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Markers of fibrosis, inflammation, and remodeling pathways in heart failure

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ABSTRACT

Ventricular remodeling occurs progressively in untreated patients after large myocardial infarction and in those with cardiomyopathy. The pathologic changes of increased left ventricular (LV) volume and perturbation in the LV chamber geometry involve not only the myocytes, but also the non-myocyte cells and the extracellular matrix. Inflammation, fibrosis, neuro-hormonal activation, and ongoing myocardial damage are the mechanisms underlying remodeling. The detection of an ongoing remodeling process by means of biomarkers such as cytokines, troponins, neurohormones, metalloproteinases, galectin-3, ST-2 and others, may hold a clinical value and could, to some extent, drive the therapeutical strategy in patients after a myocardial infarction or with heart failure. For this reason, there is an increasing interest in the development of new biomarkers and a great number of laboratory tests have been recently proposed, whose clinical usefulness, however, is not fully established yet.

1. Introduction

Heart failure (HF) has long been considered as an irreversible disease, willing only to receive palliative therapy. However, the idea of chronic HF as an irreversible, end-stage process has been challenged by experimental and clinical evidence that early pharmacological intervention may lead to improvement in the function and structure of the failing heart [1]. Several biohumoral markers have been proposed for the diagnosis of HF so far [2], natriuretic peptides and troponins being the most widely tested and validated in this clinical setting. Besides early diagnosis, evaluation of the ongoing remodeling process has challenged clinicians and a specific, accurate, and effective biomarker of this process is still an unmet need (Fig. 1). For this reason, there is an increasing interest in the development of novel biomarkers and a great number of laboratory tests have been recently proposed, whose clinical usefulness, however, is not fully established yet [2].

As a matter of fact, in the last international guidelines on the management of HF only 3 groups of biomarkers were taken into account: natriuretic peptides (in particular BNP and NT-proBNP both for diagnostic and prognostic purposes with class I recommendation), markers of myocardial injury (i.e., cardiac troponin I and T, with class I recommendation), and markers of myocardial fibrosis (such as galectin-3 and sST2, mainly for risk stratification with class IIb recommendation) [3] (Table 1). Of course, these recommendations are supported mainly by scientific evidences based on the results of well-designed randomized clinical trials, which demonstrated the good diagnostic and prognostic efficiency, as well as the favorable cost/benefit ratio for HF patients and community of these biomarkers [2,4,5]. However, some methodological considerations should also be taken into account, when a novel biomarker is recommended for clinical laboratory practice or large population screening. As an example, a list of some desirable characteristics for an ideal biomarker, recommended for the routine use in a clinical laboratory, are reported in Table 2.

Another aspect that should be preliminarily underscored is the heterogeneity of the HF syndrome, in terms of etiology, pathophysiology and clinical presentation: this may account for the wide differences in response to treatment and, therefore, in survival among patients who received a diagnosis of HF. As an example, HF may be associated with reduced (i.e. <40%) ejection fraction (HFrEF) or with preserved ejection fraction (HFpEF), resulting in similar symptoms and signs, but with profound differences in pathophysiology and response to treatment [3]. Patients with HFrEF have a higher risk of death than patients with HFpEF, [6], but absolute mortality is still high in the latter group. Randomized controlled trials have mainly enrolled patients with HFrEF, and it is only in these patients that efficacious therapies have been demonstrated to date [3]. In addition the diagnosis of HFpEF is challenging and generally posed after excluding other potential noncardiac causes of symptoms

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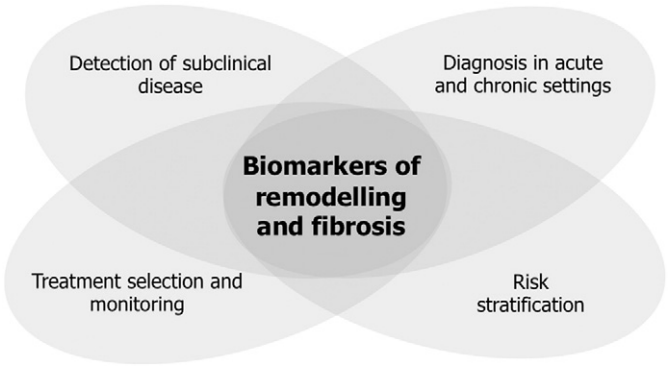

Fig. 1. Potential clinical usefulness of biomarkers of fibrosis and remodeling.

suggestive of HF. To date, efficacious therapies have not been identified for HFpEF [7,8].

The aim of this review article is to provide a general overview on the biomarkers of the different pathways involved in the remodeling process.

2. Cardiac remodeling

HF is the final result of several etiologies (ischemic heart disease accounting for roughly half of cases) and includes heterogeneous patients with diverse propensity to ventricular remodeling and clinical outcome [9]. Despite optimized medical therapy and technologically advanced device treatment, the majority of patients affected by HF experience progressive left ventricular dysfunction, worsening of symptoms and life-threatening arrhythmias. Cardiac death occurs because of arrhythmic event or pump failure, and mid and long term survival is still disappointing (9) (Fig. 2). Following the initial decline of left ventricular (LV) contractility, patients with HF can remain asymptomatic (stage B of ACCF/AHA classification) or paucisymptomatic (stage C) for years, as the result of the compensatory mechanisms sustaining cardiovascular function. However, these mechanisms promote complex structural and functional abnormalities of the myocyte and non-myocyte cells, contributing to LV enlargement and dysfunction (adverse remodeling). In particular, biomolecular remodeling [10], cardiomyocyte hypertrophy and extensive extracellular matrix production [11–13] may be promoted not only by the original noxa (i.e. necrosis, virus, toxics, autoimmunity), but also by chronic mechanical overload, myocardial ischemia due to microvascular dysfunction [14–16], and sustained activation of neurohormonal and cytokine systems [17]. From a clinical point of view, it is crucial to identify the subgroup of asymptomatic patients at higher risk, who need a more strict follow-up and an enhanced therapeutic effort, especially in the early HF stages (A and B) of disease, when the clinical status and LV function are yet poor predictors of disease evolution and clinical outcomes [18].

