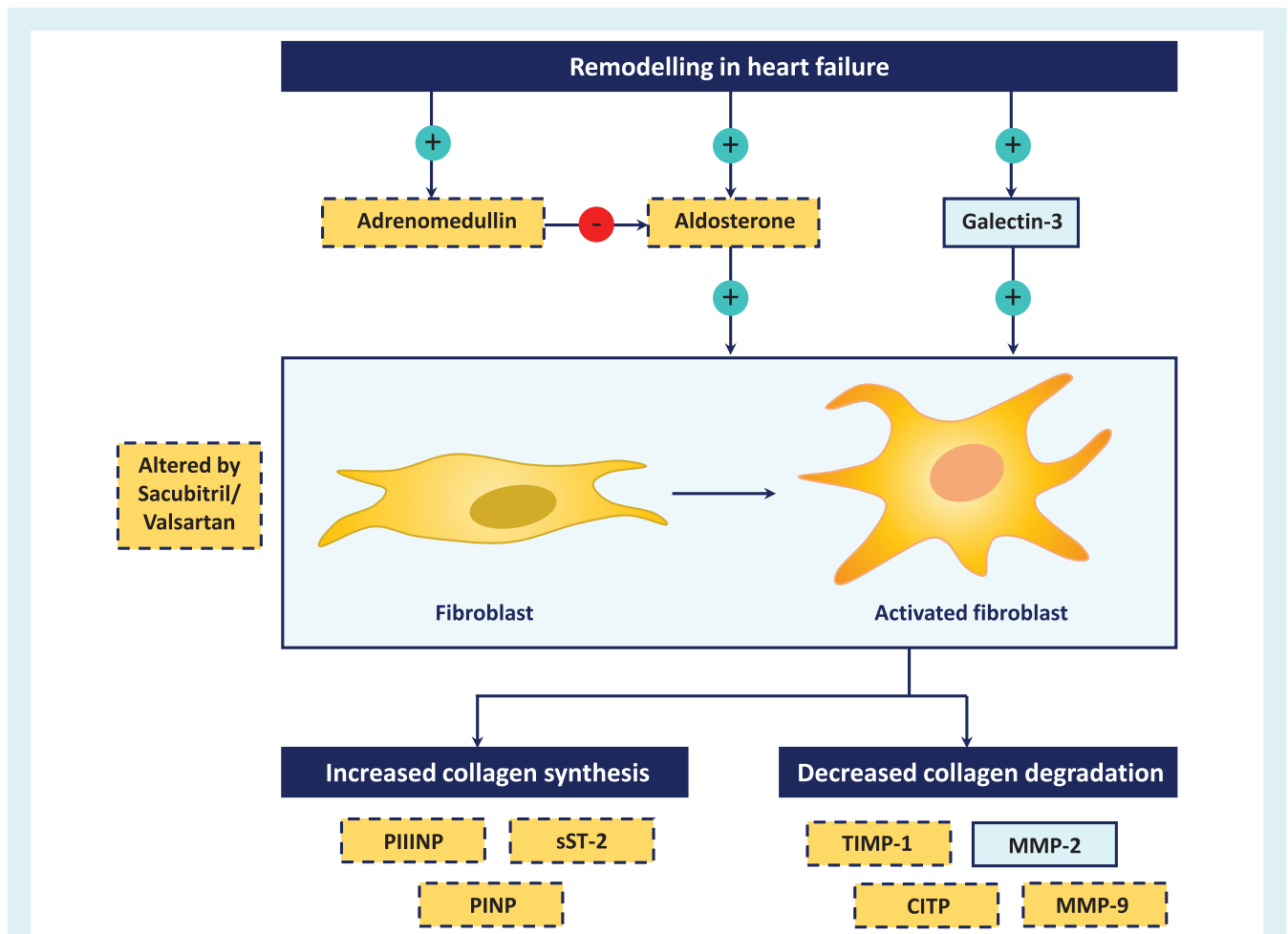


Figure 3 Effect of sacubitril/valsartan on fibrotic markers in heart failure. Changes in sacubitril/valsartan-treated patients are indicated by yellow dashed boxes. Adapted from Zile et al.¹⁶⁰ and Cunningham et al.¹⁶¹ CITP, collagen type-1 C-terminal telopeptide; MMP, matrix metalloproteinase; PINP, 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 adre-cizumab, 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 adreuzumab, an anti-ADM antibody, is being conducted in patients hospitalized for acute HF (NCT04252937). This study will serve as a safety trial for adreuzumab 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 aptamers 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
Nephrilysin	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) trial²¹² 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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References

- Ponikowski P, Voors AA, Anker SD, Bueno H, Cleland JGF, Coats AJS, Falk V, González-Juanatey JR, Harjola VP, Jankowska EA, Jessup M, Linde C, Nihoyannopoulos P, Parissis JT, Pieske B, Riley JP, Rosano GMC, Ruilope LM, Ruschitzka F, Rutten FH, van der Meer P; ESC Scientific Document Group. 2016 ESC guidelines for the diagnosis and treatment of acute and chronic heart failure: The Task Force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC). Developed with the special contribution of the Heart Failure Association (HFA) of the ESC. *Eur J Heart Fail* 2016;**18**:891–975.
- Yancy CW, Jessup M, Bozkurt B, Butler J, Casey DE, Drazner MH, Fonarow GC, Geraci SA, Horwich T, Januzzi JL, Johnson MR, Kasper EK, Levy WC, Masoudi FA, McBride PE, McMurray JJV, Mitchell JE, Peterson PN, Riegel B, Sam F, Stevenson LW, Tang WHW, Tsai EJ, Wilkoff BL; American College of Cardiology Foundation; American Heart Association Task Force on Practice Guidelines. 2013 ACCF/AHA guideline for the management of heart failure: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. *J Am Coll Cardiol* 2013;**62**:e147–e239.
- Miller WL, Hartman KA, Burritt MF, Grill DE, Rodeheffer RJ, Burnett JC, Jaffe AS. Serial biomarker measurements in ambulatory patients with chronic heart failure. *Circulation* 2007;**116**:249–257.
- Felker GM, Hasselblad V, Hernandez AF, O'Connor CM. Biomarker-guided therapy in chronic heart failure: a meta-analysis of randomized controlled trials. *Am Heart J* 2009;**158**:422–430.
- Maisel A, Hollander JE, Guss D, McCullough P, Nowak R, Green G, Saltzberg M, Ellison SR, Bhalla MA, Bhalla V, Clopton P, Jesse R; Rapid Emergency Department Heart Failure Outpatient Trial Investigators. Primary results of the Rapid Emergency Department Heart Failure Outpatient Trial (REDHOT). A multicenter study of B-type natriuretic peptide levels, emergency department decision making, and outcomes in patients presenting with shortness of breath. *J Am Coll Cardiol* 2004;**44**:1328–1333.
- Omar HR, Guglin M. Discharge BNP is a stronger predictor of 6-month mortality in acute heart failure compared with baseline BNP and admission-to-discharge percentage BNP reduction. *Int J Cardiol* 2016;**221**:1116–1122.
- Piek A, Meijers WC, Schrotten NF, Gansevoort RT, de Boer RA, Silljé HHW. HE4 serum levels are associated with heart failure severity in patients with chronic heart failure. *J Card Fail* 2017;**23**:12–19.
- Meijers WC, de Boer RA, van Veldhuisen DJ, Jaarsma T, Hillege HL, Maisel AS, Di Somma S, Voors AA, Peacock WF. Biomarkers and low risk in heart failure. Data from COACH and TRIUMPH. *Eur J Heart Fail* 2015;**17**:1271–1282.
- Meijers WC, van der Velde AR, Muller Kobold AC, Dijk-Brouwer J, Wu AH, Jaffe A, de Boer RA. Variability of biomarkers in patients with chronic heart failure and healthy controls. *Eur J Heart Fail* 2017;**19**:357–365.
- Harjola VP, Mullens W, Banaszewski M, Bauersachs J, Brunner-La Rocca HP, Chioncel O, Collins SP, Doehner W, Filippatos GS, Flammer AJ, Fuhrmann V, Lainscak M, Lassus J, Legrand M, Masip J, Mueller C, Papp Z, Parissis J, Platz E, Rudiger A, Ruschitzka F, Schäfer A, Seferovic PM, Skouri H, Yilmaz MB, Mebazaa A. Organ dysfunction, injury and failure in acute heart failure: from pathophysiology to diagnosis and management. A review on behalf of the Acute Heart Failure Committee of the Heart Failure Association (HFA) of the European Society of Cardiology (ESC). *Eur J Heart Fail* 2017;**19**:821–836.
- de Boer RA, Daniels LB, Maisel AS, Januzzi JL. State of the art: newer biomarkers in heart failure. *Eur J Heart Fail* 2015;**17**:559–569.
- Mueller C, McDonald K, de Boer RA, Maisel A, Cleland JGF, Kozhuharov N, Coats AJS, Metra M, Mebazaa A, Ruschitzka F, Lainscak M, Filippatos G, Seferovic PM, Meijers WC, Bayes-Genis A, Mueller T, Richards M, Januzzi JL; Heart Failure Association of the European Society of Cardiology. Heart Failure Association of the European Society of Cardiology practical guidance on the use of natriuretic peptide concentrations. *Eur J Heart Fail* 2019;**21**:715–731.
- Thygesen K, Alpert JS, Jaffe AS, Chaitman BR, Bax JJ, Morrow DA, White HD, Thygesen K, Alpert JS, Jaffe AS, Chaitman BR, Bax JJ, Morrow DA, White HD, Micley H, Crea F, Van de Werf F, Bucciarelli-Ducci C, Katus HA, Pinto FJ, Antman EM, Hamm CW, De Caterina R, Januzzi JL, Apple FS, Alonso Garcia MA, Underwood SR, Cauty JM, Lyon AR, Devereaux PJ. Fourth universal definition of myocardial infarction (2018). *Eur Heart J* 2019;**40**:237–269.
- Januzzi JL, Filippatos G, Nieminen M, Gheorghide M. Troponin elevation in patients with heart failure: on behalf of the Third Universal Definition Of Myocardial Infarction Global Task Force: Heart Failure Section. *Eur Heart J* 2012;**33**:2265–2271.
- Sandoval Y, Jaffe AS. Type 2 myocardial infarction: JACC review topic of the week. *J Am Coll Cardiol* 2019;**73**:1846–1860.
- Aimo A, Januzzi JL, Vergaro G, Ripoli A, Latini R, Masson S, Magnoli M, Anand IS, Cohn JN, Tavazzi L, Tognoni G, Graving J, Ueland T, Nymo SH, Brunner-La Rocca HP, Genis AB, Lupón J, de Boer RA, Yoshida A, Takeishi Y, Egstrup M, Gustafsson I, Gaggin HK, Eggers KM, Huber K, Tentzeris I, Tang WHW, Grodin J, Passino C, Erndin M. Prognostic value of high-sensitivity troponin T in chronic heart failure. *Circulation* 2018;**137**:286–297.
