Cardiac Remodelling Part 1: From Cells and Tissues to Circulating Biomarkers

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A review from the Study Group on Biomarkers of the Heart Failure Association of the ESC

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ABSTRACT (246/250)

Cardiac remodelling refers to changes in left ventricular (LV) structure and function over time, with a progressive deterioration that may lead to heart failure (HF) development (adverse remodelling) or *vice versa* a recovery in response to HF treatment. Adverse remodelling predicts a worse outcome, whilst reverse remodelling predicts a better prognosis. The geometry, systolic and diastolic function and electric activity of the left ventricle are affected, as well as the left atrium and on the long term even right heart chambers. At a cellular and molecular level, remodelling involves all components of cardiac tissue: cardiomyocytes, fibroblasts, endothelial cells and leukocytes. The molecular, cellular and histological signatures of remodelling may differ according to the cause and severity of cardiac damage, and clearly to the global trend toward worsening or recovery. These processes cannot be routinely evaluated through endomyocardial biopsies, but may be reflected by circulating levels of several biomarkers. Different classes of biomarkers (e.g., proteins, non-coding RNAs, metabolites and/or epigenetic modifications) and many biomarkers of each class might inform on some aspects on HF development, progression and long-term outcomes, but most have failed to enter clinical practice. This may be due to the biological complexity of remodelling, so that no single biomarker could provide great insight on remodelling when assessed alone. Another possible reason is a still incomplete understanding of the role of biomarkers in the pathophysiology of cardiac remodelling. Such role will be investigated in the first part of review paper on biomarkers of cardiac remodelling.

Adverse cardiac remodelling occurs in response to an acute myocardial injury or to chronic systemic stressors and comorbidities (e.g., hypertension, diabetes, chronic kidney disease) imposing a chronic stress on the heart via mechanical or hyperdynamic overload, neurohumoral activation, inflammation, metabolic disturbances and potentially many other factors. It is the consequence of a complex series of transcriptional, signalling, structural, and functional events at the cellular and tissue level that affect cardiac size, mass, geometry, function and electrical activity[1]. At first, these responses may be adaptive, but become maladaptive and cause further myocardial damage in a vicious circle when sustained over time. Cardiac remodelling may occur across the entire spectrum of heart failure (HF) and in several cardiac pathologies, representing a rationale target for therapy. Of note, some features of cardiac remodelling differ depending on the triggering injury as well as between patients with preserved vs. reduced left ventricular (LV) ejection fraction (HFpEF and HFrEF), being associated with different biomarker profiles; however, evaluating changes associated with specific HF phenotypes or cardiomyopathies is beyond the scope of this review.

Adverse remodelling is associated with progressive cardiac structural abnormalities and an impairment of cardiac function. Adverse remodelling can be - partially or completely - reversed by treatments controlling risk factors such as hypertension, drugs acting on some of the neurohumoral factors involved in cardiac remodelling (e.g. inhibitors of the renin-angiotensin-aldosterone system), as well as by percutaneous or surgical interventions and device implantation. Reverse remodelling is frequently accompanied by beneficial changes in molecular, metabolic and extracellular matrix (ECM) properties of the myocardium[2,3].

Cardiac remodelling affects all cell types in the heart (cardiomyocytes, fibroblasts, endothelial cells [ECs] and leukocytes), and entails a metabolic switch and alterations in mitochondrial bioenergetics[10,11]. Cardiomyocytes may display contractile dysfunction, hypertrophy or death. Activation of cardiac fibroblasts leads to excessive collagen deposition and fibrosis, either as a direct response to damage (reactive fibrosis) or to replace the loss of cardiomyocytes (replacement fibrosis). Microvascular endothelial dysfunction increase reactive oxygen species (ROS) production, promote leukocyte infiltration, and lead to capillary rarefaction and tissue hypoxia. Moreover, in addition to recruited immune cells, resident macrophages may modify their profile and interact with both myocytes and non-myocytes promoting cellular and molecular alterations. Indeed,

there is a complex intercellular cross-talk among the different cell types present in the myocardium through cytokines, growth factors, non-coding RNAs and mechano-transduction pathways[12]. Some of these changes in the myocardial tissue may be monitored by measuring circulating biomarkers.

The National Institute of Health defined a biomarker (abbreviation of "biological marker") as "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention"[4]. Circulating biomarkers (measured in body fluids) are easily available and reproducible tools for large-scale trials and clinical practice. Given that cardiac tissue is not usually available for direct evaluation, circulating biomarkers measured in different body fluids such as serum, plasma, urine, pericardial fluid or coronary sinus can provide useful information[5,6]. Cells and mechanisms involved change dynamically over time depending on the triggering factor leading to remodelling and cardiac dysfunction, and cardiac remodelling involves alterations in different cell types and components. Therefore, a panel of multiple biomarkers is better suited to characterize the remodelling process than any single biomarker.

A large number of circulating biomarkers has been proposed to refine the management of HF patients[6,7], but very few biomarkers have been thoroughly studied, and most have failed to enter clinical practice[6,8]. Understanding the mechanisms of release, the main source of systemic concentrations (cardiac versus non-cardiac), and the pathophysiological significance of each marker is essential for their optimal interpretation. For example, almost all currently available circulating biomarkers other than cardiac natriuretic peptides and troponins are not cardiac-specific, and their circulating levels may reflect systemic as well as cardiac perturbations [8,9].

This two-part review from the Study Group on Biomarkers of the Heart Failure Association (HFA) of the ESC is devoted to biomarkers of cardiac remodelling. This first part will focus on the main biomarkers related to the cellular and molecular alterations involved in cardiac remodelling. We will review the biomarkers derived from the major cardiac cell types (i.e., cardiomyocytes, fibroblasts, EC and immune cells) in response to injury and or stress. We will then discuss novel biomarkers involved in autocrine and paracrine cellular communication such as non-coding RNAs or extracellular vesicles. Finally, we will address novel potential biomarkers identified through high throughput "-omic" approaches including transcriptomics, proteomics and metabolomics.

Cardiomyocytes

Myocardial stretch, death, oxidative stress and inflammation affecting cardiomyocytes are central mechanisms in cardiac remodelling and HF. Assessing biomarkers related to these processes may allow to explore the mechanisms and progression of myocardial disease (Figure 1).

In response to increased atrial or ventricular wall tension, cardiomyocytes secrete natriuretic peptides. The mature biologically active natriuretic peptides (NP) are obtained after the release of their amino-terminal portion (i.e., pro-B-type natriuretic peptide [BNP] is split into NT-proBNP and BNP). We will focus on BNP and NT-proBNP as the most studied NPs, since atrial natriuretic peptide (ANP) is more difficult to measure. When secreted, NPs promote myocardial relaxation, natriuresis and vasodilatation and blunt the activity of both the renin-angiotensin-aldosterone (RAS) and sympathetic nervous systems[13]. Plasma concentrations of all circulating NPs and their second messenger (cyclic guanosine monophosphate; cGMP) increase in response to increased atrial or ventricular wall tension and proportionally to the extent and severity of LV systolic and diastolic dysfunction[13]. This is related to the mechanisms that control proBNP expression, including stimulation by hormones such as endothelin and angiotensin II, as well as by cytokines and myocardial hypoxia[13]. Importantly, longitudinal changes in NT-proBNP are associated with left atrial and LV reverse remodelling in HF patients with reduced ejection fraction[14–16]. Thus, BNP and NT-proBNP are currently accepted as hallmark diagnostic and prognostic biomarkers for HF across the whole spectrum of LV ejection fraction. Their measurement is included in the all guidelines for HF management[17], and they are frequently utilized as inclusion criteria and surrogate endpoints in early-phase clinical trials.