Myocardial remodeling in ischemic and nonischemic cardiomyopathies involves not only the myocytes, but also the non-myocyte cells and the extracellular matrix (ECM). ECM constitutes around 6% of the normal heart and includes fluid, collagen and glycoproteins. In particular, collagen is secreted by fibroblasts as procollagen into the ECM, where protease enzymes remove amino and carboxy-propeptide terminals, and is then broken down by matrix metalloproteinase enzymes, which are in turn regulated by their tissue inhibitors. In pathological conditions, the cardiac interstitium increases as a result of diffuse interstitial (microscopic) fibrosis, post-necrotic replacement (macroscopic) fibrosis, myocardial edema (as result of inflammatory processes) or pathological infiltration (e.g. amyloid). The activation of the renin–angiotensin–aldosterone system plays a central role in fibroblast activation and collagen deposition, with the transforming growth factor β (TGF β) as the downstream signal mediator. Endomyocardial biopsy still represents the current reference method for the evaluation of the remodeling process at a cellular level, although routine endomyocardial biopsy is not recommended in all cases of HF [3], but some circulating cardiac biomarkers may provide unique information regarding cardiovascular remodeling. Indeed, along the complex path from risk to fully developed HF, there are increasing numbers of injury, remodeling and neurohormonal activation substances discovered, whose assays might provide important information about HF. Some, as natriuretic peptides and troponins, are well validated and established according to evidence-based laboratory medicine principles [3–5], while several other biomarkers are still being explored for potential use in the clinical practice.

Table 1
Established biomarkers for HF management.
Adapted from 2013 ACCF/AHA Heart Failure Guidelines [3].

Biomarker	Setting	Application	Class	Evidence
BNP/NT-proBNP	Acute/chronic	Diagnosis	I	A
BNP/NT-proBNP	Acute/chronic	Risk stratification	I	A
BNP/NT-proBNP	Chronic	Guide for treatment	IIa	B
BNP/NT-proBNP	Acute	Guide for treatment	IIb	C
Troponins	Chronic	Risk stratification	I	A
Soluble ST2	Acute/chronic	Risk stratification	IIb	A/B
Galectin-3	Acute/chronic	Risk stratification	IIb	A/B

3. The pathophysiological role of cytokines in myocardial fibrosis

Inflammation mechanisms should be considered as an essential component of the normal wound healing process [19–22]. However, when the injury cannot be repaired in a short time, a chronic inflammatory response may be established. In this case, a chronic inflammatory response allows a pathological wound repair, with accumulation of permanent fibrotic tissue at the site of injury. The final result of this dysregulated inflammatory process is the impossibility for the tissue to restore the normal function.

Fibrosis can affect any organ including the lung, skin, heart, kidney and liver and it is estimated that 45% of deaths in the western world can now be attributed to diseases where fibrosis plays a major pathophysiological role [19]. In particular, the clinical syndrome of HF is characterized by a systemic inflammatory response that contributes to end organ damage in the heart and circulation and thus, can lead to progressive worsening of cardiovascular function. The inflammatory mediators in HF patients include pro-inflammatory cytokines and their cognate receptors, as well as molecules secreted/released by macrophages (such as galectin-3 and pentraxin-3-PTX3) [21]. Inflammatory biomarkers usually correlate with disease severity and prognosis across the broad spectrum of HF syndromes [21–23].

Levine et al. [23] reported for the first time that HF patients usually show elevated circulating levels of tumor necrosis factor (TNF). Further studies have then expanded this observation by demonstrating that proinflammatory cytokines and their receptors, cell adhesion molecules, and chemokines are elevated in patients with HF with a decreased ejection fraction [9]. In addition, the most important pathophysiological mechanisms underlying HF with a preserved ejection fraction are fibrosis and reduced ventricular compliance, which in turn cause the development of left ventricular diastolic dysfunction. In Tables 3 and 4 we reported a list of these inflammatory agents, more frequently suggested as possible biomarkers for HF [19–24].

Inflammation is one of the earliest events in cardiac stress situations such as pressure and/or volume overload and involves elevated levels of endothelial/vascular (VCAM) and intercellular adhesion molecules (ICAM), as well as increased production and release of inflammatory cytokines and chemokines in the tissue [18–21,23]. Cytokines and chemokines recruit activated inflammatory cells, particularly monocytes, from circulation into the cardiac tissue. Increased monocyte infiltration is seen in the early and late stages of HF [23]. Once inside the cardiac tissue, monocytes differentiate into macrophages and promote inflammation, tissue injury, and fibrosis of myocardial tissue. Activated

Table 2
Desirable features fitted by biomarkers measured by the laboratory test.

Desirable feature of biomarker	Tests fitting the feature	Tests not fitting the feature or no available data	References
Evaluation of in vivo and in vitro stability	cTnI, cTnT, NT-proBNP, BNP	MMP assay, cytokines assays, galectin-3, sST2	[60,64,77,121]
Evaluation of analytical performance according to the EBLM criteria	cTnI, cTnT, NT-proBNP, BNP, galectin-3, sST2	MMP assay, cytokine assays	[2,3,5,21,77,118–121,123,124]
Complete automation	cTnI, cTnT, NT-proBNP, BNP, galectin-3	MMP assay, cytokine assays, sST2	[2,60,64,77,118–120,122–125]
Acceptable harmonization between methods	cTnT, NT-proBNP, galectin-3, sST2	BNP, MMP assay, cytokine assays	[60,64,77,121]
Evaluation of biological variation	cTnI, cTnT, BNP, NT-proBNP	MMP assay, cytokines assays, galectin-3, sST2	[75,77]
Cardiac specificity	cTnI, cTnT, BNP, NT-proBNP	MMP assay, cytokines assays, galectin-3, sST2	[2,21,22,77]
Evaluation of reference interval tested for gender, age and ethnicity dependence	cTnI, cTnT, BNP, NT-proBNP	MMP assay, cytokines assays, galectin-3, sST2	[3,17,77,87]
Diagnostic and prognostic accuracy tested by large randomized clinical trials according to the EBLM criteria (level of evidence IA)	cTnI, cTnT, BNP, NT-proBNP	MMP assay, cytokines assays, galectin-3, sST2	[2–4,21,22,77]
Cost–benefit ratio favorable tested by randomized clinical trials	cTnI, cTnT, BNP, NT-proBNP	MMP assay, cytokines assays, galectin-3, sST2	[2–4,77]

Cytokine assays include all the immunoassays for the interleukin/cytokine superfamilies (such TNF, IL-1, IL-2, IL-6).

macrophages produce and secrete several inflammatory mediators, such as monocyte chemotactic protein-1 (MCP1) and TNF-alpha (TNF-α), and fibrogenic activators, such as TGF-β, and in this way support pro-inflammatory and pro-fibrotic processes [24,25]. Activated macrophages also secrete galectin-3, which may induce cardiac fibroblast proliferation, collagen deposition, and ventricular dysfunction [26,27]. PTX3 is a novel inflammatory marker and member of the pentraxin superfamily of cytokines, which has also recently been identified in patients with HF [21]. PTX3 synthesis is produced/released by endothelial cells, macrophages, myeloid cells, and dendritic cells stimulated by cytokines and endotoxins such as bacterial products, interleukin-1 (IL-1), and TNF [21,28].

