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Review Article — *Special issue: Heart Failure*

New biomarkers and heart failure

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ABSTRACT

Heart failure is a major health problem with an increasing incidence and prevalence of the disease. The role of both established natriuretic peptides: B-type natriuretic peptide (BNP) and N-terminal prohormone pro-brain natriuretic peptide (NT-proBNP) in acute and chronic heart failure (HF) has been intensively studied. Its testing is routine in clinical practice for diagnosis and prognosis in HF. However, increased clarification and understanding of the interplay in the pathophysiology of HF revealed several new potential cardiac biomarkers. These novel biomarkers soluble ST2, galectin, copeptin and, mid-regional fragment of pro-adrenomedullin (MR-proADM) may aid in the diagnostic and prognostic evaluation of acute and chronic heart failure.

Contents

1. Introduction

Heart failure (HF) represents an increasing problem worldwide and has an advancing trend, predominantly in elderly patients. The prevalence rate in the general population is 0.4–2.0% and rapidly increases with age [1]. At the age of 50, the prevalence rate is about 1%, whilst at the age of 80 and above, almost one out of 10 people will suffer from heart failure [2].

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Table 1 – Properties of biomarkers.

1.	Accurate, reproducible, standardized measurements
2.	Reasonable cost, short setting time
3.	Biomarkers provide information that is not available from clinical assessment
4.	Knowing the measured level to aid in medical decision making
5.	High sensitivity and specificity

Table 2 – Classification of biomarkers.

Biomarkers in heart failure
Inflammation
• C-reactive protein
• Tumour necrosis factor
• Interleukins 1, 6 and 18
Oxidative stress
• Oxidized low-density lipoproteins
• Myeloperoxidase
• Urinary biopyrrins
• Urinary and plasma isoprostanes
• Plasma malondialdehyde
Hormones
• Norepinephrine
• Renin
• Angiotensin II.
• Aldosterone
• Copeptin
• Endothelin
Myocyte injury
• Troponins T and I
• Creatine kinase MB fraction
• Heart-type fatty-acid protein
• Myosin light-chain kinase I
Myocyte stress
• Brain natriuretic peptide
• N-terminal pro-brain natriuretic peptide
• Midregional fragment of proadrenomedullin
Matrix remodelling
• ST2
• Galectin

After establishing a HF diagnosis, nearly 60% of men and 45% of women will die within 5 years [3].

HF is the clinical syndrome, in which the heart fails to pump blood at a rate commensurate with the requirements of the metabolizing tissues or is able to do so only with an elevated diastolic filling pressure. HF is often a clinically silent process, with progressive cardiac remodelling that eventually leads to symptomatic presentation late in the course of disease progression.

Clinical assessment and management is the keystone of patients with HF, but has its limitations. Physicians have used additional tools to aid clinical assessment, which are helpful in making an accurate and prompt diagnosis, and effectively prognosticate, treat and better identify high-risk subjects. Biomarkers are one such tool.

A biomarker is a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention. A biomarker should fulfil certain criteria to be useful clinically [4] (Table 1).

In current guidelines for heart failure it is recommended to test BNP or its precursor NT-proBNP. [5]. But measurement of BNP and NT-proBNP has its own limitations. A variety of clinical factors influence level of natriuretic peptides including the age and sex of the individual [6,7,8], renal function [9,10], body mass index [11], thyroid function [12] and anaemia [10]. In respect to these drawbacks, research and trials are needed to find convenient biomarkers suitable for clinical practice. In the article below we discuss more novel cardiac biomarkers in HF and their applications in clinical practice (Table 2).

1.1. *Biomarkers of inflammation*

The presence of inflammatory processes in HF has been verified [13]. Clinical studies revealed that many inflammatory cytokines such as tumour necrosis factor alpha (TNF-α), and at least three interleukins 1, 6 and 18 are elevated in HF. These cytokines are produced by nucleated cells in the heart [14]. Experimental studies have shown that each cytokine has various effects on cardiac function [15,16].