- Pascual-Figal DA, Casas T, Ordonez-Llanos J, Manzano-Fernández S, Bonaque JC, Boronat M, Muñoz-Esparza C, Valdés M, Januzzi JL. Highly sensitive troponin T for risk stratification of acutely destabilized heart failure. *Am Heart J* 2012;**163**:1002–1010.
- Weil BR, Suzuki G, Young RF, Iyer V, Cauty JM. Troponin release and reversible left ventricular dysfunction after transient pressure overload. *J Am Coll Cardiol* 2018;**71**:2906–2916.
- Feng J, Schaus BJ, Fallavollita JA, Lee TC, Cauty JM. Preload induces troponin I degradation independently of myocardial ischemia. *Circulation* 2001;**103**:2035–2037.
- Xue Y, Clopton P, Peacock WF, Maisel AS. Serial changes in high-sensitive troponin I predict outcome in patients with decompensated heart failure. *Eur J Heart Fail* 2011;**13**:37–42.
- Twerenbold R, Boeddinghaus J, Nestelberger T, Wildi K, Rubini Gimenez M, Badertscher P, Mueller C. Clinical use of high-sensitivity cardiac troponin in patients with suspected myocardial infarction. *J Am Coll Cardiol* 2017;**70**:996–1012.
- Mair J, Lindahl B, Hammarsten O, Müller C, Giannitsis E, Huber K, Möckel M, Plebani M, Thygesen K, Jaffe AS. How is cardiac troponin released from injured myocardium? *Eur Heart J Acute Cardiovasc Care* 2018;**7**:553–560.
- Thygesen K, Mair J, Giannitsis E, Mueller C, Lindahl B, Blankenberg S, Huber K, Plebani M, Biasucci LM, Tubaro M, Collinson P, Venge P, Hasin Y, Galvani M, Koenig W, Hamm C, Alpert JS, Katus H, Jaffe AS; Study Group on Biomarkers in Cardiology of ESC Working Group on Acute Cardiac Care. How to use high-sensitivity cardiac troponins in acute cardiac care. *Eur Heart J* 2012;**33**:2252–2257.
- Apple FS, Collinson PO; IFCC Task Force on Clinical Applications of Cardiac Biomarkers. Analytical characteristics of high-sensitivity cardiac troponin assays. *Clin Chem* 2012;**58**:54–61.
- Wu AHB, Christenson RH, Greene DN, Jaffe AS, Kavsak PA, Ordonez-Llanos J, Apple FS. Clinical laboratory practice recommendations for the use of cardiac troponin in acute coronary syndrome: expert opinion from the Academy of the American Association for Clinical Chemistry and the Task Force on Clinical Applications of Cardiac Bio-Markers of the International Federation of Clinical Chemistry and Laboratory Medicine. *Clin Chem* 2018;**64**:645–655.
- Clerico A, Zaninotto M, Ripoli A, Masotti S, Prontera C, Passino C, Plebani M; Study Group on Cardiovascular Risk Biomarkers of the Italian Society of Clinical Biochemistry (SIBioC). The 99th percentile of reference population for cTnI and cTnT assay: methodology, pathophysiology and clinical implications. *Clin Chem Lab Med* 2017;**55**:1634–1651.
- Clerico A, Ripoli A, Zaninotto M, Masotti S, Musetti V, Ciaccio M, Aloe R, Rizzardi S, Dittadi R, Carrozza C, Fasano T, Perrone M, de Santis A, Prontera C, Riggio D, Guiocto C, Migliardi M, Bernardini S, Plebani M. Head-to-head comparison of plasma cTnI concentration values measured with three high-sensitivity methods in a large Italian population of healthy volunteers and patients admitted to emergency department with acute coronary syndrome: a multi-center study. *Clin Chim Acta* 2019;**496**:25–34.
- Apple FS, Ler R, Murakami MM. Determination of 19 cardiac troponin I and T assay 99th percentile values from a common presumably healthy population. *Clin Chem* 2012;**58**:1574–1581.
- de Filippi CR, de Lemos JA, Christenson RH, Gottdiener JS, Kop WJ, Zhan M, Seliger SL. Association of serial measures of cardiac troponin T using a sensitive assay with incident heart failure and cardiovascular mortality in older adults. *JAMA* 2010;**304**:2494–2502.
- McEvoy JW, Chen Y, Ndumele CE, Solomon SD, Nambi V, Ballantyne CM, Blumenthal RS, Coresh J, Selvin E. Six-year change in high-sensitivity cardiac

- troponin T and risk of subsequent coronary heart disease, heart failure, and death. *JAMA Cardiol* 2016;**1**:519–528.
31. Wang TJ, Wollert KC, Larson MG, Coglianese E, McCabe EL, Cheng S, Ho JE, Fradley MG, Ghorbani A, Xanthakis V, Kempf T, Benjamin EJ, Levy D, Vasani RS, Januzzi JL. Prognostic utility of novel biomarkers of cardiovascular stress: the Framingham Heart Study. *Circulation* 2012;**126**:1596–1604.
 32. Yan I, Börschel CS, Neumann JT, Sprünker NA, Makarova N, Kontto J, Kuulasmaa K, Salomaa V, Magnussen C, Iacoviello L, Di Castelnuovo A, Costanzo S, Linneberg A, Söderberg S, Zeller T, Ojeda-Echevarria FM, Blankenberg S, Westermann D. High-sensitivity cardiac troponin I levels and prediction of heart failure: results from the BiomarCaRE consortium. *JACC Heart Fail* 2020;**8**:401–411.
 33. Pascual-Figal DA, Manzano-Fernández S, Boronat M, Casas T, Garrido IP, Bonaque JC, Pastor-Perez F, Valdés M, Januzzi JL. Soluble ST2, high-sensitivity troponin T- and N-terminal pro-B-type natriuretic peptide: complementary role for risk stratification in acutely decompensated heart failure. *Eur J Heart Fail* 2011;**13**:718–725.
 34. Grodin JL, Butler J, Metra M, Felker GM, Voors AA, McMurray JJ, Armstrong PW, Hernandez AF, O'Connor C, Starling RC, Tang WHW. Circulating cardiac troponin I levels measured by a novel highly sensitive assay in acute decompensated heart failure: insights from the ASCEND-HF trial. *J Card Fail* 2018;**24**:512–519.
 35. Felker GM, Mentz RJ, Teerlink JR, Voors AA, Pang PS, Ponikowski P, Greenberg BH, Filippatos G, Davison BA, Cotter G, Prescott MF, Hua TA, Lopez-Pintado S, Severin T, Metra M. Serial high sensitivity cardiac troponin T measurement in acute heart failure: insights from the RELAX-AHF study. *Eur J Heart Fail* 2015;**17**:1262–1270.
 36. Felker GM, Hasselblad V, Tang WHW, Hernandez AF, Armstrong PW, Fonarow GC, Voors AA, Metra M, McMurray JJV, Butler J, Heizer GM, Dickstein K, Massie BM, Atar D, Troughton RW, Anker SD, Califf RM, Starling RC, O'Connor CM. Troponin I in acute decompensated heart failure: insights from the ASCEND-HF study. *Eur J Heart Fail* 2012;**14**:1257–1264.
 37. Demissei BG, Postmus D, Cleland JG, O'Connor CM, Metra M, Ponikowski P, Teerlink JR, Cotter G, Davison BA, Givertz MM, Bloomfield DM, van Veldhuisen DJ, Dittrich HC, Hillege HL, Voors AA. Plasma biomarkers to predict or rule out early post-discharge events after hospitalization for acute heart failure. *Eur J Heart Fail* 2017;**19**:728–738.
 38. O'Connor CM, Fiuzat M, Lombardi C, Fujita K, Jia G, Davison BA, Cleland J, Bloomfield D, Dittrich HC, Delucca P, Givertz MM, Mansoor G, Ponikowski P, Teerlink JR, Voors AA, Massie BM, Cotter G, Metra M. Impact of serial troponin release on outcomes in patients with acute heart failure: analysis from the PROTECT pilot study. *Circ Heart Fail* 2011;**4**:724–732.
 39. Latini R, Masson S, Anand IS, Missov E, Carlson M, Vago T, Angelici L, Barlera S, Parrinello G, Maggioni AP, Tognoni G, Cohn JN; Val-HeFT Investigators. Prognostic value of very low plasma concentrations of troponin T in patients with stable chronic heart failure. *Circulation* 2007;**116**:1242–1249.
 40. Masson S, Anand I, Favero C, Barlera S, Vago T, Bertocchi F, Maggioni AP, Tavazzi L, Tognoni G, Cohn JN, Latini R; Valsartan Heart Failure Trial (Val-HeFT) and Gruppo Italiano per lo Studio della Sopravvivenza nell'Insufficienza Cardiaca-Heart Failure (GISSI-HF) Investigators. Serial measurement of cardiac troponin T using a highly sensitive assay in patients with chronic heart failure: data from 2 large randomized clinical trials. *Circulation* 2012;**125**:280–288.
 41. Myhre PL, O'Meara E, Claggett BL, de Denus S, Jarolim P, Anand IS, Beldhuis IE, Fleg JL, Lewis E, Pitt B, Rouleau JL, Solomon SD, Pfeffer MA, Desai AS. Cardiac troponin I and risk of cardiac events in patients with heart failure and preserved ejection fraction. *Circ Heart Fail* 2018;**11**:e005312.
 42. Motiwala SR, Gaggin HK, Gandhi PU, Belcher A, Weiner RB, Baggish AL, Szymonifka J, Januzzi JL. Concentrations of highly sensitive cardiac troponin-I predict poor cardiovascular outcomes and adverse remodeling in chronic heart failure. *J Cardiovasc Transl Res* 2015;**8**:164–172.
 43. Packer M, McMurray JJV, Desai AS, Gong J, Lefkowitz MP, Rizkala AR, Rouleau JL, Shi VC, Solomon SD, Swedberg K, Zile M, Andersen K, Arango JL, Arnold JM, Böhlhávek J, Böhm M, Boytsov S, Burgess LJ, Cabrera W, Calvo C, Chen CH, Dukat A, Duarte YC, Erglis A, Fu M, Gomez E, González-Medina A, Hágé AA, Huang J, Katova T, Kiatchoosakun S, Kim KS, Kozan O, Llamas EB, Martínez F, Merkely B, Mendoza I, Mosterd A, Negrusz-Kawecka M, Peuhkurinen K, Ramires FJ, Refsgaard J, Rosenthal A, Senni M, Sibulo AS Jr, Silva-Cardoso J, Squire IB, Starling RC, Teerlink JR, Vanhaecke J, Vinereanu D, Wong RC; PARADIGM-HF Investigators and Coordinators. Angiotensin receptor neprilysin inhibition compared with enalapril on the risk of clinical progression in surviving patients with heart failure. *Circulation* 2015;**131**:54–61.