According to the pathophysiological mechanisms reported above, circulating levels of the inflammatory mediators and agents are found with increasingly higher concentrations in the blood from HF patients with asymptomatic left ventricular dysfunction [29] to those with more severe disease according to the NYHA functional class [30]. In particular, soluble type 1 and type 2 TNF receptors (sTNFR1 and sTNFR2, respectively) and soluble transmembrane glycoprotein 130 (gp130, one of the receptors related to the IL-6) are increased according to worsening HF functional class [21,22]. Furthermore, elevated circulating levels of some pro-inflammatory cytokines correlate not only with disease severity, but also with increased mortality in HF patients [21,22]. In particular, TNF, IL-6, sTNFR1, and sTNFR2 have been reported to be associated to increased mortality [31,32]. There are relatively few studies, which evaluated the prognostic relevance of pro-inflammatory cytokine levels in HF patients with preserved ejection fraction: only TNF levels have been shown to correlate with increased mortality in this setting [33].

Growth differentiation factor 15 (GDF15), a member of the transforming growth factor-beta cytokine superfamily, is another marker of cell injury and inflammation that has been shown to circulate in higher concentrations in patients with HFrEF with reduced ejection fraction [34,35] and also in HFpEF [36], compared with controls. In a more recent study, GDF15 was shown to be able to discriminate HFpEF from controls at least as well as NT-proBNP [37] and the ratio of NT-proBNP to GDF15 provided the best discriminatory ability between HFpEF and HFrEF [37].

Finally, increased levels of some inflammatory mediators were found to be significantly associated with disease severity and prognosis also in patients with acute decompensated HF [3]. In particular, C-reactive Protein (CRP), ST-2, galectin-3, and IL-6 showed a significant association with an increased mortality rate in patients with acute HF [38–44]. However, inflammatory biomarkers alone show much lower diagnostic and prognostic accuracy than natriuretic peptides in HF patients admitted with acute dyspnea in emergency department [21,42].

4. Biomarker of ECM

The ECM in the healthy heart is dynamic and can adapt to differing environmental factors [45]. In pathological states, it can increase as a result of diffuse myocardial fibrosis (reactive or interstitial fibrosis, secondary to mechanical, toxic, infective or autoimmune insults) or of

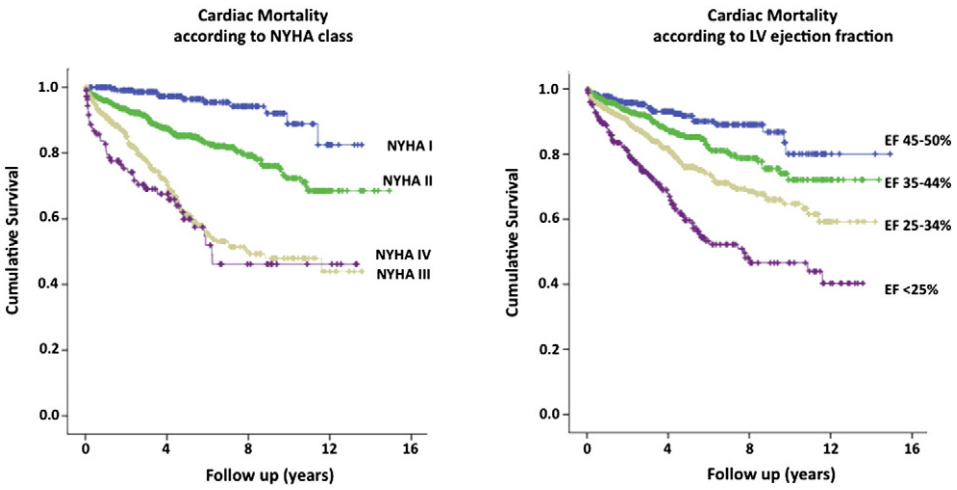

Fig. 2. Survival plots in the Pisa cohort of systolic heart failure patients (N = 1546 patients) on optimal medical therapy, according to the New York Heart Association (NYHA) classification (left) or to the left ventricular ejection fraction (right); cardiac death was considered as an end-point.

Table 3
Some biochemical and physiological characteristics of TNF superfamily cytokines and other pro-inflammatory and regulatory cytokines suggested as biomarkers for heart failure.