IL-6, TNF- α and IL-18 activate intracellular signalling pathways and have various effects on remodelling, hypertrophy and apoptosis in HF [16]. IL-6 binds to plasma membrane receptor complexes containing the receptor chain gp130. The IL-6-gp130 complex is known to activate two major signalling pathways, and these play important roles in cardiac development, hypertrophy, protection and remodelling in response to physiological and pathological stimuli. It has been reported that elevated serum levels of IL-6 cytokines and gp130 proteins are strong prognostic markers for morbidity and mortality in patients with HF or after a myocardial infarction [17,18]. Matsumoto et al. [16] described that serum IL-6 and high-sensitivity C-reactive protein (hs-CRP) concentrations were more elevated in acute cardiac decompensation in patients with left ventricular (LV) systolic dysfunction compared to patients with preserved LV ejection fraction. Further, it has been reported that concentrations of IL-6 are higher in patients with asymptomatic LV systolic dysfunction compared to patients with normal LV function. IL-6 negatively correlated with LV ejection fraction and the diagnostic value of IL-6 (in this study) is able to predict the progress of heart failure [19].

The effect of TNF-α on cardiomyocytes is mediated by two types of TNF-α cell surface receptors, namely TNF receptor (TNFR) 1 and TNFR 2. In HF activation, TNFR 1 has proapoptotic effects—facilitated cardiac remodelling and apoptosis in cardiomyocytes, while stimulation of TNFR 2 has an anti-apoptotic effect [20]. The fragment of the extracellular part of both receptors is possible to detect in blood as soluble forms, sTNFR-1 and sTNFR-2, and their blood levels are elevated in patients with severe HF [21]. These soluble cytokine receptors

modulate the activity of TNF-α, namely they neutralize the effect of TNF- α.

Rivera et al. [22] determined urinary levels of sTNFR-1 and sTNFR-2 in patients with HF. This study showed that plasma and urinary levels of TNF receptors were increased in patients with higher NYHA classes. In clinical trials of severe systolic dysfunction, elevated TNF- α and IL-6 were associated with increased mortality [23].

Dunlay et al. [24] studied the level of TNF- α in patients with both preserved and reduced ejection fraction (EF). The results showed that elevated TNF-α is independently associated with mortality in patients with heart failure regardless of EF. This biomarker is suitable for risk prediction in all categories of HF.

HF is linked with inflammatory response and hs-CRP as a marker of inflammation modulates the disease process. CRP is synthesized by the liver in response to numerous stimuli, for example proinflammatory cytokine IL-6. CRP attenuates nitric oxide production in endothelial cells, increases production of endothelin-1 and the expression of endothelial adhesion molecules [14,25]. These results show that CRP directly negatively influences vascular endothelium. Multivariate analysis indicated that an increased hs-CRP level is an independent predictor of adverse outcomes in patients with acute or chronic heart failure [26].

1.2. *Oxidative stress*

Oxidative stress is characterized by an imbalance between the formation of reactive oxygen species (ROS) and endogenous antioxidant mechanisms. Under physiological conditions, low concentrations of ROS (including superoxide anion, hydrogen peroxide and hydroxyl radical) have beneficial effects. ROS influence processes of cellular response to infectious agents, participate in cell signalling and as a stimulus of mitogenesis response [27,28]. Overproduction of ROS may result in pathological consequences such as damage to cell structures, including lipids, DNA and proteins.

Oxidative stress plays a role in the aetiology of cardiovascular diseases such as atherosclerosis, ischaemic heart disease, hypertension and heart failure [27]. An obvious mechanism through which myocardial oxidative stress might impair cardiac function is oxidative damage to cellular proteins and membranes, thereby inducing cellular dysfunction or death through apoptosis and necrosis. In the heart, further effects of ROS can influence extracellular matrix remodelling through the activation of the matrix metalloproteinases (MMPs) [29].

MMPs are a family of protease enzymes capable of degrading all the matrix components of the heart. ROS modulate fibroblast proliferation, collagen synthesis, activate MMPs and also increase MMPs expression [29].

The superoxide anions contribute to vascular endothelial dysfunction. Superoxide anions are a potent inactivator of nitric oxide (NO), it results in the reduction of NO bioavailability. Furthermore, the reaction between NO and superoxide generates peroxynitrite, which is itself a potent and very reactive ROS [30].