 44. Schuetz P, Christ-Crain M, Thomann R, Falconnier C, Wolbers M, Widmer I, Neidert S, Fricker T, Blum C, Schild U, Regez K, Schoenenberger R, Henzen C, Bregenzer T, Hoess C, Krause M, Bucher HC, Zimmerli W, Mueller B; ProHOSP Study Group. Effect of procalcitonin-based guidelines vs standard guidelines on antibiotic use in lower respiratory tract infections: the ProHOSP randomized controlled trial. *JAMA* 2009;**302**:1059–1066.
 45. Christ-Crain M, Jaccard-Stolz D, Bingisser R, Gencay MM, Huber PR, Tamm M, Müller B. Effect of procalcitonin-guided treatment on antibiotic use and outcome in lower respiratory tract infections: cluster-randomised, single-blinded intervention trial. *Lancet* 2004;**363**:600–607.
 46. Kubo S, Iwasaki M, Horie M, Matsukane A, Hayashi T, Tanaka Y, Hase H, Joki N. Biological variation of procalcitonin levels in hemodialysis patients. *Clin Exp Nephrol* 2019;**23**:402–408.
 47. Alavi SM, Shokri S. Can serum procalcitonin measurement help monitor the treatment of acute bacterial meningitis? A prospective study. *Caspian J Intern Med* 2012;**3**:382.
 48. Alba GA, Truong QA, Gaggin HK, Gandhi PU, Berardinis B De, Magrini L, Bajwa EK, Di Somma S, Januzzi JL; Global Research on Acute Conditions Team (GREAT) Network. Diagnostic and prognostic utility of procalcitonin in patients presenting to the emergency department with dyspnea. *Am J Med* 2016;**129**:96–104.e7.
 49. Maisel A, Neath SX, Landsberg J, Mueller C, Nowak RM, Peacock WF, Ponikowski P, Möckel M, Hogan C, Wu AHB, Richards M, Clopton P, Filippatos GS, Di Somma S, Anand I, Ng LL, Daniels LB, Christenson RH, Potocki M, McCord J, Terracciano G, Hartmann O, Bergmann A, Morgenthaler NG, Anker SD. Use of procalcitonin for the diagnosis of pneumonia in patients presenting with a chief complaint of dyspnoea: results from the BACH (Biomarkers in Acute Heart Failure) trial. *Eur J Heart Fail* 2012;**14**:278–286.
 50. Schuetz P, Kutz A, Grolimund E, Haubitz S, Demame D, Vögeli A, Hitz F, Christ-Crain M, Thomann R, Falconnier C, Hoess C, Henzen C, Marlowe RJ, Zimmerli W, Mueller B, ProHOSP Study Group. Excluding infection through procalcitonin testing improves outcomes of congestive heart failure patients presenting with acute respiratory symptoms: results from the randomized ProHOSP trial. *Int J Cardiol* 2014;**175**:464–472.
 51. Möckel M, Slagman A, Vollert JO, Ebmeyer S, Wiemer JC, Searle J, Giannitsis E, Kellum JA, Maisel A. Rationale and design of the IMPACT EU-trial: improve management of heart failure with procalcitonin biomarkers in cardiology (BIC)-18. *Biomarkers* 2018;**23**:97–103.
 52. Möckel M, Boer RA, Slagman AC, Haehling S, Schou M, Vollert JO, Wiemer JC, Ebmeyer S, Martín-Sánchez FJ, Maisel AS, Giannitsis E. Improve Management of acute heart failure with ProCaLciTonin in EUROpe: results of the randomized clinical trial IMPACT EU Biomarkers in Cardiology (BIC) 18. *Eur J Heart Fail* 2020;**22**:267–275.
 53. Wussler D, Kozuharov N, Tavares Oliveira M, Bossa A, Sabti Z, Nowak A, Murray K, Fay de Lavallaz J, Badertscher P, Twerenbold R, Shrestha S, Flores D, Nestelberger T, Walter J, Boeddinghaus J, Zimmermann T, Koechlin L, von Eckardstein A, Breidhardt T, Mueller C. Clinical utility of procalcitonin in the diagnosis of pneumonia. *Clin Chem* 2019;**65**:1532–1542.
 54. McAlister FA, Ezekowitz J, Tonelli M, Armstrong PW. Renal insufficiency and heart failure. *Circulation* 2004;**109**:1004–1009.
 55. de Silva R, Nikitin NP, Witte KKA, Rigby AS, Goode K, Bhandari S, Clark AL, Cleland JGF. Incidence of renal dysfunction over 6 months in patients with chronic heart failure due to left ventricular systolic dysfunction: contributing factors and relationship to prognosis. *Eur Heart J* 2006;**27**:569–581.
 56. Gottlieb SS, Abraham W, Butler J, Forman DE, Loh E, Massie BM, O'Connor CM, Rich MW, Stevenson LW, Young J, Krumholz HM. The prognostic importance of different definitions of worsening renal function in congestive heart failure. *J Card Fail* 2002;**8**:136–141.
 57. Krumholz HM, Chen YT, Vaccarino V, Wang Y, Radford MJ, Bradford WD, Horwitz RJ. Correlates and impact on outcomes of worsening renal function in patients ≥ 65 years of age with heart failure. *Am J Cardiol* 2000;**85**:1110–1113.
 58. McCallum W, Tighiouart H, Kiernan MS, Huggins GS, Sarnak MJ. Relation of kidney function decline and NT-proBNP with risk of mortality and readmission in acute decompensated heart failure. *Am J Med* 2020;**133**:115–122.
 59. McCallum W, Tighiouart H, Testani JM, Griffin M, Konstam MA, Udelson JE, Sarnak MJ. Acute kidney function declines in the context of decongestion in acute decompensated heart failure. *JACC Heart Fail* 2020;**8**:537–547.
 60. Wettersten N, Horiuchi Y, van Veldhuisen DJ, Mueller C, Filippatos G, Nowak R, Hogan C, Kontos MA, Cannon CM, Müller GA, Birkhahn R, Taub P, Vilke GM, Barnett O, McDonald K, Mahon N, Nuñez J, Briguori C, Passino C, Murray PT, Maisel A. B-type natriuretic peptide trend predicts clinical significance of worsening renal function in acute heart failure. *Eur J Heart Fail* 2019;**21**:1553–1560.
 61. Makris K, Spanou L. Acute kidney injury: definition, pathophysiology and clinical phenotypes. *Clin Biochem Rev* 2016;**37**:85–98.
 62. Rosen S, Stillman IE. Acute tubular necrosis is a syndrome of physiologic and pathologic dissociation. *J Am Soc Nephrol* 2008;**19**:871–875.
 63. McCullough PA, Shaw AD, Haase M, Bouchard J, Waikar SS, Siew ED, Murray PT, Mehta RL, Ronco C. Diagnosis of acute kidney injury using functional and

- injury biomarkers: workgroup statements from the tenth Acute Dialysis Quality Initiative Consensus Conference. *Contrib Nephrol* 2013;**182**:13–29.
64. Massie BM, O'Connor CM, Metra M, Ponikowski P, Teerlink JR, Cotter G, Weatherley BD, Cleland JGF, Givertz MM, Voors A, DeLucca P, Mansoor GA, Salerno CM, Bloomfield DM, Dittrich HC, PROTECT Investigators and Committees. Rolofylline, an adenosine A1-receptor antagonist, in acute heart failure. *N Engl J Med* 2010;**363**:1419–1428.
 65. Mehta RL, McDonald B, Gabbai F, Pahl M, Farkas A, Pascual MTA, Zhuang S, Kaplan RM, Chertow GM. Nephrology consultation in acute renal failure: does timing matter? *Am J Med* 2002;**113**:456–461.
 66. Endre ZH, Pickering JW, Walker RJ. Clearance and beyond: the complementary roles of GFR measurement and injury biomarkers in acute kidney injury (AKI). *Am J Physiol Renal Physiol* 2011;**301**:F697–707.
 67. Firth JD, Raine AE, Ledingham JG. Raised venous pressure: a direct cause of renal sodium retention in oedema? *Lancet* 1988;**1**:1033–1035.
 68. McCullough PA, Bouchard J, Waikar SS, Siew ED, Endre ZH, Goldstein SL, Koyner JL, Macedo E, Doi K, Somma S Di, Lewington A, Thadhani R, Chakravarthi R, Ice C, Okusa MD, Duranteau J, Doran P, Yang L, Jaber BL, Meehan S, Kellum JA, Haase M, Murray PT, Cruz D, Maisel A, Bagshaw SM, Chawla LS, Mehta RL, Shaw AD, Ronco C. Implementation of novel biomarkers in the diagnosis, prognosis, and management of acute kidney injury: executive summary from the tenth consensus conference of the Acute Dialysis Quality Initiative (ADQI). *Contrib Nephrol* 2013;**182**:5–12.
 69. Pickering JW, Endre ZH. Linking injury to outcome in acute kidney injury: a matter of sensitivity. *PLoS One* 2013;**8**:e62691.
 70. Stevens PE, Levin A, Kidney Disease: Improving Global Outcomes Chronic Kidney Disease Guideline Development Work Group Members. Evaluation and management of chronic kidney disease: synopsis of the kidney disease: improving global outcomes 2012 clinical practice guideline. *Ann Intern Med* 2013;**158**:825, 830.
 71. Pianta TJ, Buckley NA, Peake PW, Endre ZH. Clinical use of biomarkers for toxicant-induced acute kidney injury. *Biomark Med* 2013;**7**:441–456.
 72. Vanmassenhove J, Vanholder R, Nagler E, Biesen W Van. Urinary and serum biomarkers for the diagnosis of acute kidney injury: an in-depth review of the literature. *Nephrol Dial Transplant* 2013;**28**:254–273.
 73. Aghel A, Shrestha K, Mullens VV, Borowski A, Tang WHW. Serum neutrophil gelatinase-associated lipocalin (NGAL) in predicting worsening renal function in acute decompensated heart failure. *J Card Fail* 2010;**16**:49–54.
 74. Shrestha K, Shao Z, Singh D, Dupont M, Tang WHW. Relation of systemic and urinary neutrophil gelatinase-associated lipocalin levels to different aspects of impaired renal function in patients with acute decompensated heart failure. *Am J Cardiol* 2012;**110**:1329–1335.
 75. Shrestha K, Borowski AG, Troughton RW, Thomas JD, Klein AL, Tang WHW. Renal dysfunction is a stronger determinant of systemic neutrophil gelatinase-associated lipocalin levels than myocardial dysfunction in systolic heart failure. *J Card Fail* 2011;**17**:472–478.