Biomarker	MW (kDa)[a]	Biochemical structure	Biological characteristics
TNF superfamily			The TNF superfamily currently consists of 19 ligands and 29 receptors in humans. Most TNF ligands are type II transmembrane proteins whose extracellular domains can be cleaved by specific metalloproteinases to generate soluble cytokines. TNF superfamily ligands and receptors play a role in normal developmental processes, apoptosis, regulation of immune cell functions, and also in cancer and autoimmune diseases.
TNFa	About 17 kDa (recombinant, mouse)	156 aa protein (recombinant mouse)	Adipokine/cytokine involved in systemic inflammation and the acute phase reaction
TWEAK (*TNFSF12*)	About 17 kDa soluble protein (recombinant, human)	249 aa membrane protein, 156 aa soluble protein	Transmembrane and soluble (cytokine) protein of the TNF ligand superfamily.
FasL (*TNFSF6* or *CD95L*)	About 40 kDa as a tramsmenbrane protein (human) About 18 kDa as a soluble protein (recombinant, human)	157 aa soluble protein (recombinant, human)	Transmembrane and soluble protein of the TNF ligand superfamily
LIGHT (*TNFSF14* or *CD258*)	About 23 kDa (recombinant, human)	183 aa (recombinant, human)	Member of the TNF ligand superfamily, which acts as a ligand for TNFRSF14
Pro-inflammatory and regulatory cytokines			A pro-inflammatory cytokines are agents promoting systemic inflammation (such as IL-1 and TNF). Regulatory cytokines include IL-2, IL-4, IL-7, IL-9, IL-15 and IL-21, which play a role in the maturation of lymphocytes.
IL-1β	17.4 kDa (recombinant, mouse) 17.4 kDa (recombinant, human)	152 aa (recombinant, mouse) 153 aa (recombinant, human)	IL-1β is a member of the interleukin 1 family of cytokines, which is an important mediator of the inflammatory response, and is involved in a variety of cellular activities (such as cell proliferation, differentiation, and apoptosis).
IL-2	15.3 kDa (recombinant, human)	About 134 aa	IL-2 is a regulatory cytokine necessary for the growth, proliferation, and differentiation of thymic-derived lymphocytes (T cells) to become 'effector' T cells.
IL-6	23.7 kDa (human)	212 aa (human)	IL-6 is a pro-inflammatory cytokine, secreted by macrophages and T cells to stimulate immune response during infection, trauma, and burns.
IL-18	18 kDa (recombinant, human)	157 aa (recombinant, human)	IL-18, also known as interferon-gamma inducing factor, is a proinflammatory cytokine of the IL-1 superfamily.
IL-33	18 kDa (recombinant, human)	159 aa (recombinant, human)	IL-33 is a proinflammatory cytokine expressed on a wide variety of cell types, including fibroblasts, mast cells, dendritic cells, macrophages, osteoblasts, endothelial cells, and epithelial cells.

FasL: Fas ligand; *LIGHT*, an acronym derived from: homologous to Lymphotoxins, Inducible expression, competes with HSV Glycoprotein D for HVEM, a receptor expressed on T-lymphocytes; *IL-1β*: interleukin-1β; *IL-2*: interleukin-2; *IL-6*: interleukin-6; *IL-18*: interleukin-18; *IL-33*: interleukin-33; *TNF*: tumor necrosis factor; *TNFSF*: tumor necrosis factor superfamily; *TWEAK*: TNF-like weak inducer of apoptosis.
[a] The values of MW reported in the table are only indicative because several circulating and tissue isoforms of the same protein are present in humans.

Table 4
Biochemical and physiological characteristics of some cytokine receptors and macrophage products suggested as biomarkers for heart failure.

Biomarker	MW (kDa)[a]	Biochemical structure	Biological characteristics
Cytokines receptors			
TNFR1 (*TNFRSF1A* or *CD120a*) and *sTNFR1*	18.3 kDa (sTNFR1 recombinant, human)	162 aa (sTNFR1 recombinant, human)	TNFR1 belongs to the TNFR superfamily of transmembrane proteins. sTNFR1 (the soluble form of the receptor) is capable of inhibiting TNFa and TNFb activities by acting as a decoy receptor binding the TNF ligands
TNFR2 (*TNFRAF1B* or *CD120b*) and *sTNFR2* (soluble form)	24.5 kDa (TNFR1 recombinant, human)	184 aa (TNFR1 recombinant, human)	TNFR2 is a lower activator of signaling pathways related to TNF compared to TNFR1.
gp130 (*IL6ST*, *IL6-beta* or *CD130a*) and *sgp130*	103.5 kDa (human)	918 aa (human)	gp130 is a transmembrane protein, which is the founding member of the class of all cytokine receptors.
IL-1RA (*IL-1F3*)	About 17 kDa (there are several isoforms from 16 to 18 kDa)	143 aa (16 kDa) and 152 aa (17 kDa)	IL-1RA is the natural receptor antagonist of IL-1 because it is able to bind the same specific receptor of IL-1.
ST2 (*IL-1RL1*) and *sST2*	About 63 kDa (ST2 or IL-1RL1) 39.5 kDa (recombinant, human sST2)	556 aa (ST2 or IL-1RL1) 310 aa (recombinant, human sST2)	ST2 is a member of the interleukin 1 receptor family. The ST2 protein has two isoforms: a soluble form (sST2) and a membrane-bound receptor form (ST2). The ligand for ST2 is IL-33.
Macrophage products			
Galectin-3	26 kDa (recombinant, human)	250 aa (recombinant, human)	Galectin-3 is a member of the lectin family, of which 14 mammalian galectins have been identified. Galectin-3 contains a carbohydrate-recognition-binding domain which specifically binds the β-galactosides. Galectin-3 plays a role in cell adhesion, cell activation and chemoattraction, cell growth and differentiation, cell cycle, and apoptosis.
Pentraxin-3	About 42 kDa (monomer, human)	381 aa (human)	Pentraxin-3 is a member of the pentraxin superfamily, which is characterized by cyclic multimeric structure. This protein is rapidly produced and released by several cell types, in particular by mononuclear phagocytes, dendritic cells, fibroblasts and endothelial cells in response to primary inflammatory signals.

gp130: glycoprotein 130; *sgp130*: soluble gp130; *IL1-F*: interleukin-1 family; *IL-1RA*: interleukin-1 receptor antagonist; *IL-1RL1*: interleukin-1 receptor-like-1; *sST2*: soluble ST2 receptor; *sTNFR1*: soluble TNF type1 receptor; *sTNFR2*: soluble TNF receptor type 2; *TNFSF*: tumor necrosis factor superfamily; *TNFSFR*: tumor necrosis factor superfamily receptor.
[a] The values of MW reported in the table are only indicative because several circulating and tissue isoforms of the same protein are present in humans.

replacement fibrosis (e.g. in response to a large loss of myocytes, such as in myocardial infarction) (Table 5). The ECM can also increase because of myocardial edema (in myocarditis) or infiltration (for example with amyloid protein), and its expansion has been shown to correlate with arrhythmias, sudden cardiac death and HF in a number of cardiac diseases [46].