Inflammatory cells, mitochondria, xanthine oxidase and the family complex of enzymes termed NADPH oxidases are sources of ROS. Several factors which are involved in pathophysiology of HF, such as angiotensin II, α-adrenergic agonists, endothelin-1, tumour necrosis factor-α can stimulate ROS production by NADPH oxidases [31]. In vitro studies revealed a pivotal role of NADPH oxidase in angiotensin II-induced cardiac hypertrophy and interstitial fibrosis by using gene-modified mice with defective NADPH oxidase activity [32]. But it should be noted that ROS production by the higher activity of an enzymatic source can modulate or trigger the activity of other ROS sources (e.g., NADPH oxidase can drive ROS production by NO synthase).

Direct markers of oxidative stress have not been fully satisfactorily established and their measurement is difficult. In clinical practice we can determine indirect markers of oxidative stress of heart failure, including plasma levels of myeloperoxidase [33] and levels of isoprostane—specifically 8-iso-PGF2α evaluated in the pericardial fluid [34]. The levels of both markers correlate with the severity of heart failure. In addition, with impaired hemodynamics in patients with HF [35] and independently predict an adverse prognosis in patients with moderate-to-severe heart failure [36]. Elevated levels of uric acid are associated with increased xanthine oxidase activity, which is a source of ROS. So uric acid is a simple, useful, but nonspecific indicator of enhanced oxidative stress. ROS in fact exert multiple effects in the pathophysiology of HF and the elucidation of all these processes should be helpful in the therapeutic approach. Statins appear to have a “cardioprotective” role not only by their effect of lowering cholesterol but also by improving NO-dependent vasodilation through attenuation of endothelial superoxide anion radical formation [37]. On the basis of this information statin administration can decelerate pathological processes conditioned by ROS.

2. Hormones, copeptin

Copeptin is cosynthesized with vasopressin (AVP), also known as antidiuretic hormone. Copeptin is a glycopeptide consisting of 39 amino-acids. It is the C-terminal part of pro-AVP, synthesized with AVP in the hypothalamus and released from the neurohypophysis upon hemodynamic or osmotic stimuli [38]. Physiologic functions of AVP are mediated through different receptors (R) subtypes V1, V2, V3, oxytocin receptor (OTR) and P2-purinergic receptor (P2R). V1 receptors are found in vascular smooth muscle, cardiac myocytes and cause vasoconstriction and a positive ionotropic effect. The antidiuretic effect of vasopressin occurs via the activation of V2R and the activation of V3R causes the secretion of adrenocorticotropic hormone (ACTH) from the anterior pituitary cells.

Oxytocin receptors (OTR) are nonselective vasopressin receptors. The OTR has an equal affinity for vasopressin and oxytocin, whereas the V1R has a 30-fold higher affinity for vasopressin than for oxytocin [39]. OTR have been localized in many reproductive and nonreproductive tissues [40]. Importantly, OTR exist in a high density on vascular endothelium and are responsible for vasodilatation mediated by releasing nitric oxide. Whether vasopressin causes vasoconstriction or vasodilatation depends on the vascular beds

studied and therefore depends on the receptor density (V1 versus OTR) [41]. OTR are also in the heart and their activation stimulates the release of natriuretic peptide, which is involved in natriuresis, regulation of blood pressure and cell growth [42].

It has been shown that vasopressin exerts cardiac effects also through the activation of P2Rs expressed on endothelium. This animal in vitro study showed a negative ionotropic effect and coronary vasoconstriction [43,39]. Influences of V1R in the heart result in a positive ionotropic effect [44] so studies of ionotropic effects of vasopressin and its effect on coronary arteries are controversial. Clinical studies of low-doses of vasopressin do not demonstrate adverse cardiac effects of vasopressin [45]. It has been supposed that the vasoconstrictor effect of vasopressin on coronary vessels, as well as its effect on myocardium, may be dependent on oxygen tension—which is significantly attenuated during hypoxia [46].

Recently reported studies have shown, that copeptin levels predict outcomes in several medical conditions such as acute exacerbation of chronic obstructive pulmonary disease [47], ischaemic stroke [48] and even predict neurological outcomes and mortality in cardiac arrest survivors [49]. Vasopressin concentrations are increased in patients with heart failure [50]. Vasopressin is involved in the adverse cardiac remodelling by acting V1R. Stimulation of this receptor leads to increased myocyte contractile protein synthesis, development of myocardial hypertrophy [51] and then stimulates cardiac fibroblast, resulting in increased myocardial fibrosis [52].