 76. Alvelos M, Lourenço P, Dias C, Amorim M, Rema J, Leite AB, Guimarães JT, Almeida P, Bettencourt P. Prognostic value of neutrophil gelatinase-associated lipocalin in acute heart failure. *Int J Cardiol* 2013;**165**:51–55.
 77. Murray PT, Wettersten N, van Veldhuisen DJ, Mueller C, Filippatos G, Nowak R, Hogan C, Kontos MC, Cannon CM, Müller GA, Birkhahn R, Horiuchi Y, Clopton P, Taub P, Vilke GM, Barnett O, McDonald K, Mahon N, Nuñez J, Briguori C, Passino C, Maisel A. Utility of urine neutrophil gelatinase-associated lipocalin for worsening renal function during hospitalization for acute heart failure: primary findings of the urine N-gal Acute Kidney Injury N-gal Evaluation of Symptomatic Heart Failure Study (AKINESIS). *J Card Fail* 2019;**25**:654–665.
 78. Kashani K, Al-Khafaji A, Ardiles T, Artigas A, Bagshaw SM, Bell M, Bihorac A, Birkhahn R, Cely CM, Chawla LS, Davison DL, Feldkamp T, Furni LG, Gong MN, Gunnerson KJ, Haase M, Hackett J, Honore PM, Hoste EAJ, Joannes-Boyau O, Joannidis M, Kim P, Koyner JL, Laskowitz DT, Lissauer ME, Marx G, McCullough PA, Mullaney S, Ostermann M, Rimmelé T, Shapiro NI, Shaw AD, Shi J, Sprague AM, Vincent JL, Vinsonneau C, Wagner L, Walker MG, Wilkerson RG, Zacharowski K, Kellum JA. Discovery and validation of cell cycle arrest biomarkers in human acute kidney injury. *Crit Care* 2013;**17**:R25.
 79. Ng LL, Squire IB, Jones DJL, Cao TH, Chan DCS, Sandhu JK, Quinn PA, Davies JE, Struck J, Hartmann O, Bergmann A, Mebazaa A, Gayat E, Arrigo M, Akiyama E, Sabti Z, Lohmann J, Twerenbold R, Herrmann T, Schumacher C, Kozuharov N, Mueller C; GREAT Network. Proenkephalin, renal dysfunction, and prognosis in patients with acute heart failure: a GREAT Network Study. *J Am Coll Cardiol* 2017;**69**:56–69.
 80. Ambros V. The functions of animal microRNAs. *Nature* 2004;**431**:350–355.
 81. Mendell JT, Olson EN. MicroRNAs in stress signaling and human disease. *Cell* 2012;**148**:1172–1187.
 82. Schena FP, Serino G, Sallustio F. MicroRNAs in kidney diseases: new promising biomarkers for diagnosis and monitoring. *Nephrol Dial Transplant* 2014;**29**:755–763.
 83. Saikumar J, Hoffmann D, Kim T-M, Gonzalez VR, Zhang Q, Goering PL, Brown RP, Bijol V, Park PJ, Waikar SS, Vaidya VS. Expression, circulation, and excretion profile of microRNA-21, -155, and -18a following acute kidney injury. *Toxicol Sci* 2012;**129**:256–267.
 84. Lan YF, Chen HH, Lai PF, Cheng CF, Huang YT, Lee YC, Chen TW, Lin H. MicroRNA-494 reduces ATF3 expression and promotes AKI. *J Am Soc Nephrol* 2012;**23**:2012–2023.
 85. Lorenzen JM, Kielstein JT, Hafer C, Gupta SK, Kumpers P, Faulhaber-Walter R, Haller H, Fliser D, Thum T. Circulating miR-210 predicts survival in critically ill patients with acute kidney injury. *Clin J Am Soc Nephrol* 2011;**6**:1540–1546.
 86. Hojs R, Bevc S, Ekart R, Gorenjak M, Puklavec L. Serum cystatin C as an endogenous marker of renal function in patients with mild to moderate impairment of kidney function. *Nephrol Dial Transplant* 2006;**21**:1855–1862.
 87. Vinge E, Lindergård B, Nilsson-Ehle P, Grubb A. Relationships among serum cystatin C, serum creatinine, lean tissue mass and glomerular filtration rate in healthy adults. *Scand J Clin Lab Invest* 1999;**59**:587–592.
 88. Stevens LA, Schmid CH, Greene T, Li L, Beck GJ, Joffe MM, Froissart M, Kusek JW, Zhang YL, Coresh J, Levey AS. Factors other than glomerular filtration rate affect serum cystatin C levels. *Kidney Int* 2009;**75**:652–660.
 89. Newman DJ. Cystatin C. *Ann Clin Biochem* 1999;**59**:589–104.
 90. Muntner P, Mann D, Winston J, Bansilal S, Farkouh ME. Serum cystatin C and increased coronary heart disease prevalence in US adults without chronic kidney disease. *Am J Cardiol* 2008;**102**:54–57.
 91. Ix JH, Shlipak MG, Chertow GM, Whoolo MA. Association of cystatin C with mortality, cardiovascular events, and incident heart failure among persons with coronary heart disease: data from the Heart and Soul Study. *Circulation* 2007;**115**:173–179.
 92. Woitas RP, Kleber ME, Meintzer A, Grammer TB, Silbernagel G, Pilz S, Tomaschitz A, Wehrauch G, Dobnig H, März W, Scharnagl H. Cystatin C is independently associated with total and cardiovascular mortality in individuals undergoing coronary angiography. The Ludwigshafen Risk and Cardiovascular Health (LURIC) study. *Atherosclerosis* 2013;**229**:541–548.
 93. Keller T, Messow CM, Lubos E, Nicaud V, Wild PS, Rupprecht HJ, Bickel C, Tzikas S, Peetz D, Lackner KJ, Tiret L, Münzel TF, Blankenberg S, Schnabel RB. Cystatin C and cardiovascular mortality in patients with coronary artery disease and normal or mildly reduced kidney function: results from the AtheroGene study. *Eur Heart J* 2009;**30**:314–320.
 94. van der Laan SW, Fall T, Soumaré A, Teumer A, Sedaghat S, Baumert J, Zabaneh D, van Setten J, Isgum I, Galesloot TE, Arpegård J, Amouyel P, Trompet S, Waldenberger M, Dörr M, Magnusson PK, Giedraitis V, Larsson A, Morris AP, Felix JF, Morrison AC, Franceschini N, Bis JC, Kavousi M, O'Donnell C, Drenos F, Tragante V, Munroe PB, Malik R, Dichgans M, Worrall BB, Erdmann J, Nelson CP, Samani NJ, Schunkert H, Marchini J, Patel RS, Hingorani AD, Lind L, Pedersen NL, de Graaf J, Kiemeny LALM, Baumeister SE, Franco OH, Hofman A, Uitterlinden AG, Koenig W, Meisinger C, Peters A, Thorand B, Jukema JW, Eriksen BO, Toft I, Wilsgaard T, Onland-Moret NC, van der Schouw YT, Debette S, Kumari M, Svensson P, van der Harst P, Kivimaki M, Keating BJ, Sattar N, Dehghan A, Reiner AP, Ingelsson E, den Ruijter HM, de Bakker PIW, Pasterkamp G, Ärnlöv J, Holmes MV, Asselbergs FW. Cystatin C and cardiovascular disease: a Mendelian randomization study. *J Am Coll Cardiol* 2016;**68**:934–945.
 95. Dupont M, Wu Y, Hazen SL, Tang WHW. Cystatin C identifies patients with stable chronic heart failure at increased risk for adverse cardiovascular events. *Circ Heart Fail* 2012;**5**:602–609.
 96. Lassus J, Harjola VP, Sund R, Siirilä-Waris K, Melin J, Peuhkurinen K, Pulkki K, Nieminen MS; FINN-AKVA Study Group. Prognostic value of cystatin C in acute heart failure in relation to other markers of renal function and NT-proBNP. *Eur Heart J* 2007;**28**:1841–1847.
 97. Du W, Piek A, Schouten EM, van de Kolk CWA, Mueller C, Mebazaa A, Voors A, de Boer RA, Silljé HHW. Plasma levels of heart failure biomarkers are primarily a reflection of extracardiac production. *Thrombosis* 2018;**8**:4155–4169.
 98. Kaye DM, Mariani JA, van Empel V, Maeder NT. Determinants and implications of elevated soluble ST2 levels in heart failure. *Int J Cardiol* 2014;**176**:1242–1243.
 99. Pascual-Figal DA, Pérez-Martínez MT, Asensio-Lopez MC, Sanchez-Más J, García-García ME, Martínez CM, Lencina M, Jara R, Januzzi JL, Lax A. Pulmonary production of soluble ST2 in heart failure. *Circ Heart Fail* 2018;**11**:e005488.
 100. Bajwa EK, Volk JA, Christiani DC, Harris RS, Matthay MA, Thompson BT, Januzzi JL; National Heart, Lung and Blood Institute Acute Respiratory Distress Syndrome Network. Prognostic and diagnostic value of plasma soluble suppression of tumorigenicity-2 concentrations in acute respiratory distress syndrome. *Crit Care Med* 2013;**41**:2521–2531.

101. Suthahar N, Meijers WC, Silljé HHW, Ho JE, Liu FT, de Boer RA. Galectin-3 activation and inhibition in heart failure and cardiovascular disease: an update. *Theranostics* 2018;**8**:593–609.
102. Meijers WC, López-Andrés N, de Boer RA. Galectin-3, cardiac function, and fibrosis. *Am J Pathol* 2016;**186**:2232–2234.
103. Meijers WC, van der Velde AR, Pascual-Figal DA, de Boer RA. Galectin-3 and post-myocardial infarction cardiac remodeling. *Eur J Pharmacol* 2015;**763**:115–121.
104. Kempf T, Zarbock A, Widera C, Butz S, Stadtmann A, Rossaint J, Bolomini-Vittori M, Korf-Klingebiel M, Napp LC, Hansen B, Kanwischer A, Bavendiek U, Beutel G, Hapke M, Sauer MG, Laudanna C, Hogg N, Vestweber D, Wollert KC. GDF-15 is an inhibitor of leukocyte integrin activation required for survival after myocardial infarction in mice. *Nat Med* 2011;**17**:581–588.
105. Kempf T, von Haehling S, Peter T, Allhoff T, Ciccoira M, Doehner W, Ponikowski P, Filippatos GS, Rozentryt P, Drexler H, Anker SD, Wollert KC. Prognostic utility of growth differentiation factor-15 in patients with chronic heart failure. *J Am Coll Cardiol* 2007;**50**:1054–1060.