Some enzymes and molecules involved in ECM metabolism may be assayed in the peripheral blood and are therefore promising biomarkers for the assessment of cardiac fibrosis and clinical management in HF patients. Collagen network is the main structural component of ECM, and collagen types I and III are the most abundantly expressed in the heart [47]. Collagen concentration is affected by different stimuli, such as ischemia, autocrine/paracrine factors, myocardial stretch, or inflammation, through the regulation of the expression of matrix metalloproteinases (MMPs) and tissue inhibitor of matrix metalloproteinases (TIMPs). In particular, MMP-1 and TIMP-1 are co-expressed in cardiac fibroblasts and are tightly regulated to maintain the architecture of the ECM. Indeed higher levels of both MMP-1 and TIMP-1 are detected in the coronary sinus compared to the peripheral blood in patients with cardiovascular diseases (e.g. hypertension) [48]. The balance between MMPs and TIMPs likely reflects the extent of collagen turnover and may therefore influence the progression of cardiac remodeling. Consistently, the serum MMP-1:TIMP-1 ratio is associated with the degree of left ventricular dilatation and systolic dysfunction. [48].

Carboxy-terminal and amino-terminal propeptides of collagens I (PICP and PINP) and III (PIIICP and PIIINP), are cleaved during the conversion of procollagen molecules into mature collagen and are related to ECM synthesis. On the other hand MMPs – in particular MMP-1, MMP-2 and MMP-9 – are responsible for collagen digestion, collagen I carboxy-terminal telopeptide (ICTP) being the principal by-product [49,50].

All the aforementioned molecules reach the blood and their circulating levels have been tested as biomarkers of ventricular fibrosis and remodeling in HF settings. In some biopsy studies about the association between serum biomarkers and myocardial collagen content, serum concentrations of PICP, PIIINP and ICTP were correlated with the fibrillar collagen fraction in the myocardium [51,52]. Among community-living older adults, PIIINP was associated with cardiovascular disease and heart failure [53]; both ICTP and PIIINP have been shown to be significantly associated with an adverse outcome in individuals at risk of developing HF (stages A–B) [54]. Serum PIIINP correlates well with its tissue analogue (i.e. collagen type III) [51] and significantly associated with clinical status and outcome in cohorts of patients with dilated cardiomyopathy of both ischemic and non-ischemic etiologies [51,55]. A prognostic role for ICTP has been also demonstrated in both the acute and chronic phases following myocardial infarction [56] and elevated ICTP plasma levels have been associated with a worse prognosis in HF [57]. The PICP/ICTP serum level ratio reflects the extent of collagen accumulation. Izawa and colleagues have reported that patients with dilated cardiomyopathy and a high PICP/ICTP ratio show more abundant perivascular fibrosis and interstitial fibrosis, as assessed by collagen-specific staining on cardiac bioptic samples [58]. Interestingly, in the same study an increased diastolic left ventricular stiffness was also reported in patients with an elevated PICP/ICTP ratio. It could be hypothesized that ECM remodeling may indeed underlie the transition from subclinical cardiac damage and early stages (A/B) to clinically overt HF (stage C) either with or without reduced ejection fraction. Differential balances between MMP-1 and TIMP-1 (evaluated by the ratio of their circulating levels) seem to be specific of normotensive patients compared to hypertensive subjects who have developed HFpEF or HFrEF [59]. The MMP-1 to TIMP-1 ratio, measured in the peripheral vein blood, is correlated with left ventricular ejection fraction and end-diastolic diameter in an inverse and direct fashion, respectively. Further, plasma TIMP-1 was positively associated with left ventricular mass and wall thickness in a cohort of more than 1000 patients from the Framingham Heart Study [60], and predicts the presence of symptoms of congestive HF in hypertensive patients [61]. In recent years, the relationship between circulating ECM-associated molecules and left ventricular fibrosis and remodeling has been more and more often investigated by means of cardiac magnetic resonance; given the in vivo histological information this technique is able to provide. Eschalier et al. reported a correlation between early cardiac structural abnormalities, detected by magnetic resonance imaging, and PIIINP circulating concentration in obese patients [62].

Interestingly, antifibrotic treatment with mineralocorticoid receptor antagonists influences peripheral levels of biomarkers of ECM metabolism. In a subset of patients from the Randomized Aldactone Evaluation Study (RALES), PIIINP levels were decreased by treatment with spironolactone and identified those subjects with a significant prognostic benefit from the use of aldosterone antagonists [63], suggesting a potential value of this assay for tailoring medical treatment.

Although several studies support the potential clinical utility of ECM related biomarkers, their circulating levels are altered in several other extra-cardiac conditions, including cancer, bone diseases and inflammatory diseases, likely acting as confounding factors. Moreover, preanalytical issues should be taken into account in interpreting the assays of ECM related molecules. For example, significant higher values have been reported for serum compared to plasma MMP-9, due to the release of MMP-9 by polymorphonuclears during clot formation [64]. Finally, some difficulties still exist in comparing the results obtained using different commercial kits, given the lack of standardized assay procedures [60].

Table 5
Phenotypes of the cardiac fibrous tissue.

Myocardial fibrosis
The fibrous tissue infiltrating the myocardial can be divided into two distinct types: 1. Replacement fibrosis, which occurs throughout the myocardium and is associated with the loss of cardiomyocyte mass (myocardial scar). 2. Reactive myocardial fibrosis, which originates from areas surrounding the microvasculature (perivascular fibrosis subtype) and spreads throughout the myocardium (interstitial fibrosis subtype).
Valvular fibrosis
Valvular fibrosis is an inappropriate proliferation of fibroblasts causing thickening and fibrosis of the tricuspid valve, but also occurring on the pulmonary valve. The thickening and loss of flexibility eventually may lead to valvular dysfunction and right-sided heart failure.

5. The renin–angiotensin-aldosterone system, the transforming growth factor beta and cardiac fibrosis

While myocyte hypertrophy has been demonstrated to be load-dependent, the activation of the renin–angiotensin–aldosterone system is a major determinant of fibroblast activation and collagen deposition [11], with the TGF-β as the downstream signal mediator [65]. For instance, in animal models of high-output HF (e.g. arterio-venous fistula model of HF), the remodeling of the myocardium involves exclusively myocytes without relevant interstitial fibrosis, whereas in low-output HF models (e.g. pacing-induced HF), characterized by enhanced neurohormonal activation, extensive interstitial fibrosis is documented [66]. Angiotensin II partially mediates its effects through TGF-β, resulting in the upregulation of myocardial procollagens 1 and 3 production [67]: along with alterations in MMP breakdown enzymes, this leads to an excess of collagen in the extracellular space and hence fibrosis (Fig. 3).