Stimulation of V1R in vascular smooth muscle increases systemic vascular resistance, increasing impedance to ventricular emptying (afterload) and thereby negatively affecting ventricular function. Sustained increased afterload also affects myocardial remodelling. Activation of V2R increases water retention. Upregulation of these receptors results in an increased movement of water from kidneys into the plasma, which leads to increased water retention. This effect, if sustained, may contribute to volume expansion that exacerbates diastolic wall stress, ventricular remodelling and dysfunction. V2R—mediated water reabsorption may participate in hyponatremia, depending on the balance between regulated water and sodium intake and excretion [53].

AVP has more limitations when determining its level in plasma. More than 90% of vasopressin in the circulation is bound to platelets and there is a possibility of underestimating the amount of AVP, since AVP is rapidly cleared from the circulation (half-life 24 min). AVP is unstable in isolated plasma, even when stored at −20 °C.

Copeptin has fewer limitations and is therefore an ideal mirror reflecting AVP release [54,55]. In several studies copeptin has been reported to be a useful biomarker, not only in patients with chronic HF but also in patients with post-acute myocardial infarction (MI).

Stoiser et al. [56] first showed that copeptin is an excellent predictor of outcome in advanced heart failure patients. Its value is superior to that of BNP in predicting death and a combined endpoint (death, re-hospitalization due to heart failure), although BNP was still suitable for predicting chronic heart failure (CHF) re-hospitalization.

Nuehold et al. [57] reported that copeptin levels escalate with NYHA. In patients with NYHA II and III, copeptin was not only found to be the most potent single predictor of mortality but was superior to BNP and NT-proBNP. For patients with NYHA IV, copeptin provided independent additional information, but was inferior to sodium levels and especially glomerular filtration.

In post-acute MI cases, copeptin was a significant independent predictor of death or heart failure compared to event-free survivors. Copeptin levels were higher in STEMI versus non-STEMI patients and in those with Killip class above 1. Plasma copeptin correlated with NT-proBNP. In the present study, 706 healthy volunteers were recruited from a local HF screening study. Participants with a history of cardiovascular disease were excluded from the study. Copeptin levels were significantly higher in the male volunteers compared with the females. In males, copeptin was correlated with eGFR (estimated glomerular filtration rate). In females, the correlation of copeptin with eGFR was weak [58].

Kelly et al. [59] in a study with subjects with myocardial infarction confirmed the association between copeptin and left ventricular ejection fraction, volumes, remodelling and clinical heart failure post-acute MI.

Mason et al. [60] measured the plasma concentrations of four stable precursor fragments of neurohormonal systems in patients with chronic HF. 1237 patients with chronic and stable HF were involved in the study. The following four precursor fragments, mid-regional pro-atrial natriuretic peptide (MR-proANP), mid-regional pro-adrenomedullin (MR-proADM), C-terminal pro-endothelin-1 (CT-proET-1) and C-terminal pro-vasopressin (CT-proAVP or copeptin), were measured at randomization and after 3 months. It examined the prognostic performance of these biomarkers which were compared with the well-established B-type natriuretic peptides (BNP and NT-proBNP). Measurement of stable precursor fragments of vasoactive peptides provided prognostic information independent of natriuretic peptides which are currently the best biomarkers for risk stratification.

In summary, elevated plasma levels of AVP have been associated with a poor prognosis in patients with chronic HF and its measurement improves the prognostic evaluation of these patients.

2.1. *Myocyte stress, adrenomedullin*

Adrenomedullin (AM) is a potent vasoactive peptide, originally isolated from human pheochromocytoma cells [61]. Subsequently AM has been discovered in many tissues including adrenal medulla, brain, lung, kidney, gastrointestinal organs, or heart—cardiomyocyte, fibroblasts and its mRNA are highly expressed in endothelial cells [62,63].