106. Au Yeung SL, Luo S, Schooling CM. The impact of GDF-15, a biomarker for metformin, on the risk of coronary artery disease, breast and colorectal cancer, and type 2 diabetes and metabolic traits: a Mendelian randomisation study. *Diabetologia* 2019;**62**:1638–1646.
107. Mueller T, Dieplinger B. Soluble ST2 and galectin-3: what we know and don't know analytically. *EJIFCC* 2016;**27**:224–237.
108. Wollert KC, Kempf T, Wallentin L. Growth differentiation factor 15 as a biomarker in cardiovascular disease. *Clin Chem* 2017;**63**:140–151.
109. Meijers WC, van der Velde AR, de Boer RA. The ARCHITECT galectin-3 assay: comparison with other automated and manual assays for the measurement of circulating galectin-3 levels in heart failure. *Expert Rev Mol Diagn* 2014;**14**:257–266.
110. Dieplinger B, Egger M, Gegenhuber A, Haltmayer M, Mueller T. Analytical and clinical evaluation of a rapid quantitative lateral flow immunoassay for measurement of soluble ST2 in human plasma. *Clin Chim Acta* 2015;**451**:310–315.
111. Mueller T, Leitner I, Egger M, Haltmayer M, Dieplinger B. Association of the biomarkers soluble ST2, galectin-3 and growth-differentiation factor-15 with heart failure and other non-cardiac diseases. *Clin Chim Acta* 2015;**445**:155–160.
112. Aimo A, Vergaro G, Ripoli A, Bayes-Genis A, Pascual Figal DA, de Boer RA, Lassus J, Mebazaa A, Gayat E, Breidhardt T, Sabti Z, Mueller C, Brunner-La Rocca HP, Tang WHW, Grodin JL, Zhang Y, Bettencourt P, Maisel AS, Passino C, Januzzi JL, Emdin M. Meta-analysis of soluble suppression of tumorigenicity-2 and prognosis in acute heart failure. *JACC Heart Fail* 2017;**5**:287–296.
113. Aimo A, Vergaro G, Passino C, Ripoli A, Ky B, Miller WL, Bayes-Genis A, Anand I, Januzzi JL, Emdin M. Prognostic value of soluble suppression of tumorigenicity-2 in chronic heart failure. *JACC Heart Fail* 2017;**5**:280–286.
114. Chen H, Chen C, Fang J, Wang R, Nie W. Circulating galectin-3 on admission and prognosis in acute heart failure patients: a meta-analysis. *Heart Fail Rev* 2020;**25**:331–341.
115. Chen YS, Gi WT, Liao TY, Lee MT, Lee SH, Hsu WT, Chang SS, Lee CC. Using the galectin-3 test to predict mortality in heart failure patients: a systematic review and meta-analysis. *Biomark Med* 2016;**10**:329–342.
116. Cotter G, Voors AA, Prescott MF, Felker GM, Filippatos G, Greenberg BH, Pang PS, Ponikowski P, Milo O, Hua TA, Qian M, Severin TM, Teerlink JR, Metra M, Davison BA. Growth differentiation factor 15 (GDF-15) in patients admitted for acute heart failure: results from the RELAX-AHF study. *Eur J Heart Fail* 2015;**17**:1133–1143.
117. Schopfer DW, Ku IA, Regan M, Whooley MA. Growth differentiation factor 15 and cardiovascular events in patients with stable ischemic heart disease (The Heart and Soul Study). *Am Heart J* 2014;**167**:186–192.e1.
118. Bouwens E, Brankovic M, Mouthaan H, Baart S, Rizopoulos D, van Boven N, Caliskan K, Manintveld O, Germans T, van Ramshorst J, Umans V, Akkerhuis KM, Kardys I. Temporal patterns of 14 blood biomarker candidates of cardiac remodeling in relation to prognosis of patients with chronic heart failure – the Bio-SHiFT Study. *J Am Heart Assoc* 2019;**8**:e009555.
119. Lok DJ, Klip IT, Lok SI, Bruggink-André de la Porte PW, Badings E, van Wijngaarden J, Voors AA, de Boer RA, van Veldhuisen DJ, van der Meer P. Incremental prognostic power of novel biomarkers (growth-differentiation factor-15, high-sensitivity C-reactive protein, galectin-3, and high-sensitivity troponin-T) in patients with advanced chronic heart failure. *Am J Cardiol* 2013;**112**:831–837.
120. Yancy CW, Jessup M, Bozkurt B, Butler J, Casey DE, Colvin MM, Drazner MH, Filippatos GS, Fonarow GC, Givertz MM, Hollenberg SM, Lindenfeld J, Masouli FA, McBride PE, Peterson PN, Stevenson LW, Westlake C. 2017 ACC/AHA/HFSA Focused update of the 2013 ACCF/AHA guideline for the management of heart failure: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines and the Heart Failure Society of America. *J Am Coll Cardiol* 2017;**70**:776–803.
121. Lupón J, Simpson J, McMurray JJ V, de Antonio M, Vila J, Subirana I, Barallat J, Moliner P, Domingo M, Zamora E, Bayes-Genis A. Barcelona Bio-HF Calculator Version 2.0: incorporation of angiotensin II receptor blocker neprilysin inhibitor (ARNI) and risk for heart failure hospitalization. *Eur J Heart Fail* 2018;**20**:938–940.
122. Pacho C, Domingo M, Núñez R, Lupón J, Núñez J, Barallat J, Moliner P, de Antonio M, Santesmas J, Cediel G, Roura S, Pastor MC, Tor J, Bayes-Genis A. Predictive biomarkers for death and rehospitalization in comorbid frail elderly heart failure patients. *BMC Geriatr* 2018;**18**:109.
123. van Vark LC, Lesman-Leegte I, Baart SJ, Postmus D, Pinto YM, Orsel JG, Westenbrink BD, Brunner-La Rocca HP, van Miltenburg AJM, Boersma E, Hillege HL, Akkerhuis KM; TRIUMPH Investigators. Prognostic value of serial ST2 measurements in patients with acute heart failure. *J Am Coll Cardiol* 2017;**70**:2378–2388.
124. Pascual-Figal DA, Ordoñez-Llanos J, Tornel PL, Vázquez R, Puig T, Valdés M, Cinca J, de Luna AB, Bayes-Genis A; MUSIC Investigators. Soluble ST2 for predicting sudden cardiac death in patients with chronic heart failure and left ventricular systolic dysfunction. *J Am Coll Cardiol* 2009;**54**:2174–2179.
125. Weir RAP, Miller AM, Murphy GEJ, Clements S, Steedman T, Connell JMC, McInnes IB, Dargie HJ, McMurray JJV. Serum soluble ST2: a potential novel mediator in left ventricular and infarct remodeling after acute myocardial infarction. *J Am Coll Cardiol* 2010;**55**:243–250.
126. Gaggin HK, Motiwala S, Bhardwaj A, Parks KA, Januzzi JL. Soluble concentrations of the interleukin receptor family member ST2 and β -blocker therapy in chronic heart failure. *Circ Heart Fail* 2013;**6**:1206–1213.
127. Maisel A, Xue Y, van Veldhuisen DJ, Voors AA, Jaarsma T, Pang PS, Butler J, Pitt B, Clopton P, de Boer RA. Effect of spironolactone on 30-day death and heart failure rehospitalization (from the COACH Study). *Am J Cardiol* 2014;**114**:737–742.
128. Huet F, Nicoleau J, Dupuy AM, Curinier C, Breuker C, Castet-Nicolas A, Lotierzo M, Kalmanovich E, Zerkowski L, Akodad M, Adda J, Agullo A, Leclercq F, Pasquie JL, Battistella P, Roubille C, Fesler P, Mercier G, Bourel G, Cristol JP, Roubille F. STADE-HF (sST2 as a help for management of HF): a pilot study. *ESC Heart Fail* 2020;**7**:774–778.
129. de Boer RA, Lok DJ, Jaarsma T, van der Meer P, Voors AA, Hillege HL, van Veldhuisen DJ. Predictive value of plasma galectin-3 levels in heart failure with reduced and preserved ejection fraction. *Ann Med* 2011;**43**:60–68.
130. Meijers WC, Januzzi JL, de Filippi C, Adourian AS, Shah SJ, van Veldhuisen DJ, de Boer RA. Elevated plasma galectin-3 is associated with near-term rehospitalization in heart failure: a pooled analysis of 3 clinical trials. *Am Heart J* 2014;**167**:853–860.e4.
131. Ho JE, Liu C, Lyass A, Courchesne P, Pencina MJ, Vasan RS, Larson MG, Levy D. Galectin-3, a marker of cardiac fibrosis, predicts incident heart failure in the community. *J Am Coll Cardiol* 2012;**60**:1249–1256.
132. van der Velde AR, Meijers WC, Ho JE, Brouwers FP, Rienstra M, Bakker SJL, Muller Kobold AC, van Veldhuisen DJ, van Gilst WH, van der Harst P, de Boer RA. Serial galectin-3 and future cardiovascular disease in the general population. *Heart* 2016;**102**:1134–1141.
133. Imran TF, Shin HJ, Mathenge N, Wang F, Kim B, Joseph J, Gaziano JM, Djoussé L. Meta-analysis of the usefulness of plasma galectin-3 to predict the risk of mortality in patients with heart failure and in the general population. *Am J Cardiol* 2017;**119**:57–64.
134. Miller WL, Saenger AK, Grill DE, Slusser JP, Bayes-Genis A, Jaffe AS. Prognostic value of serial measurements of soluble suppression of tumorigenicity 2 and galectin-3 in ambulatory patients with chronic heart failure. *J Card Fail* 2016;**22**:249–255.
135. van der Velde AR, Gullestad L, Ueland T, Aukrust P, Guo Y, Adourian A, Muntendam P, van Veldhuisen DJ, de Boer RA. Prognostic value of changes in galectin-3 levels over time in patients with heart failure: data from CORONA and COACH. *Circ Heart Fail* 2013;**6**:219–226.
136. French B, Wang L, Ky B, Brandimarto J, Basuray A, Fang JC, Sweitzer NK, Cappola TP. Prognostic value of galectin-3 for adverse outcomes in chronic heart failure. *J Card Fail* 2016;**22**:256–262.
137. Gandhi PU, Motiwala SR, Belcher AM, Gaggin HK, Weiner RB, Baggish AL, Fandz M, Brunner-La Rocca HP, Januzzi JL. Galectin-3 and mineralocorticoid receptor antagonist use in patients with chronic heart failure due to left ventricular systolic dysfunction. *Am Heart J* 2015;**169**:404–411.e3.
138. Fiuzat M, Schulte PJ, Felker M, Ahmad T, Neely M, Adams KF, Donahue MP, Kraus WE, Piña IL, Whellan DJ, O'Connor CM. Relationship between galectin-3 levels and mineralocorticoid receptor antagonist use in heart failure: analysis from HF-ACTION. *J Card Fail* 2014;**20**:38–44.