Cardiac troponins (cTnI and cTnT) are released upon cardiomyocyte injury, necrosis, or apoptosis, and may also leak from injured but still viable cardiomyocytes due to increased plasma membrane permeability[18]. The high-sensitivity troponins assays can predict the incidence of cardiac events (including HF hospitalization) and cardiovascular and all-cause mortality[19,20]. Increased high-sensitivity cardiac troponin T (hs-cTnT) is associated with poor outcomes (e.g., cardiovascular death and HF rehospitalization) in patients with either HFpEF or HFrEF[21]. Combined with NT-proBNP, hs-cTnT can be used to identify maladaptive asymptomatic LV hypertrophy, associated with a high risk of incident HF

and cardiovascular death[22]. Moreover, longitudinal changes in hs-cTnT are associated with left atrial and LV reverse remodelling in patients with HFrEF[14].

Cardiomyocyte hypertrophy is one of the main features of cardiac remodelling and is associated with the expression of foetal gene isoforms. Several signalling pathways are responsible for the coordinated control of the hypertrophic response. These include the adrenergic system, RAS, NP, ROS, adhesion and cytoskeletal proteins, the interleukin-6 (IL-6) cytokine family, MEK-ERK1/2 signalling, histone acetylation and calcium-mediated modulation[23]

Numerous inflammatory cytokines are released in response to myocardial injury, as discussed in the following sections. Some pro-inflammatory cytokines can stimulate the production of cardiomyocyte-derived factors that hold potential as biomarkers of cardiac remodelling. This is the case of the soluble form of ST2 or interleukin-1 receptor-like 1, a molecule released by the lungs and the endothelium in response to hemodynamic stress and inflammation, which is also produced by cardiomyocytes in response to IL-1β and mechanical strain[24]. sST2 is then an indicator of cardiac and pulmonary vascular overload.

Growth-differentiation factor-15 (GDF-15) is involved in several of the mechanisms underlying cardiac remodelling [25]. GDF-15 shares some mechanisms of regulation with BNP, being produced by cardiomyocytes and other cells in response to myocardial injury, stretch, proinflammatory cytokines and neurohormones, such as angiotensin II[26]. GDF-15 might play a role in cardiac hypertrophy, endothelial dysfunction and fibrosis, although whether GDF-15 is a protective or detrimental factor is debated[25]. GDF-15 is associated with incident HF in the general population and poor outcomes in HF patients[27]. For example, higher GDF-15 levels were associated with a higher risk of cardiovascular death and HF hospitalization in patients with either HFpEF or HFrEF[28]. Of note, it adds prognostic information over NT-proBNP in patients with acute coronary syndrome[29]. The effects of drug treatments on GDF-15 concentrations in chronic HF remain elusive, while LV assist-device implantation can lead to a significant decrease of circulating GDF-15 in patients with advanced HFrEF[27,30].

Cardiomyocytes also release extracellular vesicles and non-coding RNAs that are potential biomarkers and act as mediators via autocrine and paracrine actions in cardiomyocytes and other cardiac cells. These aspects will be further discussed in the following sections.

Fibroblasts and the extracellular matrix

Activated cardiac fibroblasts and myofibroblasts secrete high amounts of structural (e.g., collagens and fibronectin) and matricellular proteins, as well as enzymes, growth factors, cytokines, and non-coding RNAs, leading to an intense deposition of collagen fibres and subsequent myocardial fibrosis[31]. In addition, molecules secreted by cardiac fibroblasts can influence other cardiac cells including cardiomyocytes, macrophages and endothelial cells[32,33]. Cardiac fibroblasts can be activated through different pathways[34] in response to local cardiac injury and systemic alterations such as neurohormonal activation or to extra-cardiac comorbidities (e.g., diabetes or chronic kidney disease).

Cardiac fibroblasts do not constitute a homogenous population, and diverse subsets with specific functions have been identified in different conditions. For instance, single-cell transcriptomic analyses in mice after myocardial infarction (MI) led to the identification of clusters of pro- or anti-fibrotic myofibroblasts[35]. In conditions of pressure overload, a subset of activated fibroblasts may preserve an adequate collagen scaffold and suppress macrophage-related inflammation[36].

Myocardial fibrosis is the result of collagen synthesis exceeding degradation, leading to excessive formation and deposition of collagen fibres, with predominance of type I over type III collagen fibres[31]. There are 2 key steps in the process of collagen fibre formation and maturation (shown in Figure 2): first, the release of C- and N-terminal propeptides from procollagen to render collagen molecules able to form fibrils; second, the stabilization of collagen fibrils through cross-links (mostly enzymatically mediated by enzymes of the lysyl oxidase [LOX] family, including LOX and LOX-like2). The increased collagen cross-linking makes fibres stiffer and more resistant to degradation, contributing to the detrimental impact of myocardial fibrosis on cardiac function and prognosis[37–41]. Moreover, increasing ECM stiffness may further activate cardiac fibroblasts perpetuating the pro-fibrotic response[42]. Of note, the proportion between collagen type I and III is also relevant, as they have different physico-chemical properties, with collagen type I fibres being thicker and stiffer[31].

Collagen degradation is regulated by matrix metalloproteinases (MMPs) and their specific tissue inhibitors (TIMPs). MMP-1, a collagenase, is the first enzyme acting on collagen, which is then further degraded by gelatinases (e.g., MMP-2 and MMP-9). The pathophysiological interpretation of circulating MMPs is complex as they should be

considered in the context of the balance between collagen synthesis and degradation. Moreover, excessive collagen degradation may be detrimental and lead to LV dilatation in some settings (e.g., after MI). On the other hand, some of the peptides derived form MMP-mediated collagen degradation, the matricryptins, may also contribute to increase myocardial fibrosis[43]. Nevertheless, elevated circulating levels of several MMPs are associated with poor outcomes in HF patients (e.g., higher risk of mortality and HF hospitalization), especially in those with HFrEF[44]. Similarly, increased serum TIMP-1, the inhibitor of MMP-1, potentially reflecting an impaired collagen degradation, is also associated with poor clinical outcomes in both HFrEF and HFpEF patients[44,45]. It is important to remember that none of the fibrosis-related biomarkers is cardiac-specific, and that they may also reflect the overall profibrotic response associated with some conditions like HFpEF, or altered collagen metabolism related to other comorbidities.

Biomarkers related to collagen type I quantity and cross-linking have been identified. The C-terminal propeptide of procollagen type I (PICP) is directly associated with the extent of collagen deposition in myocardial biopsies in HF of different aetiologies including hypertensive heart disease[46] and dilated cardiomyopathy[47] On the other hand, the ratio of the C-terminal telopeptide collagen type I (CITP) to matrix metalloproteinase-1 (MMP-1) is inversely correlated with myocardial collagen cross-linking, given that the higher the degree of cross-linking the less CITP is released by MMP-1[38]. Additionally, cardiac and serum LOXL2 are upregulated in the myocardium of HF patients, correlating with the degree of collagen cross-linking[41].

High serum PICP is associated with a higher risk of hospitalization and mortality in patients with dilated cardiomyopathy[47] (Figure 2). Likewise, the CITP:MMP-1 ratio is inversely associated with the risk of HF hospitalization in patients with HFpEF or HFrEF of hypertensive origin[38] (Figure 2), and can identify patients at risk of HF[53] and HF patients with diastolic dysfunction[54 53] who will respond to mineralocorticoid receptor antagonists (MRA) treatment[54]. The combination of PICP and CITP:MMP-1 ratio also offers incremental prognostic value for HF hospitalization or cardiovascular death beyond that offered by established risk factors in HF patients of hypertensive aetiology[40]. Of interest, serum PICP decreased in response to treatment with drugs interfering with the RAS such as angiotensin II type 1 receptor blockers (i.e., losartan)[48] or MRAs[49–51], or with the loop diuretic torasemide[52] in patients with HF of ischemic and hypertensive aetiologies or in patients at risk for HF. On the other hand, the N-terminal pro-peptide of

procollagen type III (PIIINP) is the only other marker correlated with collagen deposition as seen on myocardial biopsies in HF patients with idiopathic or ischemic dilated cardiomyopathy [55], and it is also modified by treatment with MRAs, mainly in HFrEF patients[56].