AM is a member of the calcitonin gene-related peptide (CGRP) superfamily, which includes calcitonin, calcitonin-gene related peptides α and β (CGRP α, CGRP β), amylin and intermedin, called adrenomedullin 2. This group of peptide hormones is necessary for haemostasis in diverse tissues [64]. The adrenomedullin gene is located on human chromosome 11, encoding a 185-amino acid pre-pro-hormone, pre-pro-adrenomedullin. Following the cleavage of the 21-residue N-terminal signal peptide, a 164-aminoacid pro-AM peptide is

generated. Next fission originates in two particular biologically active peptides: AM and proAM N-terminal 20 peptide (PAMP) [65]. PAMP evokes hypotension by inhibiting peripheral sympathetic nerve activity and reduces sympathetic tone [66].

The mature molecule of AM is a 52-amino acid peptide. AM possess many important physiological properties and mediates its activities through binding to a complex receptor composed of the calcitonin receptor like-receptor (CRLR) associated with receptor activity modifying proteins RAMP-2 and RAMP-3. RAMP isoforms determine the ligand selectivity [67].

AM has pleiotropic effects: vasodilatory, positive ionotropic, antiapoptotic, suppresses the renin–angiotensin–aldosterone system, in kidney induces diuresis and natriuresis, protects against oxidative stress as described by Ishimitsu et al. [68]. Considerable evidence exists for the previously described positive ionotropic effect of AM mediated by increased intracellular cAMP [69]. Infusion of AM increases cardiac output and reduces pulmonary wedge pressure with little effect on heart rate and blood pressure, thereby resulting in increases in urine volume and natriuresis [70]. These positive effects may be mediated by decreased afterload due to peripheral vasodilatation and by the positive ionotropic effect.

Adrenomedullin has been found to be increased in patients with essential hypertension, renal failure, cardiac hypertrophy [71], heart failure [72], acute myocardial infarction [73,74], sepsis [75] and community-acquired pneumonia [76]. Plasma ADM concentration has a positive correlation with circulating ADM and plasma creatinine levels [71]. These pathological conditions increase production of AM and its measurement should be useful in clinical practice.

The production of AM has been shown to be stimulated by both cardiac pressure and overload.

Pousset et al. [72] assessed plasma adrenomedullin in ambulatory patients ($n=117$) with chronic heart failure and found increasing plasma levels of AM with increasing NYHA class. Immunoreactive AM plasma levels were similar in men and women, did not correlate with age and were not influenced by the aetiology of heart failure (ischaemic vs. idiopathic aetiology). No correlation has been found between ejection fraction and plasma levels of AM.

AM was an independent predictor of prognosis in predominantly mild to moderate chronic HF.

Reliable quantification of AM is impaired by the fact that mature AM has a short plasma half-life of only ~22 min [77], creates a complex with complement factor H and is rapidly cleared from the circulation.

In clinical practice, the more stable mid-regional fragment of pro-adrenomedullin (MR-proADM) is determined, which directly reflects the levels of the rapidly degraded active peptide AM [78].

Klip et al. [79] evaluated the prognostic value of MR-proADM in a subset of 214 patients with heart failure after an acute MI from the OPTIMAAL study and compared this with BNP and NT-proBNP. MR-proADM was a promising biomarker and had a strong prognostic value for mortality and morbidity in patients with HF after an acute MI. In this study, MR-proADM had stronger predictive value than BNP and NT-proBNP. Kahn et al. [74] assessed the prognostic impact of MR-proADM after an acute MI and compared it with NTproBNP.

The AM system is activated after MI and is a powerful predictor of death and heart failure, especially in combination with an elevated NTproBNP giving additive prognostic information.

In patients with chronic HF MR-proADM is an independent predictor of mortality, which adds prognostic information to NT-proBNP. In this study MR-proADM correlated with age, creatinine and NYHA class, but not with LVEF. In contrast a strong correlation was observed between NT-proBNP and LVEF [80].

The prognostic value of MR-proADM for predicting 90-day mortality in patients with acute heart failure was confirmed in the prospective trial BACH (Biomarkers in Acute Heart Failure) [81]. Then Shah et al. [82] confirmed the prognostic value of MR-proADM in patients with acute heart failure.

In summary, measurement of MR-proADM provided a reliable predictor of cardiovascular death and heart failure.