139. Pellicori P, Ferreira JP, Mariottoni B, Brunner-La Rocca HP, Ahmed FZ, Verdonchot J, Collier T, Cuthbert JJ, Petutschnigg J, Mujaj B, Girerd N, González A, Clark AL, Cosmi F, Staessen JA, Heymans S, Latini R, Rossignol P, Zannad F,

- Cleland JGF. Effects of spironolactone on serum markers of fibrosis in people at high risk of developing heart failure: rationale, design and baseline characteristics of a proof-of-concept, randomised, precision-medicine, prevention trial. *The Heart OMics in AGing (HO)*. *Eur J Heart Fail* 2020;**22**:1711–1723.
140. Cleland JGF, Pellicori P, González A. A novel treatment for heart failure targets myocardial fibrosis. *Nat Med* 2021;**27**:1343–1344.
 141. Cleland JGF, Ferreira JP, Mariottoni B, Pellicori P, Cuthbert J, Verdonschot JA, Petutschnigg J, Ahmed FZ, Cosmi F, Brunner La Rocca HP, Mamas MA, Clark AL, Edelmann F, Pieske B, Khan J, McDonald K, Rouet P, Staessen JA, Mujaj B, González A, Diez J, Hazebroek M, Heymans S, Latini R, Grojean S, Pizard A, Girerd N, Rossignol P, Collier TJ, Zannad F. HOMAGE Trial Committees and Investigators. The effect of spironolactone on cardiovascular function and markers of fibrosis in people at increased risk of developing heart failure: the heart 'OMics' in AGEing (HOMAGE) randomized clinical trial. *Eur Heart J* 2021;**42**:684–696.
 142. Demissei BG, Cotter G, Prescott MF, Felker GM, Filippatos G, Greenberg BH, Pang PS, Ponikowski P, Severin TM, Wang Y, Qian M, Teerlink JR, Metra M, Davison BA, Voors AA. A multimarker multi-time point-based risk stratification strategy in acute heart failure: results from the RELAX-AHF trial. *Eur J Heart Fail* 2017;**19**:1001–1010.
 143. Sharma A, Stevens SR, Lucas J, Fiuza M, Adams KF, Whellan DJ, Donahue MP, Kitzman DW, Piña IL, Zannad F, Kraus WE, O'Connor CM, Felker GM. Utility of growth differentiation factor-15, a marker of oxidative stress and inflammation, in chronic heart failure. *JACC Heart Fail* 2017;**5**:724–734.
 144. Bouabdallaoui N, Claggett B, Zile MR, McMurray JJV, O'Meara E, Packer M, Prescott MF, Swedberg K, Solomon SD, Rouleau JL; PARADIGM-HF Investigators and Committees. Growth differentiation factor-15 is not modified by sacubitril/valsartan and is an independent marker of risk in patients with heart failure and reduced ejection fraction: the PARADIGM-HF trial. *Eur J Heart Fail* 2018;**20**:1701–1709.
 145. Hongisto M, Kataja A, Tarvasmäki T, Holopainen A, Javanainen T, Jurkko R, Jääntti T, Kimmoun A, Levy B, Mebazaa A, Pulkki K, Sionis A, Tolppanen H, Wollert KC, Harjola VP, Lassus J; CardShock Investigators. Levels of growth differentiation factor 15 and early mortality risk stratification in cardiogenic shock. *J Card Fail* 2019;**25**:894–901.
 146. Lok SI, Winkens B, Goldschmeding R, van Geffen AJP, Nous FMA, van Kuik J, van der Weide P, Klöpping C, Kirkels JH, Lahpor JR, Doevendans PA, de Jonge N, de Weeger RA. Circulating growth differentiation factor-15 correlates with myocardial fibrosis in patients with non-ischaemic dilated cardiomyopathy and decreases rapidly after left ventricular assist device support. *Eur J Heart Fail* 2012;**14**:1249–1256.
 147. Ahmad T, Wang T, O'Brien EC, Samsky MD, Pura JA, Likhnygina Y, Rogers JG, Hernandez AF, Craig D, Bowles DE, Milano CA, Shah SH, Januzzi JL, Felker GM, Patel CB. Effects of left ventricular assist device support on biomarkers of cardiovascular stress, fibrosis, fluid homeostasis, inflammation, and renal injury. *JACC Heart Fail* 2015;**3**:30–39.
 148. Lankeit M, Kempf T, Dellas C, Cuny M, Tapken H, Peter T, Olschewski M, Konstantinides S, Wollert KC. Growth differentiation factor-15 for prognostic assessment of patients with acute pulmonary embolism. *Am J Respir Crit Care Med* 2008;**177**:1018–1025.
 149. Nickel N, Kempf T, Tapken H, Tongers J, Laenger F, Lehmann U, Golpon H, Olsson K, Wilkins MR, Gibbs JSR, Hoepfer MM, Wollert KC. Growth differentiation factor-15 in idiopathic pulmonary arterial hypertension. *Am J Respir Crit Care Med* 2008;**178**:534–541.
 150. Kimmoun A, Cotter G, Davison B, Takagi K, Addad F, Celutkienė J, Chioncel O, Solal AC, Diaz R, Damasceno A, Duengen H-D, Filippatos G, Goncalvesova E, Merai I, Metra M, Ponikowski P, Privalov D, Sliwa K, Sani MU, Voors AA, Shogenov Z, Mebazaa A. Safety, tolerability and efficacy of rapid optimization, helped by NT-proBNP and GDF-15, of heart failure therapies (STRONG-HF): rationale and design for a multicentre, randomized, parallel-group study. *Eur J Heart Fail* 2019;**21**:1459–1467.
 151. Arrigo M, Truong QA, Onat D, Szymonifka J, Gayat E, Tolppanen H, Sadoune M, Demmer RT, Wong KY, Launay JM, Samuel JL, Cohen-Solal A, Januzzi JL, Singh JP, Colombo PC, Mebazaa A. Soluble CD146 is a novel marker of systemic congestion in heart failure patients: an experimental mechanistic and transcatheter clinical study. *Clin Chem* 2017;**63**:386–393.
 152. Van Aelst LNL, Arrigo M, Placido R, Akiyama E, Girerd N, Zannad F, Manivet P, Rossignol P, Badoz M, Sadoune M, Launay JM, Gayat E, Lam CSP, Cohen-Solal A, Mebazaa A, Seronde MF. Acutely decompensated heart failure with preserved and reduced ejection fraction present with comparable haemodynamic congestion. *Eur J Heart Fail* 2018;**20**:738–747.
 153. Arrigo M, Von Moos S, Gerritsen K, Sadoune M, Tangvoraphonkhai K, Davenport A, Mebazaa A, Segerer S, Cippà PE. Soluble CD146 and B-type natriuretic peptide dissect overhydration into functional components of prognostic relevance in haemodialysis patients. *Nephrol Dial Transplant* 2018;**33**:2035–2042.
 154. Bayes-Genis A, Barallat J, Richards AM. A test in context: neprilysin: function, inhibition, and biomarker. *J Am Coll Cardiol* 2016;**68**:639–653.
 155. McMurray JJV, Packer M, Desai AS, Gong J, Lefkowitz MP, Rizkala AR, Rouleau JL, Shi VC, Solomon SD, Swedberg K, Zile MR; PARADIGM-HF Investigators and Committees. Angiotensin-neprilysin inhibition versus enalapril in heart failure. *N Engl J Med* 2014;**371**:993–1004.
 156. Velazquez EJ, Morrow DA, DeVore AD, Duffy CI, Ambrosy AP, McCague K, Rocha R, Braunwald E; PIONEER-HF Investigators. Angiotensin-neprilysin inhibition in acute decompensated heart failure. *N Engl J Med* 2019;**380**:539–548.
 157. Januzzi JL, Prescott MF, Butler J, Felker GM, Maisel AS, McCague K, Camacho A, Piña IL, Rocha RA, Shah AM, Williamson KM, Solomon SD; PROVE-HF Investigators. Association of change in N-terminal pro-B-type natriuretic peptide following initiation of sacubitril-valsartan treatment with cardiac structure and function in patients with heart failure with reduced ejection fraction. *JAMA* 2019;**322**:1085–1095.
 158. Desai AS, Solomon SD, Shah AM, Claggett BL, Fang JC, Izzo J, McCague K, Abbas CA, Rocha R, Mitchell GF; EVALUATE-HF Investigators. Effect of sacubitril-valsartan vs enalapril on aortic stiffness in patients with heart failure and reduced ejection fraction: a randomized clinical trial. *JAMA* 2019;**322**:1077–1084.
 159. Solomon SD, McMurray JJV, Anand IS, Ge J, Lam CSP, Maggioni AP, Martinez F, Packer M, Pfeffer MA, Pieske B, Redfield MM, Rouleau JL, van Veldhuisen DJ, Zannad F, Zile MR, Desai AS, Claggett B, Jhund PS, Boytsov SA, Comin-Colet J, Cleland J, Düngen HD, Goncalvesova E, Katova T, Kerr Saraiva JF, Lelonek M, Merkely B, Senni M, Shah SJ, Zhou J, Rizkala AR, Gong J, Shi VC, Lefkowitz MP; PARAGON-HF Investigators and Committees. Angiotensin-neprilysin inhibition in heart failure with preserved ejection fraction. *N Engl J Med* 2019;**381**:1609–1620.
 160. Zile MR, O'Meara E, Claggett B, Prescott MF, Solomon SD, Swedberg K, Packer M, McMurray JJV, Shi V, Lefkowitz M, Rouleau J. Effects of sacubitril/valsartan on biomarkers of extracellular matrix regulation in patients with HFpEF. *J Am Coll Cardiol* 2019;**73**:795–806.
 161. Cunningham JW, Claggett BL, O'Meara E, Prescott MF, Pfeffer MA, Shah SJ, Redfield MM, Zannad F, Chiang LM, Rizkala AR, Shi VC, Lefkowitz MP, Rouleau J, McMurray JJV, Solomon SD, Zile MR. Effect of Sacubitril/Valsartan on Biomarkers of Extracellular Matrix Regulation in Patients With HFpEF. *J Am Coll Cardiol* 2020;**76**:503–514.
 162. Ochoa-Callejero L, Pozo-Rodríguez A, Martínez-Murillo R, Martínez A. Lack of adrenomedullin in mouse endothelial cells results in defective angiogenesis, enhanced vascular permeability, less metastasis, and more brain damage. *Sci Rep* 2016;**6**:33495.