Other ECM molecules such as osteopontin or thrombospondins are potential biomarkers of pro-fibrotic pathway activation in new-onset and chronic HF[57,58]. Similarly, other factors related to fibroblast biology such as fibroblasts growth factor-23 (FGF-23) were associated with cardiac remodelling after MI[59]. In addition, more sensitive methodologies for the analysis of plasma proteome have allowed to identify pro-fibrotic proteomic profiles in patients at risk of HF[60], as explained below.

Microvascular remodelling and endothelial dysfunction

The microvascular endothelium accounts for one third of all cardiac cells and is a direct target of all the major cardiovascular risk factors[61]. The epithelial layer not only serves as a barrier between the blood and myocardial tissue, but is also involved in the immune response and communicates with adjacent cells through the release of peptides, proteins, extracellular vesicles and microRNAs[62]. Indeed, endothelial dysfunction has been proposed as one of the main pathophysiological pathways involved the development and progression of HF, in particular in the setting of HFpEF, as it can induce cardiomyocyte hypertrophy and stiffness, trigger fibrosis and facilitate the infiltration of immune cells [61,63]. Figure 3 depicts the most relevant biomarkers released by dysfunctional endothelial cells and their autocrine and paracrine effects. As mentioned above for fibrosis biomarkers, none of the biomarkers discussed in this section is specific of cardiac EC. Circulating levels of indicators of EC activation like adhesion molecules involved in leukocyte trafficking (e.g., E-selectin, ICAM-1 or VCAM-1) or von Willebrand factor are proposed as biomarkers of endothelial dysfunction in HF[64,65].

Besides damage to vascular ECs, microvascular remodelling is characterized by structural abnormalities including medial hypertrophy, intimal hyperplasia, interstitial fibrosis and disarray of vascular smooth muscle cells. These changes lead to impaired coronary flow reserve[66], which can be associated with specific circulating proteomic profiles[67,68], and narrowing of resistance vessels and capillary rarefaction[69–71]. Vascular remodelling may compromise local tissue oxygen supply, impair fluid homoeostasis and alter LV wall compliance[61,72].

ECs produce nitric oxide (NO) through the endothelial isoform of the nitric oxide synthase (eNOS). NO is a key regulator of vascular homeostasis, activating soluble guanylyl cyclase to generate cyclic guanosine monophosphate (cGMP), which causes vascular relaxation and regulates further effects on cardiac cells and blood, such as modulation of cardiac contraction, oxygen consumption, substrate utilization, apoptosis, hypertrophy and platelet adhesion[73]. The effects of this signalling pathway are opposed by increased ROS and inflammation-mediated prostanoids produced by the cyclo-oxygenase pathway, which promotes endothelium-dependent vasoconstriction by inducing the breakdown of NO or by acting directly on vascular smooth muscle cells. Reduced NO production and bioavailability lead to decreased cGMP, resulting in vascular smooth muscle contraction and increased cardiomyocyte stiffness[73,74]. However, no reliable biomarkers of the cGMP pathway have emerged so far. Of interest, vericiguat, a direct soluble guanylate cyclase stimulator, increases cGMP, which leads to improvements in vascular and cardiac function[75].

The peptide endothelin-1 (ET-1) is released by the endothelium. ET-1 synthesis is activated in response to cardiovascular risk factors, such as hyperglycemia, hypercholesterolemia, hypertension, aging, as well as by biochemical (e.g. angiotensin II, and several cytokines) and mechanical stimuli.[76] It is the most potent endogenous vasoconstrictor and contributes to vascular tone by binding to membrane bound G-protein coupled receptors (ET_A/ET_b)[77,78]. The utility of plasma ET-1 to predict adverse cardiovascular events has been studied in different conditions such as systemic or pulmonary hypertension and HF[76,77,79]. Although most evidence has been obtained from patients with coronary artery disease and HFrEF, pro-ET-1 was associated with a higher risk for all-cause death in patients with chronic HF irrespectively of the LV ejection fraction[80]. The ET-1 receptor inhibitor atrasentan reduced the risk of kidney failure in diabetic patients with chronic kidney disease[81], while tezosentan did not improve symptoms or clinical outcomes in patients with acute HF [82]. The cardiac benefits of ET-1 inhibition then remain to be validated in HF.

Adrenomedullin (ADM) is another regulatory peptide hormone that is produced mainly by endothelial and vascular smooth muscle cells and diffuses freely between the interstitium and the circulation. ADM is involved in the regulation of vascular integrity and is an emerging HF biomarker. ADM is synthesized by almost all tissues, but mainly by the adrenal medulla, heart, lungs and kidneys in response to pressure and volume

overload[83]. The two most important functions of ADM are vasodilatation and reduced endothelial permeability in resistance and capacitance vessels[83]. Whilst interstitial ADM is thought to cause vasodilatation by acting on vascular smooth muscle cells, intravascular ADM improves vascular integrity and decrease permeability through its effects on ECs[84]. Reliable quantification of ADM is difficult due to its short half-life and its binding to carrier proteins. The mid-regional portion of the prohormone (MR-proADM) seems to be more stable, and MR-proADM is useful to identify acute HF in conditions of severe dyspnoea and cardiogenic shock and to predict mortality[85–87]. More recently, an immunoassay that specifically measures biologically active ADM (bio-ADM) has been developed. Bio-ADM increases in acute HF patients, is associated with tissue congestion (not necessarily of cardiac origin) and increased risk of cardiovascular events[83,88]. First data suggest that bio-ADM is a biotarget of sacubitril/valsartan[89]. Clinical studies with anti-ADM antibodies are being planned[90].

Immune cells and inflammation

Macrophages are the most represented immune cell in the healthy heart, particularly in the interstitial space, while very few monocytes, dendritic cells or T-cells are present. Cardiac remodelling is characterized by an influx of immune cells that secrete a wide array of pro- and anti-inflammatory cytokines[91]. Possible phenotypes, their dynamics and the components of the immune response in cardiac remodelling and HF are extremely heterogeneous[92]. They reflect the nature of the initial trigger (e.g., MI, myocarditis, or other injury), the severity and duration of the injury, the genetic susceptibility, and the presence or absence of additional triggers and modifiers of the immune response (e.g., obesity and diabetes)[93]. The immune response after MI has been best characterized[94,95]. In this setting, immune cells including neutrophils and monocytes act as chemoattractant and adhesive cells, mobilize more cells and build up a response whereby inflammation transitions to scar formation. The post-MI response has a clear temporal pattern, with an acute response during the first days and a subsequent reparative response during the following 2-4 weeks, after which a chronic response develops and most immune cells disappear. Therefore, the profile of inflammation-related biomarkers will change dynamically along time depending on the specific underlying injury (as shown in Figure 1).

Specific proteins are produced and secreted as part of the immune response and may be measured as biomarkers. The list of ‘inflammatory biomarkers’ is very long, but we will

focus on cytokines and a few biomarkers that, although not being cardiac-specific, have been described in greater detail in the setting of cardiac remodelling and HF[96]. Figure 3 summarizes the most relevant biomarkers released by infiltrated and resident cardiac leukocytes and their paracrine actions.

Classical pro-inflammatory cytokines, including tumour necrosis factor-α (TNF-α) and IL-1 and IL-6, have been studied mainly in clinical cohorts of HFrEF being associated with less favourable outcomes[97]. These associations are not very strong and are confounded by multiple factors such as age and comorbidities. TNF-α has been proposed as a possible treatment target, but a trial with the TNF-α receptor blocker etanercept did not improve the clinical status nor prolong survival in patients with HFrEF[98].

Pro-inflammatory cytokines also trigger the release of other factors such as insulin-like growth factor binding protein-7 (IGFBP-7). Elevated serum IGFBP-7 are associated with left atrial remodelling and diastolic dysfunction in patients with acute dyspnoea (with or without HF)[99] and in HFpEF patients [100], as well as with a higher risk of cardiovascular events including mortality[100,101].