Many cells secrete TGF-β as an inactive complex, its activation being precipitated by a number of factors including MMPs and integrins. In health, it appears to play a crucial role in embryogenesis, extracellular matrix homeostasis and maybe in antiatherogenesis. In disease, it regulates the expression and function of all the cells involved in tissue repair and remodeling [68]. Mice overexpressing TGF-β have been shown to develop cardiac hypertrophy and interstitial fibrosis [69]. Myocardial TGF-β levels have been shown to be elevated in patients with dilated or hypertrophic cardiomyopathy (HCM) [47].

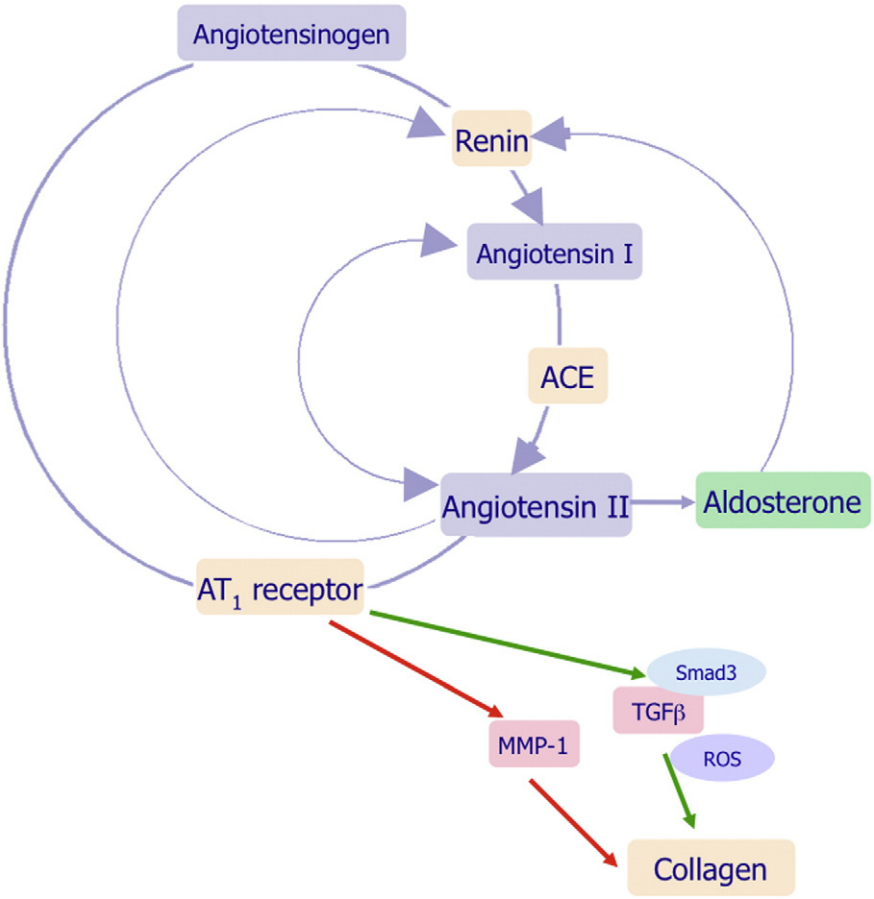

Fig. 3. Main enzymatic steps leading to the synthesis of the renin–angiotensin–aldosterone-synthesis (RAAS) effectors; interactions and feedbacks are marked by blue arrows. Angiotensin II, by binding to the angiotensin II type 1 receptor (AT1), activates the TGFbeta and SMAD3 signaling pathway and decreases the expression and activity of matrix metalloproteinase (MMP)-1. Angiotensin II also stimulates the intracellular generation of reactive oxygen species (ROS) in cardiac fibroblasts, finally contributing to the development of organ fibrosis. Green arrow: stimulatory signal; red arrow: inhibitory signal.

6. Markers of ongoing myocardial damage: troponins

Ongoing myocardial damage (OMD) has been proposed as a possible mechanism of cardiac remodeling and disease progression in chronic HF. Independent of the presence of coronary artery disease, OMD produces chronic cardiac troponin (cTn) release in patients with HF, whose plasma levels hold a prognostic value [70]. A recent study reported an association between cTnT release into the coronary circulation (by simultaneous assay of serum cTnT levels in the aortic root and coronary sinus) and the presence and extent of myocardial fibrosis assessed by late gadolinium enhancement at cardiac magnetic resonance in nonischemic patients with HF [71]. Activation of adrenergic signalling pathways [72] and mechanical stress, due to cardiac output impairment and hemodynamic overload during exercise [73], have been proposed as pathophysiological mechanisms responsible for OMD in HF.

It is well known that a relatively large fraction of patients with HF (from 25% to 45%), especially those with clinical history of coronary artery disease, share increased levels of cTnI and cTnT, even if measured by standard (not highly sensitive) methods [74]. More recent studies [71,75,76] reported that the fraction of HF patients with troponin values above the 99th percentile upper reference population limit (99th URL) greatly increase when the high sensitive immunoassay methods are used for cTnI and cTnT measurement. In particular, considering the large population of patients with chronic HF of the ValHeFT study (4053 patients randomized), only 10.4% of these patients showed measurable cTnT with the standard assay, while this fraction increased up to 92% when samples were reassessed with a more sensitive method [70]. These premises, together with the recognized prognostic role of increased troponin in HF [70], support the hypothesis that troponin-related proteolysis could somehow be also implicated in the development of cardiac damage promoting progression to HF. Indeed, it is conceivable that repetitive bouts of ischemia are able to promote cardiomyocyte death, replacement fibrosis, and ventricular remodeling, which in turn can produce a worsening of diastolic and systolic function. In addition, apoptotic cells have been described in the healthy adult heart [77] and cardiomyocytes have been shown to renew in humans [78]. Both processes, to different extent, are likely impaired in HF. At present time, there are no experimental data indicating that during apoptosis troponins are degraded within the cardiomyocytes and released into the interstitial space. There are two potential explanations for the troponin release in the absence of fatal sarcolemmal disruption: 1) cellular release of proteolytic troponin degradation products and 2) troponin leaks from reversibly damaged cardiomyocytes, as an intact non-degraded protein chain [74,79]. As these degradation mechanisms have been evaluated only in experimental studies using culture of cardiomyocytes, further studies are needed to evaluate the relevance of these pathophysiological mechanisms in vivo in patients with cardiovascular diseases. Furthermore, it is well known that a small fraction (about 4–8% of the total) of cTnI and cTnT content of cardiomyocyte is present in the monomeric form in the sarcoplasm, and so this protein fraction may be released in the circulation during a reversible myocardial injury [80]. Mechanical stretch of cardiomyocytes, as it occurs during pressure or volume overload, may activate some intracellular proteases, such as MMPs, which are able degrade cardiac troponin within the cell [81]. Overload-induced stretch at the cardiomyocyte level is sensed by integrins, which are mechanotransducer molecules that link the