 163. Tanaka M, Koyama T, Sakurai T, Kamiyoshi A, Ichikawa-Shindo Y, Kawate H, Liu T, Xian X, Imai A, Zhai L, Hirabayashi K, Owa S, Yamauchi A, Igarashi K, Taniguchi S, Shindo T. The endothelial adrenomedullin-RAMP2 system regulates vascular integrity and suppresses tumour metastasis. *Cardiovasc Res* 2016;**111**:398–409.
 164. Andaluz-Ojeda D, Nguyen HB, Meunier-Bellard N, Cicuéndez R, Quenot J-P, Calvo D, Dargent A, Zarca E, Andrés C, Nogales L, Eiros JM, Tamayo E, Gandía F, Bermejo-Martín JF, Charles PE. Superior accuracy of mid-regional proadrenomedullin for mortality prediction in sepsis with varying levels of illness severity. *Ann Intensive Care* 2017;**7**:15.
 165. Viaggi B, Poole D, Tujjar O, Marchiani S, Ognibene A, Finazzi S. Mid regional pro-adrenomedullin for the prediction of organ failure in infection. Results from a single centre study. *PLoS One* 2018;**13**:e0201491.
 166. Önal U, Valenzuela-Sánchez F, Vandana KE, Rello J. Mid-regional pro-adrenomedullin (MR-proADM) as a biomarker for sepsis and septic shock: narrative review. *Healthcare* 2018;**6**:110.
 167. Christ-Crain M, Morgenthaler NG, Struck J, Harbarth S, Bergmann A, Müller B. Mid-regional pro-adrenomedullin as a prognostic marker in sepsis: an observational study. *Crit Care* 2005;**9**:R816–R824.
 168. Müller-Redetzky HC, Will D, Hellwig K, Kummer W, Tschernig T, Pfeil U, Padenberg R, Menger MD, Kershaw O, Gruber AD, Weissmann N, Hippenstiel S, Suttrop N, Witzenrath M. Mechanical ventilation drives pneumococcal pneumonia into lung injury and sepsis in mice: protection by adrenomedullin. *Crit Care* 2014;**18**:R73.
 169. Wang P, Ba ZF, Cioffi WG, Bland KI, Chaudry IH. The pivotal role of adrenomedullin in producing hyperdynamic circulation during the early stage of sepsis. *Arch Surg* 1998;**133**:1298–1304.
 170. Struck J, Hein F, Karasch B, Bergmann A. Epitope specificity of anti-adrenomedullin antibodies determines efficacy of mortality reduction in a cecal ligation and puncture mouse model. *Intensive Care Med* 2013;**18**:122.
 171. Wagner K, Wachter U, Vogt JA, Scheuerle A, McCook O, Weber S, Gröger M, Stahl B, Georgieff M, Möller P, Bergmann A, Hein F, Calzia E, Radermacher P, Wagner F. Adrenomedullin binding improves catecholamine responsiveness and

- kidney function in resuscitated murine septic shock. *Intensive Care Med Exp* 2013;1:21.
172. Núñez J, Núñez E, Barallat J, Bodi V, Miñana G, Pastor MC, Sanchis J, Lupón J, Bayes-Genis A. Serum neprilysin and recurrent admissions in patients with heart failure. *J Am Heart Assoc* 2017;6:e005712.
 173. Claus R, Berlinier D, Bavendiek U, Vodovar N, Lichtinghagen R, David S, Patecki M, Launay J-M, Bauersachs J, Haller H, Hiss M, Balzer MS. Soluble neprilysin, NT-proBNP, and growth differentiation factor-15 as biomarkers for heart failure in dialysis patients (SONGBIRD). *Clin Res Cardiol* 2020;109:1035–1047.
 174. Weber J, Sachse J, Bergmann S, Sparwaßer A, Struck J, Bergmann A. Sandwich immunoassay for bioactive plasma adrenomedullin. *J Appl Lab Med* 2017;2:222–233.
 175. Gayat E, Caillard A, Laribi S, Mueller C, Sadoune M, Seronde MF, Maisel A, Bartunek J, Vanderheyden M, Desutter J, Dendale P, Thomas G, Tavares M, Cohen-Solal A, Samuel JL, Mebazaa A. Soluble CD146, a new endothelial biomarker of acutely decompensated heart failure. *Int J Cardiol* 2015;199:241–247.
 176. Badoz M, Arrigo M, lung B, Amioglu G, Yilmaz MB, Meneveau N, Sadoune M, Brunette A, Mebazaa A, Seronde MF. Role of cardiovascular biomarkers for the assessment of mitral stenosis and its complications. *Eur J Intern Med* 2016;34:58–62.
 177. Badoz M, Arrigo M, Mogenet AC, Sadoune M, Meneveau N, Mebazaa A, Seronde MF. Assessment of successful percutaneous mitral commissurotomy by MRproANP and sCD146. *BMC Cardiovasc Disord* 2020;20:157.
 178. Ter Maaten JM, Kremer D, Demissei BG, Struck J, Bergmann A, Anker SD, Ng LL, Dickstein K, Metra M, Samani NJ, Romaine SPR, Cleland J, Girerd N, Lang CC, van Veldhuisen DJ, Voors AA. Bio-adrenomedullin as a marker of congestion in patients with new-onset and worsening heart failure. *Eur J Heart Fail* 2019;21:732–743.
 179. Arrigo M, Vodovar N, Nougé H, Sadoune M, Pemberton CJ, Ballan P, Ludes PO, Gendron N, Carpentier A, Chollet B, Bizouarn P, Cohen-Solal A, Singh JP, Szymonifka J, Latremouille C, Samuel JL, Launay JM, Pottecher J, Richards AM, Truong QA, Smadja DM, Mebazaa A. The heart regulates the endocrine response to heart failure: cardiac contribution to circulating neprilysin. *Eur Heart J* 2018;39:1794–1798.
 180. Self WH, Storrow AB, Hartmann O, Barrett TW, Fermann GJ, Maisel AS, Struck J, Bergmann A, Collins SP. Plasma bioactive adrenomedullin as a prognostic biomarker in acute heart failure. *Am J Emerg Med* 2016;34:257–262.
 181. Arrigo M, Parenica J, Ganovska E, Pavlusova M, Mebazaa A. Plasma bio-adrenomedullin is a marker of acute heart failure severity in patients with acute coronary syndrome. *Int J Cardiol Heart Vasc* 2019;22:174–176.
 182. Pandhi P, Ter Maaten JM, Emmens JE, Struck J, Bergmann A, Cleland JG, Givertz MM, Metra M, O'Connor CM, Teerlink JR, Ponikowski P, Cotter G, Davison B, van Veldhuisen DJ, Voors AA. Clinical value of pre-discharge bio-adrenomedullin as a marker of residual congestion and high risk of heart failure hospital readmission. *Eur J Heart Fail* 2020;22:683–691.
 183. Mentz RJ, Xu H, O'Brien EC, Thomas L, Alexy T, Gupta B, Vilario J, Lala A, DeVore AD, Dhingra R, Briassoulis A, Simon MA, Stehlik J, Rodgers JE, Dunlay SM, Abshire M, Wells QS, Barringhaus KG, Eckman PM, Lowes BD, Espinoza J, Blanco R, Shen X, Duffy CI, Hernandez AF. PROVIDE-HF primary results: Patient-Reported Outcomes in Vestigation following Initiation of Drug therapy with Entresto (sacubitril/valsartan) in heart failure. *Am Heart J* 2020;230:35–43.
 184. Camaschella C. Iron-deficiency anemia. *N Engl J Med* 2015;372:1832–1843.
 185. Grote Beverborg N, Klip IT, Meijers WC, Voors AA, Vegter EL, van der Wal HH, Swinkels DW, van Pelt J, Mulder AB, Bulstra SK, Vellenga E, Mariani MA, de Boer RA, van Veldhuisen DJ, van der Meer P. Definition of iron deficiency based on the gold standard of bone marrow iron staining in heart failure patients. *Circ Heart Fail* 2018;11:e004519.
 186. Moliner P, Jankowska EA, van Veldhuisen DJ, Farre N, Rozentryt P, Enjuanes C, Polonski L, Meroño O, Voors AA, Ponikowski P, van der Meer P, Comin-Colet J. Clinical correlates and prognostic impact of impaired iron storage versus impaired iron transport in an international cohort of 1821 patients with chronic heart failure. *Int J Cardiol* 2017;243:360–366.
 187. McDonagh T, Damy T, Doehner W, Lam CSP, Sindone A, van der Meer P, Cohen-Solal A, Kindermann I, Manito N, Pfister O, Pohjantähti-Maaroos H, Taylor J, Comin-Colet J. Screening, diagnosis and treatment of iron deficiency in chronic heart failure: putting the 2016 European Society of Cardiology heart failure guidelines into clinical practice. *Eur J Heart Fail* 2018;20:1664–1672.
 188. Toft AD, Boon NA. Thyroid disease and the heart. *Heart* 2000;84:455–460.
 189. Nanchen D, Gussseklo J, Westendorp RGJ, Stott DJ, Jukema JW, Trompet S, Ford I, Welsh P, Sattar N, Macfarlane PV, Mooijaart SP, Rodondi N, de Craen AJM. Subclinical thyroid dysfunction and the risk of heart failure in older persons at high cardiovascular risk. *J Clin Endocrinol Metab* 2012;97:852–861.
 190. Kannan L, Shaw PA, Morley MP, Brandimarto J, Fang JC, Sweitzer NK, Cappola TP, Cappola AR. Thyroid dysfunction in heart failure and cardiovascular outcomes. *Circ Heart Fail* 2018;11:e005266.
 191. Jabbar A, Pingitore A, Pearce SHS, Zaman A, Iervasi G, Razvi S. Thyroid hormones and cardiovascular disease. *Nat Rev Cardiol* 2017;14:39–55.
 192. Kattoor AJ, Goel A, Mehta JL. Thiamine therapy for heart failure: a promise or fiction? *Cardiovasc Drugs Ther* 2018;32:313–317.
 193. Jain A, Mehta R, Al-Ani M, Hill JA, Winchester DE. Determining the role of thiamine deficiency in systolic heart failure: a meta-analysis and systematic review. *J Card Fail* 2015;21:1000–1007.
 194. Keith M, Quach S, Ahmed M, Azizi-Namini P, Al-Hesayen A, Azevedo E, James R, Leong-Poi H, Ong G, Desjardins S, Lee PJ, Ravamehr-Lake D, Yan AT. Thiamin supplementation does not improve left ventricular ejection fraction in ambulatory heart failure patients: a randomized controlled trial. *Am J Clin Nutr* 2019;110:1287–1295.
 195. Bayes-Genis A, Liu PP, Lanfear DE, de Boer RA, González A, Thum T, Emdin M, Januzzi JL. Omics phenotyping in heart failure: the next frontier. *Eur Heart J* 2020;41:3477–3484.