The pro-inflammatory alarmins S100A8 and S100A9 play a role in cardiac remodelling after MI[102]. Elevated plasma S100A8/S100A9 is associated with a higher risk of MI and cardiovascular death in healthy individuals[103] and with a higher risk of mortality in elderly HF patients [104].

Galectin-3 is secreted by macrophages into sites of injury[105], and circulating levels of galectin-3 are increased in patients with either HFrEF or HFpEF[106] and are associated with poor outcomes in acute[107] and chronic HF[108]. Interestingly, targeting galectin-3 may attenuate myocardial fibrosis[109]. Several compounds inhibiting galectin-3 are under evaluation in phase 2 and 3 clinical trials on several fibrotic diseases, such as pulmonary (NCT03832946) and liver (NCT02462967) fibrosis.

sST2 is another biomarker associated with tissue fibrosis, that is increased in both HFrEF and HFpEF patients[106] and associated with worse outcomes in acute and chronic HF[110,111]. Although sST2 was initially thought to be primarily produced by cardiomyocytes, more recent evidence suggests that it is produced mainly in the lungs and by the vascular endothelium in response to pro-fibrotic and pro-inflammatory stimuli, but also following vascular congestion[111]. sST2 levels change in response to guideline-recommended medical therapy[112,113]. For instance, treatment with the angiotensin receptor-neprilysin inhibitor (ARNI) sacubitril/valsartan induced a reduction in sST2 levels in HFrEF

patients from the PARADIGM-HF trial[114], whereas a substudy of the EPHESUS showed no significant effect of eplerenone on sST2[115]. However, those patients with higher sST2 treated with eplerenone showed less adverse cardiac remodelling[115]. In this context, it sST2 may be used to guide treatment[112,113]. Prospective trials are required to assess whether sST2-guided management will improve outcomes; the potential clinical relevance of sST2 will be discussed in detail in the second part of this review.

Biomarkers involved in communication between cardiac cells

Autocrine and paracrine communication between cardiac cells and immune cells plays a key role in cardiac remodelling. This communication is mediated by growth factors, cytokines and non-coding RNAs among others, and these molecules may act as both pathophysiological mediators and circulating biomarkers. A comprehensive description of all potential candidates is beyond the scope of this review, so we will focus on non-coding RNAs and extracellular vesicles, discussing some representative examples.

Non coding RNAs

Non-coding RNAs reflect the large majority of the human RNA and some are key regulators of multiple cellular and tissue remodelling processes[116,117]. However, non-coding RNAs usually have multiple targets and in some cases it is not easy to associate them with specific features of cardiac remodelling. Several microRNAs are involved in cardiac remodelling, hypertrophy and HF with diverse and sometimes opposite effects, either inhibiting or activating cardiomyocyte hypertrophy, cell death, neovascularization and fibrosis. For instance, miR-15, miR-34 and members of the miR-17-92 cluster induce cardiomyocyte apoptosis and stimulate neovascularization[118–120]. On the contrary, miR-210 has anti-apoptotic effects and protects the vasculature[121]. miR-208, a cardiac-specific microRNA, promotes hypertrophy[122]. However, miR-133, a muscle-enriched microRNA, inhibits this process[123]. Several microRNAs, such as miR-21, induce fibroblast activation and myocardial fibrosis[124]. miR-132 is actively involved in pathological cardiac remodelling, promoting cardiomyocyte hypertrophy and inhibiting autophagy[125]. A miR-132 inhibitor has shown promising results in several preclinical large animal HF models[126,127]. Recently, the first study applying a miRNA inhibitor targeting miR-132 in chronic HF patients showed a good safety profile and indicative efficacy paving the way for continued development in larger study cohorts [128].

Long non-coding RNAs (lncRNAs) comprise a large group of extremely diverse molecules which are defined by a length of more than 200 nucleotides, and regulate gene expression and miRNA function. They play an important role in cardiac remodelling. The lncRNAs "cardiac hypertrophy associated transcript" (CHAST)[129] and H19[130] promote hypertrophy and emerged as powerful treatment targets, whereas "suppression of myosin heavy chain-associated RNA transcript" (MYHEART) accelerates the progression from hypertrophy to HF[131]. In addition, both the cardiac fibroblast-enriched "Wisp2 super-enhancer-associated RNA" (WISPER), as well as the "maternally expressed gene 3" (MEG3) promote cardiac fibroblast activation and myocardial fibrosis[132,133].

Non-coding RNAs can cross the membrane barrier and can be detected in different body fluids such as urine, serum and plasma. In contrast to coding messenger RNA, they are relatively resistant to nucleolytic degradation by RNAses and some plasma non-coding RNAs are quite stable in challenging conditions (e.g., varying temperature and pH), making them appealing candidates as circulating biomarkers[134]. Their stability derives from their transportation within EVs or association with RNA-binding proteins such as nucleophosmin, argonaute protein 2 or lipoprotein complexes[135].

Some muscle-specific or cardiac-enriched microRNAs, such as miR-1, miR-133, miR-208 or miR-409, are consistently increased in the circulation following cardiac damage[134]. Circulating miR-133 and miR-208 increase shortly after MI. miR-133 increased up to 100 times within the first 9 hours after MI[136], and correlated with cTnT levels[137]. Similarly, an increase in circulating miR-208 was detected during the first hours after an MI, being correlated with cTnT, but showing a more rapid increase[138]. When compared with other cardiac- and muscle-associated miRNAs, miR-208 displayed the highest specificity and sensitivity for MI prediction[138]. Nevertheless, other studies failed to detect miR-208 in the circulation[136], probably due to low levels and quick clearance, which limits its usefulness as a biomarker in a chronic setting. miR-499 is another cardiac specific microRNA, encoded by an intron of the β-myosin heavy chain gene that is down-regulated by hypoxia/ischaemia. Circulating miR-499 is elevated already 1 hour after symptom-onset in MI and increases gradually for 9 hours, being associated with cTnT levels[139]. Interestingly, miR-208 and miR-499 have prognostic utility, being strongly associated with mortality in HF patients[140]. Alterations in circulating levels of several non-cardiac microRNAs have been reviewed elsewhere[134,141]. Notably, an 8-microRNA signature, reflecting changes in different pathological pathways, has shown an adequate

performance (AUC 0.81) to discriminate HFrEF from HFpEF[142]. The cardiac tissue-associated miR-132 is also positively correlated with NYHA class and improved risk prediction for HF hospitalization in patients with chronic HF[143].

The clinical relevance of circulating lncRNAs as biomarkers has been less studied. In transcriptomic studies of lncRNAs in plasma from patients with cardiac remodelling post-MI, the lncRNA "long-intergenic non coding RNA predicting cardiac remodelling" (LIPCAR) was identified as a potential biomarker predicting outcome post-MI and in hypertensive HF patients[144,145]. Other lncRNAs (aHIF, ANRIL, KCNQ1OT1, MIAT, MALAT) have shown some value to predict cardiac remodelling post MI, while ANRIL and KCNQ1OT1 enhanced the predictive value of a model including clinical parameters and cardiac biomarkers [146].

Some drawbacks of circulating microRNAs are the lack of cardiac specificity and variability in the proposed measurement protocols (e.g., serum vs. plasma, freezing conditions, fasting, different processing protocols)[116]. RNA yield varies significantly between different isolation procedures[147] and a low correlation has been found between the different methods used for non-coding RNA quantification (PCR, microarrays, sequencing)[148]. Moreover, normalization strategies are not yet standardized, and both endogenous and exogenous reference standards are used[149], complicating the comparison between studies. Addressing these issues is essential to facilitate the translation of non-coding RNA measurements to clinical practice[134]. In addition, further studies in randomized clinical trials are needed to elucidate their fluctuation in response to available therapeutic agents in HF.

