

# Biomarkers, myocardial fibrosis and co-morbidities in heart failure with preserved ejection fraction: an overview

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## Abstract

The prevalence of heart failure with preserved ejection fraction (HFpEF) is steadily increasing. Its diagnosis remains difficult and controversial and relies mostly on non-invasive echocardiographic detection of left ventricular diastolic dysfunction and elevated filling pressures. The large phenotypic heterogeneity of HFpEF from pathophysiological underpinnings to clinical manifestations presents a major obstacle to the development of new therapies targeted towards specific HF phenotypes. Recent studies suggest that natriuretic peptides have the potential to improve the diagnosis of early HFpEF, but they still have significant limitations, and the cut-off points for diagnosis and prognosis in HFpEF remain open to debate. The purpose of this review is to present potential targets of intervention in patients with HFpEF, starting with myocardial fibrosis and methods of its detection. In addition, co-morbidities are discussed as a means to treat HFpEF according to cut-points of biomarkers that are different from usual. Biomarkers and approaches to co-morbidities may be able to tailor therapies according to patients' pathophysiological needs. Recently, soluble source of tumorigenicity 2 (sST2), growth differentiation factor 15 (GDF-15), galectin-3, and other cardiac markers have emerged, but evidence from large cohorts is still lacking. Furthermore, the field of miRNA is a very promising area of research, and further exploration of miRNA may offer diagnostic and prognostic applications and insight into the pathology, pointing to new phenotype-specific therapeutic targets.

**Key words:** biomarkers, heart failure with preserved ejection fraction, microRNA, diagnosis.

## Introduction

### Classification of heart failure

Heart failure (HF) is a clinical syndrome characterized by substantial prevalence and mortality, frequent hospitalizations, poor quality of life, and complex treatment regimens [1]. Two main types of HF are differentiated: HF with reduced ejection fraction (HFrEF), also known as systolic HF; and HF with preserved ejection fraction (HFpEF), also known as diastolic HF [2, 3]. Very recently, the European Society of Cardiology (ESC) recommended describing a third type: HF with medium-range ejection fraction (HFmrEF) [4].

The guidelines of the ESC recommend basing the diagnosis of HFpEF on typical symptoms and signs of HF (volume overload, dyspnoea, exercise intolerance) and a left ventricular ejection fraction (LVEF) of more than 50%. More specific diagnostic criteria have evolved over time and include signs and symptoms of HF, objective evidence of diastolic dysfunction (impaired left ventricular (LV) relaxation or increased LV diastolic stiffness), disturbed LV filling, structural heart disease, and elevated B-type natriuretic peptide (BNP) or its precursor N-terminal pro-BNP [5–7]. However, it may happen that HFpEF is mimicked by restrictive cardiomyopathy, dynamic mitral regurgitation, ischaemic heart disease, pericardial disease and exercise-induced pulmonary hypertension [8]. Even though the ESC has suggested diagnostic criteria of HFpEF, enrolment criteria for HFpEF trials have varied considerably over the last decade (Table I) [9–14].

It is estimated that about half of all patients presenting with signs and symptoms of HF have HFpEF. In the past decade, the relative proportion of patients with the diagnosis of HFpEF compared with those with HFrEF has increased, suggesting the increasing importance of the syndrome, but also increasing awareness of its presence [15–18].

The aim of this review is to provide an overview of biomarkers whose assessment may be useful in patients with HFpEF, taking the pathophysiology of inflammatory mechanisms and myocardial fibrosis into consideration as well as patients' co-morbidities. Since a "one-size fits all" approach to HFpEF has proven unsuccessful in the past, the development of treatment strategies that tailor pharmacological intervention to pathophysiological needs is very promising. Biomarkers may be extremely useful tools in this undertaking.

### Pathophysiology of HFpEF

Heart failure with preserved ejection fraction is a syndrome with a number of underlying aetiologies and numerous pathophysiological alterations contributing to the heterogeneous disease state