2.2. *Matrix remodelling, ST2*

Protein ST2 has a pluripotent role, and participates importantly in immunologic processes, as well as in the fibrotic heart response to injury. ST2 belongs to the interleukin-1 (IL-1) receptor family [83]. The ST2 gene is located on human chromosome 2 and is a part of the human IL-1 gene locus. The ST2 gene encodes two isoforms of ST2 protein: transmembrane (ST2L) and soluble, circulating (sST2) isoforms [84]. These different isoforms arise by alternative modifications within the transcript of the ST2 gene [84].

Both sST2 and ST2L are induced in cardiomyocytes and fibroblasts by biomechanical stress [83]. sST2 is considered a novel biomarker for cardiac strain.

ST2L is composed of three immunoglobulin (IgG) extracellular domains, a transmembrane segment and an intracellular domain that mediates intracellular signalling [84,85]. ST2L is responsible for positive feedback in immunologic processes through activated type 2 T-helper cells (Th2) and mast cells. ST2L is expressed by Th2 cells, but not expressed by type 1 helper T cells [86].

The soluble isoform of ST2 lacks transmembrane and cytoplasmatic domain and can be detected in serum [85]. Protein interleukin 33 (IL-33) is a functional ligand for ST2 [87].

The finding of IL-33 as a ligand for ST2 has clarified the role of IL-33/ST2 signalling in the myocardium. IL-33 is induced by mechanical strain predominantly in cardiac fibroblasts.

IL-33 potently blocked cardiomyocyte hypertrophy induced by either angiotensin II or phenylephrin [85]. The sST2 protein abrogates the antihypertrophic effect of IL-33, because it operates as a soluble decoy receptor by binding IL-33 and in this way blocks preventive ST2L signalling. In studies blocking the ST2L receptor by anti-ST2L monoclonal antibodies has resulted in the blockage of the antihypertrophic effect of IL-33. Furthermore, targeted deletion of the ST2 gene in mice (ST2-/-mice) enhanced cardiac hypertrophy and fibrosis following mechanical overload and impaired contractility and survival, while administration of

recombinant IL-33 improved pathological changes and survival in wild type mice, but not in ST2-/-mice. These data show that IL33/ST2 signalling protects the myocardium under mechanical overload. IL33/ST2 signalling is a cardioprotective fibroblast–cardiomyocyte paracrine system and soluble ST2 blocked the antihypertrophic effect of IL-33 [85].

Seki et al. [88] in an experiment with rat myocytes revealed that IL-33 decreased caspase-3 activation—an important step in the apoptotic cascade and increased expression of the antiapoptotic gene Bcl-2. These antiapoptotic effects were attenuated by sST2.

In clinical practice, measurements of sST2 in subjects with HF should bring helpful insights into the biological process that leads to adverse outcomes. sST2 concentrations are positively associated with sex, age, systolic blood pressure (more notably in men) and diabetes in individuals without heart failure [89].

Januzzi et al. [90] evaluated a group of 593 patients admitted to the emergency department with acute dyspnoea with or without HF in the Pro-BNP Investigation of Dyspnoea in the Emergency Department (PRIDE) study. The patients were studied for one year. In this analysis, serum concentration of sST2 was higher in patients diagnosed with acute destabilized HF, compared with those without cardiac causes of dyspnoea. Higher concentrations of sST2 were associated with a greater likelihood of HF diagnosis. Importantly, the prognostic meaning of sST2 was considerable: concentrations of the marker were higher in patients who were dead at 1 year compared with survivors [90]. There was a dose-dependent relationship between sST2 concentrations and risk of death at 1 year, and in multivariate regression analysis for predictors of death at 1 year. An sST2 concentration greater than 0.20 ng/ml strongly predicted 1-year mortality in patients with and without HF.

In addition, the prognostic value of sST2 was additive to that of NT-proBNP, in such a way that patients with elevations in both NT-proBNP and sST2 experienced the highest rate of mortality in 1 year. Subjects with low values for both markers had the best short-term prognosis. This signification between sST2 and NT-proBNP with prognosis holds for up to 4 years from presentation.

Rehman et al. [91] in a study of 346 patients with acute HF from the PRIDE study examined the association between sST2 concentrations and clinical characteristics and prognosis. sST2 value correlated with the severity of HF assessed by NYHA, left ventricular ejection fraction, creatinine clearance, B-type natriuretic peptide, amino terminal B-type natiuretic peptide and C-reactive protein. ST2 was not associated with previous HF, age, body mass index, atrial fibrillation, or aetiology of cardiomyopathy (ischaemic vs. nonischemic).