Extracellular vesicles

Extracellular vesicles are produced by different cardiac cells and contain a variety of receptors, lipids, proteins and RNA (both mRNA and non-coding RNAs). Extracellular vesicles have emerged as potential mediators in cardiovascular disease, playing a prominent role in intercellular communication[150–152]. For instance, cardiomyocyte-derived exosomes enriched in miR-92a trigger myofibroblast activation[153], and cardiac fibroblast-derived extracellular vesicles enriched in miR21, miR-27a, miR-28-3p and miR-34a are absorbed by cardiomyocytes, leading to ROS accumulation, cardiomyocyte damage and HF development[154,155]. Under hypoxic or ischaemic conditions, cardiomyocyte-derived extracellular vesicles promote EC proliferation and vessel sprouting, with miR-122 and

miR-143 being involved in these effects[156]. In addition to miRs, extracellular vesicles contain large amounts of lncRNAs that contribute to paracrine signalling between cardiac cell types[152,157]. Extracellular vesicles from immune cells are also involved in cardiac remodelling[158]. For instance, inflammatory extracellular vesicles released after MI contain abundant alarmins (e.g., IL-1α, IL-1β, Rantes) that contribute to cardiac dysfunction[159].

Extracellular vesicles could represent new diagnostic and prognostic biomarkers as their molecular content is a fingerprint of the cell of origin and its physiological state and they are detected in easily accessible body fluids such as blood and urine[152]. Interestingly, the molecular content of extracellular vesicles, but also the number of total extracellular vesicles or vesicles from a specific cell type are altered in pathological conditions and could be used as a circulating biomarker of cellular alterations[160]. Both cardiac fibroblasts and cardiomyocytes actively secrete extracellular vesicles containing large amount of noncoding RNAs and those vesicles can be uptaken by other cardiovascular cell types thus having powerful paracrine activities opening up a new avenue of mechanistic and potentially therapeutic opportunities[155,157]. However, analysis of extracellular vesicles content requires several processing steps and low-input techniques that currently limit their applicability in clinical practice.

Novel molecular insights from "-omic" studies

HF phenotyping combining high throughput methodologies such as transcriptomics, proteomics and metabolomics might represent a new frontier in the research on cardiac remodelling and HF, representing an important step towards personalized medicine[161]. Bioinformatics and artificial intelligence platforms are essential to manage the enormous amount of data from these studies, to identify known or previously unrecognized molecular mechanisms relevant for myocardial remodelling and HF[162]. Network analyses of circulating biomarkers have been useful to identify differential phenotypes between HFpEF and HFrEF patients[163], providing mechanistic insights into cardiac remodelling[67,164] and the actions of spironolactone in patients at risk of HF[165].

Single cell transcriptomics and epigenomics

Technological advances with Next Generation Sequencing and high-throughput profiling have given insights into the molecular substrates of cardiac remodelling in HF. These include numerous single-cell transcriptomic and epigenomic studies that advance

knowledge of diverse myocardial cell types subsets, and pathologically significant changes, tracking through disease progression[166–168], confirming known, and revealing novel, pathways of the response to cardiac stress. Some findings point to new candidate biomarkers for different disease stages. For example, a single-cell transcriptomic analysis of hearts from mice subjected to angiotensin II administration to drive pathological remodelling revealed that 2 distinct fibroblast populations emerge upon stress[169]. Called Fibroblast-*Cilp* and Fibroblast-*Thbs4,* these reflect markers that were highly expressed in each cell subpopulation, but both were otherwise barely present in unstressed hearts, and only significantly increased upon angiotensin II provocation. The clinical significance of these specific subpopulations of fibroblasts will need further investigation considering the preeminent role of cardiac fibrosis in cardiac remodelling of different aetiologies. In another study, spatial transcriptomics identified 2 temporal stages in myocardial scar formation: an early scar with high hypoxia signalling and low ECM production, and a more mature scar with increased ECM production[170]. Genes including *TGFB3* and *PDGFRA* are abundantly expressed in the mature scar, while *SERPINE1* is more expressed in the early scar. The study also proposed *RUNX1* as a driver of differentiation from fibroblasts to myofibroblasts, a key process in post-MI remodelling.

Epigenetic modifications such as DNA methylation and histone modifications have also emerged as possible biomarkers in HF. Differentially methylated promoters and CpG islands represent hallmarks of failing hearts[171,172]. Genomic enhancers marked by specific histone modifications also show characteristic patterns of change when assessed in biopsies from failing LVs[173,174]. Differentially methylated loci, including demethylated *NPPA* and *NPPB,* were found in circulating blood cells from patients with dilated cardiomyopathy, making them potential biomarkers for further study[171]. Similarly, a growing body of evidence supports the utility of somatic mutations found in clonally expanded haematopoietic cells (clonal haematopoiesis of indeterminate potential: CHIP) for outcome prediction in HF[175,176].

Proteomics and metabolomics

There is a mismatch between the transcriptome and proteome due to multiple mechanisms that are dysregulated in disease conditions such as altered regulation of protein translation by non-coding RNAs or of protein degradation pathways. Therefore, proteomics is a key

complement to genomic and transcriptomic studies. Proteomic analysis implies an unbiased interrogation of multiple proteins at a genome-wide scale, but no single proteomic platform can accomplish this alone. For example, the human plasma proteome project had to combine unbiased and targeted mass spectrometric approaches, standard immunoassays and newer multiplexed affinity-capture techniques to cover the breadth and depth of the human plasma proteome (https://www.hupo.org/plasma-proteome-project). Mass spectrometry has made major advances in unbiased proteomic profiling, particularly through the application of label-free high-resolution techniques[177]. However, unbiased mass spectrometry techniques are still unable to reliably detect proteins with a very low expression, especially in complex matrices like plasma or serum where protein concentration spans across several orders of magnitude. Developments in proteomics allow sensitive and specific detection of low abundance proteins in plasma. Available approaches include mass spectrometry, protein microarray, aptamer, and proximity extension assay (PEA)-based technology[178]. For instance, plasma proteomic analyses with PEA panels identified proteins related to inflammation, ECM remodelling, angiogenesis, regulation of blood pressure and RAS activation and associated with incident HF[60]. Chan et al.[164] employed aptamer-based affinity-capture plasma proteomics to measure 1,305 plasma proteins one month after MI. They found that 212 differentially-expressed plasma proteins were significantly associated with subsequent HF events. Of these, 96 proteins correlated with LVEF measured 4 months after an MI. When these 96 proteins were cross-referenced with RNAseq data from human and murine datasets of ischaemic HF performed with single-cell resolution, the most highly-enriched mRNA-protein candidates were cardiac-specific biomarkers NT-proBNP and cTnT, as well as emergent biomarkers such as angiopoietin-2, thrombospondin-2, latent transforming growth factor-β binding protein-4 and follistatin-related protein-3, which are primarily of ECM origin. Both angiopoietin-2 and thrombospondin-2 were also found to be the circulating proteins most strongly associated with HF events in a large electronic health record-driven proteomic study (EMERGE)[179].

Comprehensive plasma metabolomic profiling shows systemic alterations in patients with cardiac hypertrophy and HF[180]. In patients from the Bogalusa Heart Study, untargeted metabolomic analysis identified pseudouridine and N-formylmethionine to be associated with cardiac hypertrophy[181]. Although promising, there are some technical and methodological challenges to the use of metabolomics for biomarker profiling. Efforts

are focused on minimizing analytical and biological variability (e.g., fasting status or duration of fasting, diet, timing of sampling, drug treatments, etc.), harmonizing metabolite data across different analytic platforms, metabolite annotation and relative quantification[182].