 196. Han X, Aslanian A, Yates JR 3rd. Mass spectrometry for proteomics. *Curr Opin Chem Biol* 2008;12:483–490.
 197. Mathivanan S, Ahmed M, Ahn NG, Alexandre H, Amanchy R, Andrews PC, Bader JS, Balgley BM, Bantscheff M, Bennett KL, Björling E, Blagoev B, Bose R, Brahmachari SK, Burlingame AS, Bustelo XR, Cagney G, Cantin GT, Cardasis HL, Celis JE, Chaerkady R, Chu F, Cole PA, Costello CE, Cotter RJ, Crockett D, DeLany JP, De Marzo AM, DeSouza LV, Deutsch EW, Dransfield E, Drewes J, Droit A, Dunn MJ, Elenitoba-Johnson K, Ewing RM, Van Eyk J, Faca V, Falkner J, Fang X, Fenselau C, Figeys D, Gagné P, Gelfi C, Gevaert K, Gimble JM, Gnad F, Goel R, Gromov P, Hanash SM, Hancock WS, Harsha HC, Hart G, Hays F, He F, Hebbbar P, Helsens K, Hermeking H, Hide W, Hjerno K, Hochstrasser DF, Hofmann O, Horn DM, Hruban RH, Ibarrola N, James P, Jensen ON, Jensen PH, Jung P, Kandasamy K, Kheterpal I, Kikuno RF, Korf U, Körner R, Kuster B, Kwon MS, Lee HJ, Lee YJ, Lefevre M, Lehvaslaiho M, Lescuyer P, Levander F, Lim MS, Lobb C, Loo JA, Mann M, Martens L, Martinez-Heredia J, McComb M, McRedmond J, Mehrle A, Menon R, Miller CA, Mischak H, Mohan SS, Mohmood R, Molina H, Moran MF, Morgan JD, Moritz R, Morzel M, Muddiman DC, Nalli A, Navarro JD, Neubert TA, Ohara O, Oliva R, Omenn GS, Oyama M, Paik YK, Pennington K, Pepperkok R, Periaswamy B, Petricoin EF, Poirier GG, Prasad TS, Purvine SO, Rahiman BA, Ramachandran P, Ramachandra YL, Rice RH, Rick J, Ronnholm RH, Salonen J, Sanchez JC, Sayd T, Seshi B, Shankari K, Sheng SJ, Shetty V, Shivakumar K, Simpson RJ, Sirdeshmukh R, Siu KW, Smith JC, Smith RD, States DJ, Sugano S, Sullivan M, Superti-Furga G, Takatalo M, Thongboonkerd V, Trinidad JC, Uhlen M, Vandekerckhove J, Vasilescu J, Veenstra TD, Vidal-Taboada JM, Viñhien R, Wait R, Wang X, Wiemann S, Wu B, Xu T, Yates JR, Zhong J, Zhou M, Zhu Y, Zurbig P, Pandey A. Human proteome data enables sharing of human protein data. *Nat Biotechnol* 2008;26:164–167.
 198. Dos Remedios CG, Liew CC, Allen PD, Winslow RL, Van Eyk JE, Dunn MJ. Genomics, proteomics and bioinformatics of human heart failure. *J Muscle Res Cell Motil* 2003;24:251–260.
 199. Wilkins MR, Appel RD, Van Eyk JE, Chung MCM, Görg A, Hecker M, Huber LA, Langen H, Link AJ, Paik YK, Patterson SD, Pennington SR, Rabilloud T, Simpson RJ, Weiss W, Dunn MJ. Guidelines for the next 10 years of proteomics. *Proteomics* 2006;6:4–8.
 200. Domanski D, Percy AJ, Yang J, Chambers AG, Hill JS, Freue GVC, Borchers CH. MRM-based multiplexed quantitation of 67 putative cardiovascular disease biomarkers in human plasma. *Proteomics* 2012;12:1222–1243.
 201. Toma M, Mak GJ, Chen V, Hollander Z, Shannon CP, Lam KKY, Ng RT, Tebbutt SJ, Wilson-McManus JE, Ignaszewski A, Anderson T, Dyck JRB, Howlett J, Ezekowitz J, McManus BM, Oudit GY. Differentiating heart failure phenotypes using sex-specific transcriptomic and proteomic biomarker panels. *ESC Heart Fail* 2017;4:301–311.
 202. Percy AJ, Michaud SA, Jardim A, Sinclair NJ, Zhang S, Mohammed Y, Palmer AL, Hardie DB, Yang J, LeBlanc AM, Borchers CH. Multiplexed MRM-based assays for the quantitation of proteins in mouse plasma and heart tissue. *Proteomics* 2017;17:1600097.
 203. Shah S, Henry A, Roselli C, Lin H, Sveinbjörnsson G, Fatemifar G, Hedman ÅK, Wilk JB, Morley MP, Chaffin MD, Helgadóttir A, Verweij N, Dehghan A, Almgren P, Andersson C, Aragam KG, Ärnlöv J, Backman JD, Biggs ML, Bloom HL, Brandimarto J, Brown MR, Buckbinder L, Carey DJ, Chasman DI, Chen X, Chen X, Chung J, Chutkow W, Cook JP, Delgado GE, Denaxas S, Doney AS, Dörr M, Dudley SC, Dunn ME, Engström G, Esko T, Felix SB, Finan C, Ford I, Ghanbari M, Ghasemi S, Giedraitis V, Giulianini F, Gottdiener JS, Gross S, Guðbjartsson DF, Gutmann R, Haggerty CM, van der Harst P, Hyde CL, Ingelsson E, Jukema JW, Kavousi M, Khaw KT, Kleber ME, Køber L, Koekemoer A,

- Langenberg C, Lind L, Lindgren CM, London B, Lotta LA, Lovering RC, Luan J, Magnusson P, Mahajan A, Margulies KB, März W, Melander O, Mordi IR, Morgan T, Morris AD, Morris AP, Morrison AC, Nagle MW, Nelson CP, Niessner A, Niiranen T, O'Donoghue ML, Owens AT, Palmer CNA, Parry HM, Perola M, Portilla-Fernandez E, Psaty BM, Regeneron Genetics Center, Rice KM, Ridker PM, Romaine SPR, Rotter JJ, Salo P, Salomaa V, van Setten J, Shalaby AA, Smelser DT, Smith NL, Stender S, Stott DJ, Svensson P, Tammesoo ML, Taylor KD, Teder-Laving M, Teumer A, Thorgeirsson G, Thorsteinsdottir U, Torp-Pedersen C, Trompet S, Tyl B, Uitterlinden AG, Veluchamy A, Völker U, Voors AA, Wang X, Wareham NJ, Waterworth D, Weeke PE, Weiss R, Wiggins KL, Xing H, Yerges-Armstrong LM, Yu B, Zannad F, Zhao JH, Hemingway H, Samani NJ, McMurray JJV, Yang J, Visscher PM, Newton-Cheh C, Malarstig A, Holm H, Lubitz SA, Sattar N, Holmes MV, Cappola TP, Asselbergs FW, Hingorani AD, Kuchenbaecker K, Ellinor PT, Lang CC, Stefansson K, Smith JG, Vasani RS, Swerdlow DI, Lumbers RT, Genome-wide association and Mendelian randomisation analysis provide insights into the pathogenesis of heart failure. *Nat Commun* 2020;**11**:163.
204. Rossing K, Bosselmann HS, Gustafsson F, Zhang ZY, Gu YM, Kuznetsova T, Nkuiipou-Kenfack E, Mischak H, Staessen JA, Koeck T, Schou M. Urinary proteomics pilot study for biomarker discovery and diagnosis in heart failure with reduced ejection fraction. *PLoS One* 2016;**11**:e0157167.
205. Farmakis D, Koeck T, Mullen W, Parissis J, Gogas BD, Nikolaou M, Lekakis J, Mischak H, Filippatos G. Urine proteome analysis in heart failure with reduced ejection fraction complicated by chronic kidney disease: feasibility, and clinical and pathogenetic correlates. *Eur J Heart Fail* 2016;**18**:822–829.
206. Horgan SJ, Watson CJ, Glezeva N, Collier P, Neary R, Tea IJ, Corrigan N, Ledwidge M, McDonald K, Baugh JA. Serum amyloid P-component prevents cardiac remodeling in hypertensive heart disease. *J Cardiovasc Transl Res* 2015;**8**:554–566.
207. Wang X, Abraham S, McKenzie JAG, Jeffs N, Swire M, Tripathi VB, Luhmann UFO, Lange CAK, Zhai Z, Arthur HM, Bainbridge JWB, Moss SE, Greenwood J. LRG1 promotes angiogenesis by modulating endothelial TGF- β signalling. *Nature* 2013;**499**:306–311.
208. Michelhaugh SA, Camacho A, Ibrahim NE, Gaggin H, D'Alessandro D, Coglianese E, Lewis GD, Januzzi JL. Proteomic signatures during treatment in different stages of heart failure. *Circ Heart Fail* 2020;**13**:e006794.
209. Khera AV, Chaffin M, Aragam KG, Haas ME, Roselli C, Choi SH, Natarajan P, Lander ES, Lubitz SA, Ellinor PT, Kathiresan S. Genome-wide polygenic scores for common diseases identify individuals with risk equivalent to monogenic mutations. *Nat Genet* 2018;**50**:1219–1224.
210. Saracyn M, Kisiel B, Bacht A, Franaszczyk M, Brodowska-Kania D, Żmudzki W, Szymański K, Sokalski A, Klatko W, Stopiński M, Grochowski J, Papliński M, Goździk Z, Niemczyk L, Bober B, Kołodziej M, Tłustochowicz W, Kamiński G, Płoski R, Niemczyk S. Value of multilocus genetic risk score for atrial fibrillation in end-stage kidney disease patients in a polish population. *Sci Rep* 2018;**8**:9284.
211. Said MA, Verweij N, van der Harst P. Associations of combined genetic and lifestyle risks with incident cardiovascular disease and diabetes in the UK Biobank study. *JAMA Cardiol* 2018;**3**:693–702.
212. Felker GM, Anstrom KJ, Adams KF, Ezekowitz JA, Fiuzat M, Houston-Miller N, Januzzi JL, Mark DB, Piña IL, Passmore G, Whellan DJ, Yang H, Cooper LS, Leifer ES, Desvigne-Nickens P, O'Connor CM. Effect of natriuretic peptide-guided therapy on hospitalization or cardiovascular mortality in high-risk patients with heart failure and reduced ejection fraction: a randomized clinical trial. *JAMA* 2017;**318**:713–720.
213. Evans JDW, Dobbin SJH, Pettit SJ, Di Angelantonio E, Willeit P. High-sensitivity cardiac troponin and new-onset heart failure: a systematic review and meta-analysis of 67,063 patients with 4,165 incident heart failure events. *JACC Heart Fail* 2018;**6**:187–197.