Shah et al. [92] described the relationship between sST2 levels and cardiac structure and function measured by echocardiography in long-term mortality of 139 patients with acute dyspnoea. Subjects had detailed 2-D echocardiography at admission (median 45 hours after admission) with a follow-up 4 years later. sST2 levels were associated with higher LV-endsystolic area and volume, end-systolic dimension but not with left atrial dimension or volume.

sST2 was inversely related to LV ejection fraction and RV fractional area change; sST2 was also associated with a higher RV systolic pressure, more severe tricuspid regurgitation, and a higher frequency of RV hypokinesis. sST2 was negatively correlated with tissue Doppler E wave peak velocity but not with other traditional markers of diastolic dysfunction. sST2 was higher in non-survivors at 4 years versus survivors. sST2 also predicted death at 4 years independently of other traditional clinical, biochemical and echocardiographic markers of risk. This small study confirmed an association between sST2 and ventricular remodelling and long-term mortality independent of other markers of risk.

In summary, products of the ST2 gene with ligand IL-33 modulate heart remodelling via effects on apoptosis, inflammation and fibrosis. sST2 appears to be a biomarker for remodelling. The circulating level of sST2 is correlated with short and long-term post-discharge mortality in acute and chronic heart failure.

2.3. *Matrix remodelling, galectin 3*

Galectin 3 (Gal-3) is a member of the lectins family. Lectins are proteins that specifically interact with carbohydrate sugars. The interactions between lectins and their target carbohydrate sugars occur via a carbohydrate recognition domain (CRD) within the lectin. Galectins are a subfamily of lectins that have a CRD that bind specifically to β-galactoside sugar molecules. CRDs typically contain 130 amino acids. Currently 15 members of the galectins family are known and, they can be divided into three subclasses, those with one CRD (galectins 1, 2, 5, 7, 10, 11, 13, 14, and 15), those with two CRDs (galectins 4, 6, 8, 9 and 12) and the third subclass contains galectin-3. Galectin-3 has a unique chemical structure for the lectin family with a non-lectin N-terminal region (about 120 amino acids) connected to CRD [93]. Their presence is necessary for the full biological activity of Gal-3, which makes it able to bind with extracellular matrix proteins and cell surface receptors [94].

Galectins can bind to cell surface—receptors and, antigens and extracellular matrix glycans [93]. It seems that galectins do not have specific individual receptors [95], but each can bind to a set of cell surface or extracellular matrix glycoproteins containing suitable oligosaccharides. It has been shown that galectins play important roles in multiple physiological and pathological processes, including tumour development and progression [96], immune and inflammatory responses [97], neural degeneration, atherosclerosis, diabetes, and wound repair as reviewed previously [93].

Galectin family members do not contain classical signal sequences, however they can be secreted and thus belong to the group of proteins that do not contain a signal sequence but can function outside cells [98]. The secretion into extracellular space can enable Gal-3 to interact with cell surface receptors and antigens to trigger transmembrane signalling cascades for different cellular functions. Expression of Gal-3 has been found in the cytoplasm and the nucleus of macrophages, eosinophils, neutrophils and mast cells [99].

In tissues, Gal-3 is plentifully detected in spleen, lung, stomach, colon, adrenal gland, uterus and ovary. It is expressed in heart, kidney, pancreas, liver, but in a lower amount [100]. However a low expression level of Gal-3 can change depending on the various pathophysiologic conditions. If the disease progresses, gal-3 is significantly up-regulated. Cardiac remodelling is an essential feature of heart

failure, and it is linked to disease progression. Recently, a role for Gal-3 in cardiac remodelling and in the pathophysiology of heart failure has been suggested. Fibrosis is a pivotal process in maladaptive cardiac response. Fibroblasts and macrophages are responsible for the initiation and progression of tissue fibrosis [101,102].

The up-regulation of Gal-3 has been found in different human pathological states, such as in liver cirrhosis [103], idiopathic lung fibrosis [104], chronic pancreatitis [105] and in cardiac fibrosis [106].