Conclusion and future perspectives

Cardiac remodelling is a dynamic process that plays a major role in HF development and progression and is characterized by a complex network of intertwined tissue, cellular and molecular alterations. An accurate assessment of these changes could be valuable for personalized risk stratification and management of HF patients. Since cardiac tissue is not usually available for study, surrogate biomarkers of tissue alterations are needed (Table 1). A large number of potential circulating biomarkers has been proposed over the last 20 years, leading to some scepticism about their clinical applicability. For several of the candidates presented, robust and reproducible assessment techniques, association with cardiac features, variations in response to disease progression or specific therapies, and validation in large-scale studies are all essential steps towards their routine clinical application. Moreover, as cardiac remodelling involves different cell types and molecules, no single biomarker is likely to provide complete information, and a multi-marker panel including biomarkers that reflect alterations in different cells and pathological pathways, and possibly also of different nature (e.g., proteins, non-coding RNAs, metabolites, epigenetic modifications) might better capture the histological and molecular signature of cardiac remodelling (Graphical abstract). The challenge remains to integrate several biomarkers and their dynamic changes associated to disease evolution or therapeutic management and capture the complexity of tissue cardiac remodelling. Algorithms obtained through machine learning strategies could help define the value of clusters of biomarkers associated with adverse or reverse cardiac remodelling. This approach is already used to identify phenotypic clusters of HF patients[183]. To this aim, multinational collaborative efforts are needed to provide large independent cohorts with the simultaneous assessment of the panel of biomarkers and develop and validate algorithms to support clinical decision-making.

Finally, the combination of circulating biomarkers with imaging findings reflecting alterations in cardiac geometry and function, as well as genetic profiles, could allow a better phenotyping of cardiac remodelling, potentially enabling a tailored approach to HF therapy.

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Table 1. Proposed circulating biomarkers of cardiac remodelling currently being measured

Biomarker	Cellular Origin	Reflects/Involved	Triggering factors	Assay performance*
BNP, NT-proBNP	Cardiomyocytes	Cardiomyocyte stress	Stretch Neurohumoral Cytokines Hypoxia	Clinical
Troponins	Cardiomyocytes	Cardiomyocyte damage	Ischemia Inflammation	Clinical
GDF-15	Macrophages, Smooth muscle Endothelium Cardiomyocytes	Hypertrophy Fibrosis Inflammation Endothelial dysfunction	Myocardial injury Stretch Neurohumoral Cytokines	Clinical
PICP, PIIINP, CITP, MMP-1	Fibroblasts	Fibrosis	Neurohumoral Pressure overload. Inflammation. Cytokines	Research
Adhesion molecules (E-selectin, VCAM-1, ICAM-1)	Endothelium	Endothelial dysfunction	Inflammation.	Research
Endothelin-1	Endothelium	Vasoconstriction	Mechanical stress Hyperglycemia, Neurohumoral Cytokines	Research
Adrenomedullin	Endothelium Smooth muscle and others	Vascular permeability Vasodilation Congestion	Pressure & volume overload	Research

Cytokines (ILs, TNF-α)	Leukocytes	Inflammation Fibrosis Endothelial dysfunction	Inflammation	Clinical & Research
Soluble ST-2	Lung Endothelium Cardiomyocytes	Inflammation Fibrosis Congestion	Hemodynamic stress Inflammation	Clinical
Galectin-3	Leukocytes and others	Inflammation and fibrosis	Inflammation Neurohumoral	Research
MicroRNA-208 MicroRNA-499	Cardiomyocytes	Cardiomyocyte injury	Ischemia	Research

BNP stands for B-type natriuretic peptide; NT-proBNP, amino-terminal fragment of proBNP; GDF-15, growth differentiation factor-15; PICP, procollagen type I carboxy-terminal propeptide; PIIINP, procollagen type III amino-terminal propepetide; CITP, collagen type I carboxy-terminal telopeptide; MMP-1, matrix metalloproteinase-1; VCAM-1, vascular cell adhesion molecule-1; ICAM-1, intercellular adhesion molecule 1; ILs, interleukins; TNF-α, tumour necrosis factor-α.

*, Those biomarkers with assays available for automatized clinical diagnostic platforms or approved for clinical diagnosis are referred to as clinical, whereas those with assays developed for research only as referred as research.

FIGURES

Figure 1: Cardiomyocyte-derived biomarkers. Cardiomyocytes release natriuretic peptides (propeptides of ANP, BNP) in response to increased left ventricular wall stress which in turn regulate cardiac hypertrophy, exerting namely anti-hypertrophic effects. Cardiac troponins are released upon cardiac injury that leads to cardiomyocyte necrosis, apoptosis or increased wall permeability. Growth-differentiation factor-15 (GDF-15) is produced by cardiomyocytes among other cells in response to cardiac damage and is involved in autocrine/paracrine effects on different cardiac cells. Similarly, cardiomyocytes release non-coding RNAs and extracellular vesicles (EVs) which are not only biomarkers of cardiomyocyte alterations but also have a pathophysiological impact on different cardiac cells.

Figure 2: Collagen-derived peptides. Collagen type I is the most abundant type of collagen in the myocardium. It is produced and secreted into the interstitium by myofibroblasts/activated fibroblasts as procollagen. Procollagen is processed by procollagen carboxy-proteinase (PCP) and procollagen amino-proteinase (PNP), releasing carboxy-terminal (PICP) and amino-terminal (PNP) propeptides. PICP must be excised to form collagen fibrils. PICP reaches the bloodstream and can be assessed as a biomarker of collagen synthesis. Collagen cross-linking (CCL) stabilizes mature collagen fibres in a process mostly mediated by lysyl oxidases (LOX). Increased collagen cross-linking makes fibres stiffer and more resistant to degradation. Therefore, the higher cross-linking the less collagen type I carboxy-terminal telopeptide (CITP) is released by matrix metalloproteinase-1 (MMP-1). MMP-1 and CITP also enter the circulation and their ratio is inversely associated with the degree of collagen cross-linking. Higher serum PICP (*upper right panel*) was associated with a worse prognosis (all-cause mortality, heart transplantation, life-threatening ventricular arrhythmias, and heart failure hospitalization) in patients with idiopathic dilated cardiomyopathy presenting cardiac late gadolinium enhancement by magnetic resonance imaging (adapted from reference [47]). Lower CITP:MMP-1 (*lower right panel*) was associated with a higher risk of heart failure hospitalization or cardiovascular death in hypertensive heart failure patients (adapted from reference [38]).

Figure 3: Endothelium and inflammation-related biomarkers. Dysfunctional endothelial cells express adhesion molecules (e.g., E-selectin, vascular cell adhesion molecule-1 [VCAM-1]), that facilitate leukocyte infiltration. Activated endothelial cells

also release von Willebrand factor. In this condition there is also a decrease in nitric oxide (NO) release and bioavailability and an increase in reactive oxygen species (ROS) that can have autocrine and paracrine effects on endothelial cells and other cardiac cells. Endothelial cells also produce C type natriuretic peptide (CNP) endothelin-1 (ET-1) and adrenomedullin (ADM). Similarly, they release non-coding RNAs and extracellular vesicles (EVs) that are not only biomarkers of endothelial cells alterations but also have a pathophysiological impact on other cardiac cells. Infiltrated leukocytes release a number of cytokines and growth factors including interleukins-1 and -6, tumour necrosis factor-α (TNF-α), transforming growth factor-β (TGF-β), galectin-3 (Gal-3) or soluble ST2 (sST2), which have pleiotropic effect on the myocardium.

Graphical abstract. Integrated view of different cell types, derived-biomarkers and their pathophysiological impact in cardiac remodelling. Integrated view of interaction of different cell types, derived-biomarkers and their pathophysiological impact in cardiac remodelling. At the histocellular level cardiac remodelling is the result of alterations in all cell types present in the myocardium, which also interact closely with each other in response to cardiac damage, injury, neurohormonal activation and stress. A number of molecules are potential circulating biomarkers of the different features of cardiac remodelling. A combination of such biomarkers will provide incremental information of the major histocellular alterations present in HF patients. BNP stands for B-type natriuretic peptide; NT-proBNP, amino-terminal fragment of proBNP; MRpro-ANP, mid-regional pro-atrial natriuretic peptide; GDF-15, growth differentiation factor-15; ncRNAS, non-coding RNAs; EVs. Extracellular vesicles; PICP, procollagen type I carboxy-terminal propeptide; PIIINP, procollagen type III amino-terminal propepetide; CITP, collagen type I carboxy-terminal telopeptide; MMP-1, matrix metalloproteinase-1; sST-2, soluble ST2; TGF-β , transforming growth factor-β; TNF-α, tumour necrosis factor-α; VCAM-1, vascular cell adhesion molecule-1; vW, von Willebrand; ADM, adrenomedullin; CNP. C-type natriuretic peptide.