extracellular matrix to the intracellular cytoskeleton [82]. Hence, this mechanism may be involved in the stretch-induced release of troponin and its degradation products [79]. Furthermore, several findings obtained in healthy individuals and even in well-trained athletes after endurance exercise support this hypothesis [83–85]. These findings suggest that the stretch stimulation of viable cardiomyocytes may lead to intact cTnI release. As discussed in detail in other recent reviews [86,87], the increased analytical sensitivity of cTnI and cTnT methods will likely help to spread more light on the process of "non-necrotic" troponin release into the blood and should be considered a powerful tool to monitor the processes related both to physiological renewal and pathologic remodeling of myocardial tissue.

7. "Novel" biomarkers of myocardial fibrosis and remodeling

7.1. ST2

ST2 is a member of the IL-1 receptor superfamily and it exists in two forms, a transmembrane (ST2L) and a soluble one (sST2), which is present in extracellular space as well as in circulation. ST2L is the specific receptor of IL-33, a cytokine generally released by myocytes after myocardial stress (e.g. pressure overload) [88], whose role is to blunt cardiac remodeling and fibrosis [89,90], as well as hypertrophy in mechanically strained tissues [91]. ST2L transduces to the cell the effects of IL-33, while sST2 acts as a decoy receptor and sequesters it, reducing its positive effects. In stress conditions sST2 increases, thus leading to a higher incidence of deleterious cardiac events, including adverse cardiac remodeling.

Due to its functional role, sST2 has been studied in various different cardiac diseases, with a special concern to myocardial infarction and HF, and it has been recently included in the ACCF/AHA guidelines for the management of HF [3]. Recent studies reported that circulating sST2 values correlate with the clinical severity of HF, LV ejection fraction, BNP and NT-proBNP [43,92]. The sST2 levels at presentation were higher among patients who died by 1 year. In a multivariable Cox model analysis containing several established clinical and biochemical predictive variables, sST2 remained an independent predictor of mortality and it showed an incremental prognostic value over natriuretic peptides [43,92]. In patients with decompensated HF, sST2 plasma concentration in the upper tertile at presentation was a strong and independent predictor of all-cause mortality after one year of follow-up [93]. Similar results have been obtained in acute myocardial infarction, with higher sST2 levels correlating to a more impaired hemodynamic, a worse ischemic profile on admission, and higher mortality rate at one month [94].

Recently sST2 has been studied as a prognostic marker together with troponins and GDF 15. Their combined measurement improved the prognostic information of the patients, independent of NT-proBNP [95], confirming the importance of a multimarker strategy when dealing with risk stratification in HF patients.

7.2. Galectin-3

Initially studied as a mediator of cancer growth and progression, galectin-3 is, among lectins, a unique chimera-like galectin, which can interact with several extracellular matrix proteins, carbohydrates and nonglycosylated proteins. Galectin-3 is localized within the cytoplasmic space of several cell types. In particular, macrophages can secrete galectin-3 in the extracellular space and activate resting fibroblasts into a matrix-producing phenotype [27,96].

Sharma and colleagues first reported a causal relationship between galectin-3 and cardiac damage in a rat model overexpressing the murine *Ren-2d* renin gene [27]. They observed a 5-fold increase in the expression of galectin-3 gene in rats with overt HF, as well as a higher degree of interstitial collagen and galectin-3 protein content, co-localized with macrophage-specific staining, compared to rats with compensated HF and to wild type [97]. They also performed intrapericardial infusion of galectin-3 in healthy rats, observing thereafter a reduction in LV ejection fraction and an increase in collagen content versus placebo. Interestingly, genetic disruption of galectin-3 produced blunted cardiac hypertrophy and dysfunction, after treatment with either angiotensin II infusion or transverse aortic constriction [98].

Although not conclusive, there is some experimental evidence that the RAAS and galectin-3 share some signaling pathways and interplay in the development of cardiac fibrosis. Azibani showed that in hypertensive mice with cardiac hyperaldosteronism, aldosterone elicited a massive macrophage infiltration and an increase in cardiac galectin-3 expression and protein content, especially in the fibrotic areas [99].

As a whole, the abovementioned experimental findings envisage a causative involvement of galectin-3, as a key mediator of maladaptive tissue response to damage, in inflammation and fibrogenesis, and finally in the pathophysiology of cardiac remodeling. Indeed, some human studies have demonstrated a correlation between galectin-3 and circulating markers of ECM metabolism and that galectin-3 elevation is an independent predictor of left ventricular adverse remodeling (defined as a percentage change in the left ventricular end-diastolic volume along a 3-month follow-up) in patients with systolic HF [100,101].

7.3. Biglycan and decorin

Biglycan is a small leucine-rich proteoglycan expressed in many tissues in vivo, including the skin, kidney and heart [102]. Several roles of biglycan have been demonstrated: ECM organization, cellular adhesion and migration [103]. Biglycan, together with other small leucine-rich proteins, can bind various collagen types (I, II, III, VI) [104,105] and plays a major role in modulating inflammatory processes by binding Toll-like receptors 2 and 4 [106–108]. Furthermore, in transgenic mice overexpressing human biglycan, the up-regulation of several proteins of the TGF-β and nitric oxide family has been described [109]. Given these physiological actions, the role of biglycan in cardiac remodeling has been further investigated, particularly after myocardial infarction. Westermann has demonstrated that biglycan induction is critical in the mechanisms of scar formation, since biglycan knock-out mice showed increased mortality, left ventricular ruptures and HF after experimental myocardial infarction [110]. Moreover, biglycan seems to be secreted in a RAAS dependent manner [111,112] and its secretion could be prevented by AT1 receptor antagonists [112].