Fibroblast activation is defined by increased expression of cytoskeletal protein α smooth muscle actin (α-SMA—an intracellular fibrosis marker) and the extracellular type 1 collagen α-1 chain (COL1A1, an extracellular fibrosis marker). Both α-SMA and COL 1 A1 are up-regulated in fibrotic tissue via Gal-3-mediated activation. It affects the synthesis of new matrix, and conversely influences degradation of extracellular matrix components by a set of tissue inhibitor metalloproteinases [107].

Gal-3 is positively associated with age and, body mass index, negatively associated with eGFR and concentrations are higher in women (14.3 ng/ml) compared with men (13.1 ng/ml) [108].

Sharma et al. [106] evaluated Gal-3 in rat and in human subjects. This group studied homozygous Ren-2 rats that exhibited overexpression of the murine Ren-2d renin gene, resulting in severe hypertension with end-organ damage. Myocardial biopsies obtained at an early stage of hypertrophy before apparent HF showed that expression of Gal-3 was increased specifically in the rats that later rapidly developed HF. Gal-3 colocalized with activated myocardial macrophages. They found Gal-3-binding sites in rat cardiac fibroblasts and the extracellular matrix. Furthermore, Sharma et al. showed that infusion with Gal-3 in the pericardial sac of normal, healthy rats led to the development of cardiac remodelling with dysfunction and elevated expression of collagens.

In human subjects, obtained biopsies from patients undergoing aortic valve replacement for aortic stenosis with preserved or depressed ejection fraction revealed that myocardial Gal-3 expression was increased in aortic stenosis patients with depressed ejection fraction [106].

Lok et al. [109] in the DEAL—Heart failure study, followed 232 patients with chronic heart failure (NYHA class III). Gal-3 was a significant predictor of mortality risk after adjustment for age and sex, and severity of HF and renal dysfunction, as assessed by NT-proBNP and estimated glomerular filtration rate, respectively. The study found neither a significant correlation between Gal-3 levels and left ventricular ejection fraction nor aetiology of HF.

In another study [110] of 599 patients with acute dyspnoea, an investigation of the utility assessment serum biomarkers alone or together with natriuretic peptide for diagnosis and short-term prognosis estimation in subjects with acute HF was performed. The NT-proBNP was superior to both apelin and Gal-3 for diagnosis of acute HF, although Gal-3 levels were significantly higher in subjects with HF compared with those without HF. An elevated level of Gal-3 was the best independent predictor of 60-day mortality or the combination of death/recurrent HF within 60 days. The Kaplan–Meier analyses showed that the combination of an elevated Gal-3 with NT-proBNP was a better predictor of mortality than either of the 2 markers alone.

Milting et al. measured plasma Gal-3 levels pre- and 30 days post-implantation of mechanical circulatory support (MCSP) in 55 patients with end stage HF. Plasma BNP levels were reduced by MCSP. Furthermore, patients who died had significantly higher plasma Gal-3 levels in comparison with those patients who were successfully bridged to transplantation. Milting et al. [111] confirmed the importance of Gal-3 level with disease progression.

In conclusion, Gal-3 is an independent marker for outcome in HF and appears to be particularly useful in HF patients with preserved LVEF [112].

Previous studies have examined the prognostic value of Gal-3 in subjects with existing HF. Recently a study [108] has been published, which reported the correlation of Gal-3 levels with risk of new onset HF in apparently healthy subjects. The conclusions are that higher circulating Gal-3 concentrations are associated with a high risk of new onset HF and all-cause mortality in the community.

We can summarize that Gal-3 appears to be a mediator of cardiac fibrosis, and is increased in acute and chronic heart failure. Measurement of Gal-3 in patients with HF may provide a novel clinical utility, because it reflects cardiac remodelling and in conjunction with BNP or NT-proBNP it could be used to better identify patients with a high risk of readmission or death.

In addition, in the future we could perhaps use the measurement of Gal-3 in asymptomatic subjects to identify patients with early evidence of cardiac fibrosis and apply targeted therapy to delay the onset of HF.

3. Conclusion

In this article we have discussed biomarker testing in patients with heart failure. It is clear that the number of these biomarkers has dramatically increased in the past several years. In addition, various applications of biomarkers have been expanded, including assessment diagnosis and prognostic evaluation. Using a multimarker strategy could confirm accurate risk stratification of patients with heart failure.

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