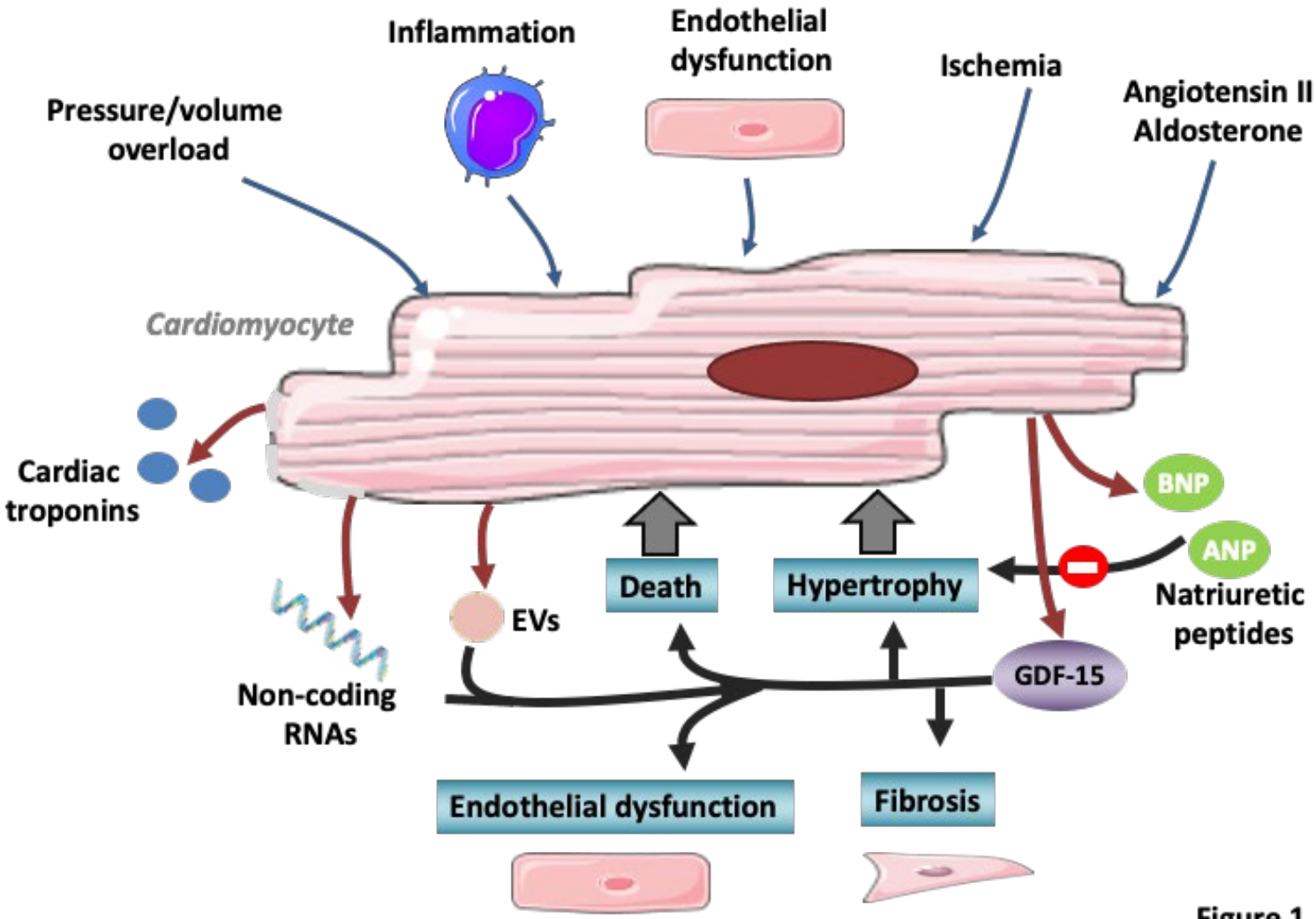


Figure 1

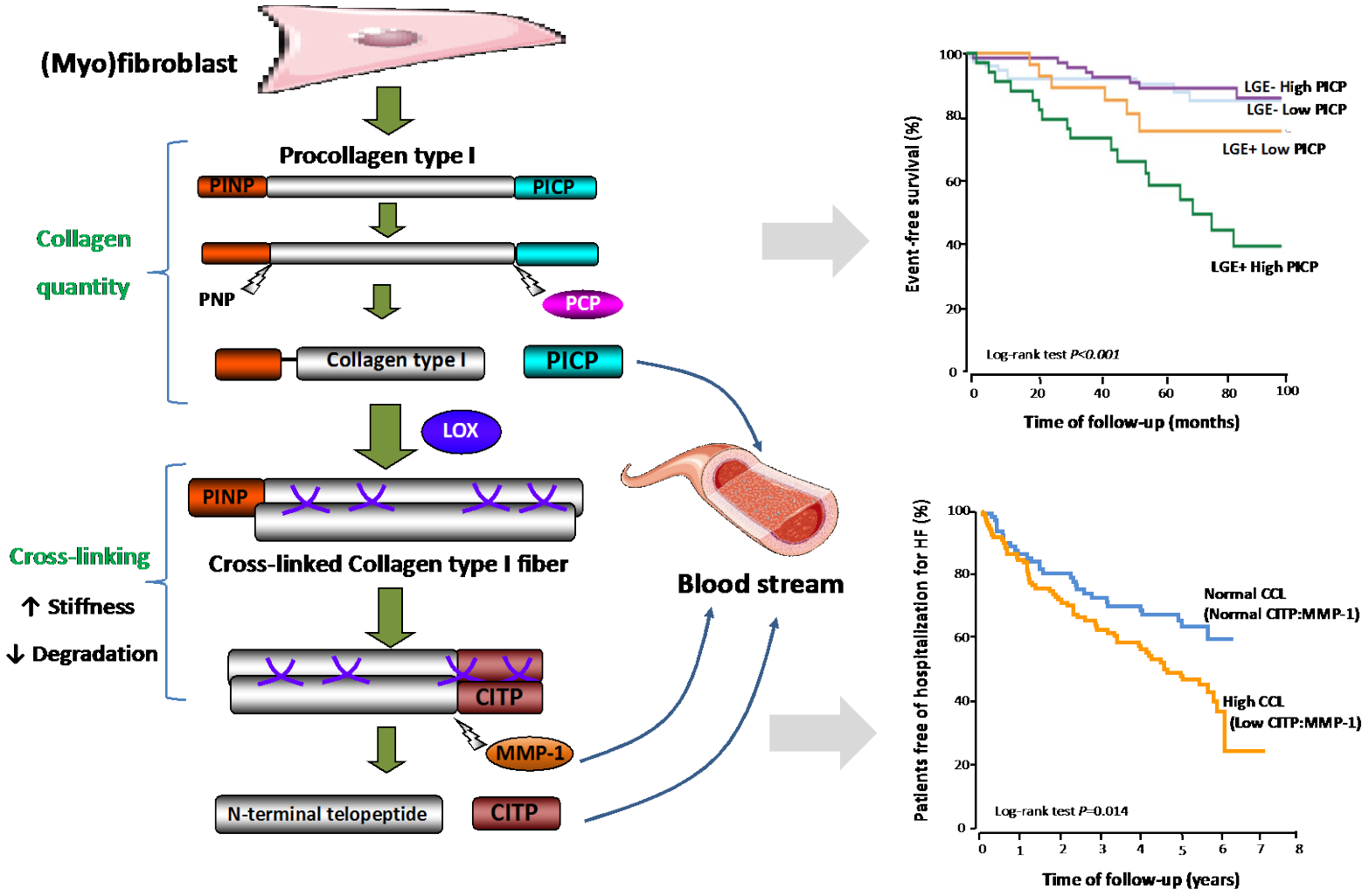


Figure 2