Like biglycan, decorin is a proteoglycan that regulates collagen formation and organization by binding to collagens I and III [113,114]. It is expressed by several tissues including the heart, and its concentration rises in cardiovascular diseases such as myocardial infarction and dilated and hypertrophic cardiomyopathy [115]. Decorin has been demonstrated to interact with the TGFβ/SMAD2 pathway. Indeed, decorin binds TGFβ [116,117] and inhibits its profibrotic effects, thus likely blunting the development of adverse remodeling.

8. Analytical performance of biomarkers for heart failure: some general considerations

From an analytical point of view, all the biomarkers suggested in the previous paragraphs are usually measured by means of immunoassay methods. However, only a small part of these biomarkers are measured with immunoassay methods using fully automated platforms (Table 2). For example, considering the 3 groups of biomarkers actually recommended by the most recent international guidelines [3], it is possible to measure BNP, troponins I and T, and galectin-3 with fully automated platforms, while, at present time, sST2, is still measured by an EIA method. Furthermore, even if the first immunoassay methods for B-type natriuretic peptides (i.e., BNP and NT-proBNP) and cardiac troponin I were set up 30 years ago, there is not an international standardization of these methods. Indeed, BNP immunoassay methods actually show systematic differences up to 50% [118–120], while even a greater bias is

Table 6
Comparison of analytical performances of galectin-3 assay methods.

Method	LoB (ng/mL)	LoD (ng/mL)	LoQ (ng/mL)	TAT	Reference
ELISA, Waltham	0.86	1.13	0.97	About 3 h	Christenson et al. [122]
ARCHITECT Platform, Abbott	0.1–0.3	1.4–2.1	3.0–3.3	About 30 min	La'ulu et al. [123]
VIDAS platform, BioMérieux	–	2.4	3.3	About 30 min	Vernet et al. [124]

LoB: limit of blank; LoD: limit of detection; LoQ: limit of quantitation; TAT: turnaround time.

on average found between cTnI immunoassay methods [121]. As an example, in Table 6, a comparison between the analytical characteristics of the immunoassay methods for galectin-3 is reported. Even if all the materials and standards used by these laboratory tests are supplied by the same company (BG Medicine, Waltham, MA), these methods show some differences in the analytical performance, as demonstrated by the data reported in Table 6. In particular, the ELISA method shows a longer turnaround time (TAT) than the two automated immunoassays, suggesting that this method has a lower practicability than those using the Architect and VIDAS automated platforms. However, the ELISA method shows an analytical sensitivity similar (if not better) than the two automated systems [122–124].

From a clinical point of view, it is important to note that tissue levels of several biomarkers (including some cytokines, chemokines, tissue proteases, and neuro-hormones, such as noradrenaline, angiotensin II and aldosterone) described in the previous paragraphs could be little (or even not at all) correlated with their respective circulating levels, thus suggesting that the measurement of plasma/serum concentrations of these biomarkers could have little pathophysiological relevance. Furthermore, these substances, if used as biomarkers, are not cardiac-specific, because they are expressed in all human tissues, when an inflammatory process or tissue damage is promoted. As a result, it is not possible, when several organs are together injured, to differentiate the fraction of biomarker circulating levels derived from cardiac tissue or other tissues. This is an important limitation of these biomarkers compared to cardiac troponins and natriuretic peptides, which can be recognized as cardiac specific biomarkers.

9. Perspectives

A large number of biomarkers with a plausible biological link with HF pathophysiology, mainly associated with immuno-inflammatory and neurohormonal response to heart damage, have been identified so far [2]. Many of these biomarkers share the ability to define the severity of the ongoing ventricular remodeling process, but often lack of cardiac specificity and levels can be influenced by both systemic inflammatory and infective processes, which occur frequently in HF patients. In this clinical context, a new generation of point-of-care testing (POCT) methods for these HF biomarkers, characterized by an improved degree of sensitivity and imprecision, could be utilized in inpatients, outpatients and emergency department settings to aid in the rapid diagnosis, risk and management of patients presenting with symptoms consistent with HF. In particular, the implementation of POCT methods for BNP/NT-proBNP into home-monitoring strategies could help patients and physicians to avoid unnecessary hospitalization or anticipate admission in the clinical ward when it is actually needed [125]. Of course, for the greater part of these novel biomarkers as well as new methodologies, usefulness in driving the clinical and therapeutic decision-making process is still to be proved.

In the recent years, some imaging techniques (cardiac magnetic resonance, cardiac ultrasound with backscatter analysis, positron emission tomography) have been also developed for in vivo tissue characterization. In particular, cardiac magnetic resonance by means of the late gadolinium enhancement technique resulted in a good means for detection of gross myocardial fibrosis in HF [41,126]. In addition, other cardiac magnetic resonance techniques, as T1 mapping, are giving encouraging results for definition and quantification of interstitial fibrosis [127], potentially providing early information on the ongoing remodeling process. Integration of circulating biomarkers with those derived from imaging techniques, as cardiac magnetic resonance, may represent an innovative and effective strategy for a thorough definition of the remodeling process.

Despite clinical guidelines [3] do not routinely recommend endomyocardial biopsies for the management of HF due to an unfavorable risk/benefit ratio, in the clinical setting there is an unmet need for a specific, accurate, and effective biomarker of the ongoing remodeling process in HF. Besides natriuretic peptides and troponins, up-to-date soluble ST2 and galectin-3 are the most studied remodeling biomarkers in the clinical setting but have not yet reached the highest class of recommendation/evidences. Indeed, further studies are needed, in selected, large populations, to test the efficacy of these and other new markers preferably within a multi-marker strategy for patient management, including diagnostic information provided by cardiovascular imaging [128]. Finally, the development of more automated, easily accessible assays, together with a careful evaluation of analytical and clinical performances as well as of their cost-effectiveness, is mandatory for a transfer to routine practices.

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