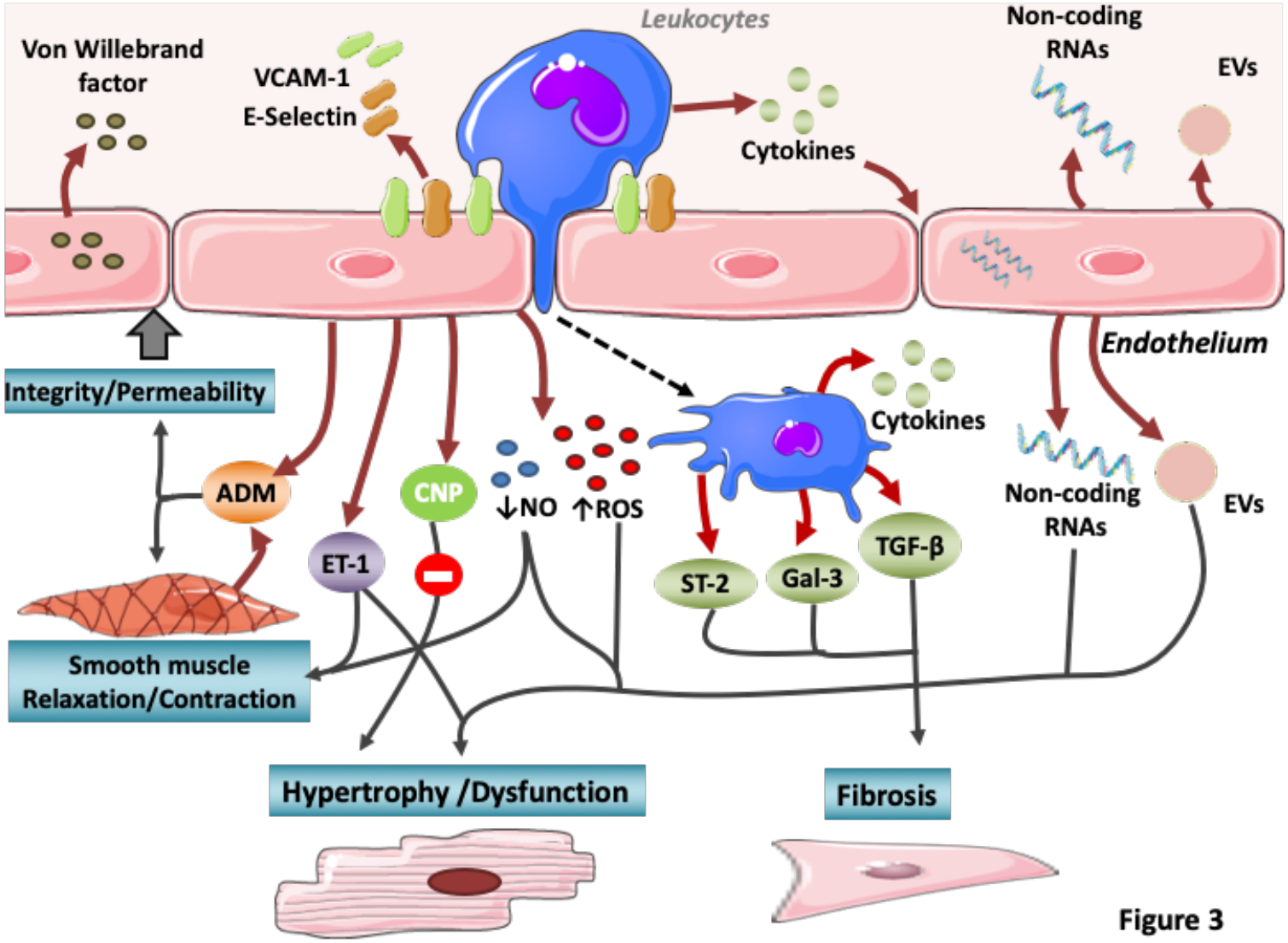
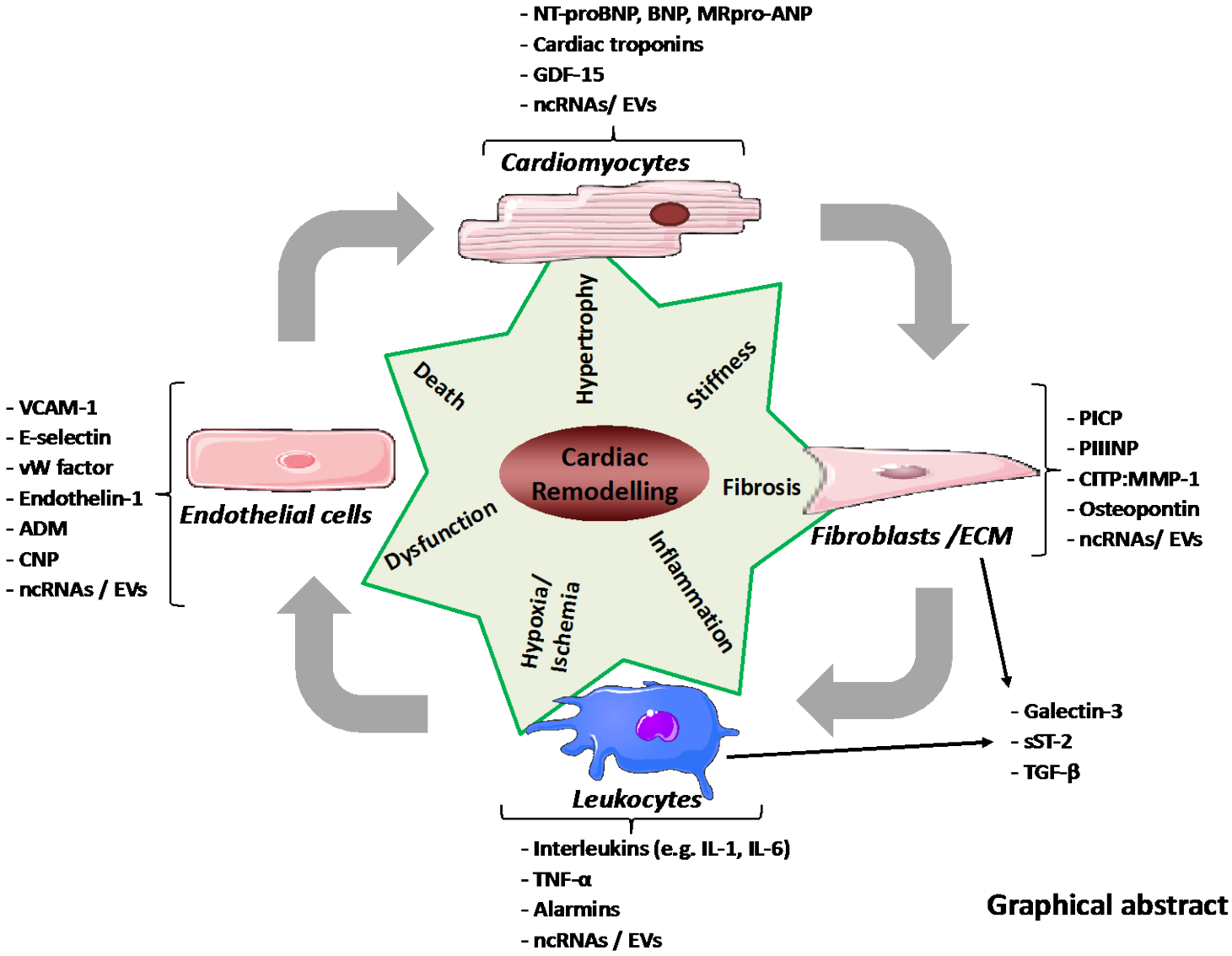


Figure 3



Graphical abstract