[19–21]. Myocardial remodelling and dysfunction in HFpEF include myocardial hypertrophy as well as progressive myocardial fibrosis and increased cardiomyocyte stiffness. These characteristics result from specific changes in transcriptional and post-translational modifications of a huge, elastic sarcomeric protein named titin [22–26]. Extracellular matrix accumulation and fibrosis are often described, and may significantly contribute to impaired LV filling, a hallmark of the disease [27]. Fibrosis is the result of an imbalance between exaggerated collagen synthesis and unchanged or depressed collagen degradation. As compared to HFrEF, patients with HFpEF are generally older, more often female and have a high prevalence of non-cardiac and cardiac co-morbidities such as hypertension, overweight/obesity, diabetes mellitus, chronic obstructive pulmonary disease, atrial fibrillation, anaemia, or chronic kidney disease even after leaving aside specific causes of HF in the context of a normal or near normal LVEF (e.g., hypertrophic cardiomyopathy, cardiac amyloidosis, Fabry disease) [28–30]. Recently, Paulus *et al.* [31] postulated a novel paradigm, which identifies a systemic pro-inflammatory state induced by co-morbidities as the origin of microvascular endothelial cell inflammation. Inflammation in response to co-morbid conditions may play a proportionally larger role in HFpEF than in HFrEF [31]. On the other hand, haemodynamic overload evidenced by increased LV diastolic pressure (LVDP) and myocardial fibrosis evidenced by increased extracellular matrix fibrillar collagen may be important mechanisms contributing to the development of HFpEF [32]. For example, Schelbert *et al.* investigated whether myocardial fibrosis is similarly prevalent in those with HFpEF and those at risk of HFpEF, and whether it is similarly associated with disease severity and outcomes [33]. Of 1174 patients identified, 250 were "at risk" of HFpEF given an elevated BNP level; 160 had HFpEF as a documented clinical diagnosis, and the remaining 745 did not have either. Patients at risk of HFpEF or with HFpEF demonstrated similarly higher prevalence/extent of myocardial fibrosis and worse prognosis compared with patients with no HFpEF. Over a median of 1.9 years of follow-up, 61 patients at risk of HFpEF or with manifest HFpEF experienced adverse events (19 hospitalizations for HF, 48 deaths, 6 with both). Regardless, myocardial fibrosis was associated with disease severity (i.e., BNP) and outcomes. Conceivably, myocardial fibrosis may precede a clinical HFpEF diagnosis [33]. Unfortunately, the measurement of LVDP and fibrosis generally requires either invasive procedures and/or complex and sophisticated imaging techniques. However, biomarkers measured in the plasma have been shown to ac-

Table 1. Inclusion criteria for patients enrolled in trials of HFpEF

Trial	No. of patients	Age at inclusion [years]	EF at inclusion (%)	NT-proBNP [pg/ml]	Hospitalization	Additional criteria
CHARM-Preserved [9]	3023	≥ 18	> 40	–	History of hospital admission for a cardiac reason	NYHA functional class II–IV of at least 4 weeks' duration
I-PRESERVE [10]	4128	≥ 60	≥ 45	–	Hospitalized for HF during the previous 6 months	NYHA functional class II–IV
PEP-CHF [11]	850	≥ 70	> 40	–	CV hospitalization within the previous 6 months	Patients treated with diuretics; at least 3 out of 9 clinical and at least 2 out of 4 echo criteria
TOPCAT [12]	3445	≥ 50	≥ 45	≥ 360	History of hospitalization within the previous 12 months	At least one sign and at least one symptom of HF; HF hospitalization within the previous 1 year, with management of HF a major component of the care provided, or an elevated NP level within 60 days before randomization
REACH-HFpEF [13]	50	≥ 18	≥ 45	–	–	At least one of the 3 symptoms at the time of screening and at least one of the 4 signs of HF prior to study entry; EF ≥ 45% obtained within 6 months prior to randomization and after any MI or other event that would affect EF
OPTIMIZE-HFpEF [14]	360	≥ 60	≥ 50	> 500 (sinus rhythm) or > 1000 (atrial fibrillation)	At least one hospitalization because of worsened HF during the past 12 months	Increased <i>p.o.</i> furosemide to 80 mg/day or need of <i>i.v.</i> diuretics because of worsened HF during the past 12 months; NYHA functional class II–IV, regarded as optimally treated and stable for at least 4 weeks; normal-sized LV; one of three echocardiographic parameters within 6 months before randomization

BNP – brain natriuretic peptide, CHARM-Preserved – Candesartan in Heart failure: Assessment of Reduction in Mortality and morbidity, CV – cardiovascular, EF – ejection fraction, HF – heart failure, HFpEF – heart failure with preserved ejection fraction, *i.v.* – intravenous, I-PRESERVE – Ibesartan in Heart Failure with Preserved Ejection Fraction Study, LV – left ventricle, MI – myocardial infarction, NP – natriuretic peptide, NYHA – New York Heart Association, OPTIMIZE-HFpEF – Optimizing the Management of Heart Failure with Preserved Ejection Fraction in the Elderly by Targeting Comorbidities, *p.o.* – per oral, PEP-CHF – Perindopril in Elderly People with Chronic Heart Failure, REACH-HFpEF – Rehabilitation Enablement in Chronic Heart Failure in patients with Heart Failure (HF) with preserved ejection fraction, TOPCAT – Treatment Of Preserved Cardiac function heart failure with an Aldosterone antagonist.

curately reflect changes in haemodynamic load and myocardial fibrosis and may have important applications to the management of patients with HFpEF [34]. For example, matrix metalloproteinases (MMPs), tissue inhibitors of MMPs (TIMPs), and the collagen processing proteins collagen propeptides and collagen telopeptides reflect changes in collagen homeostasis and the transition from antecedent diseases such as hypertension to clinically symptomatic HFpEF [34, 35]. Galectin-3 and soluble source of tumorigenicity 2 (sST2) reflect the general extent of fibrosis and the severity of HFpEF, and they are also good candidates for determining the early stage of HF development influencing fibrosis. Apart from the aforementioned markers, growth differentiation factor 15 (GDF-15) and microRNAs (miRNA) are promising new biomarkers for the detection of fibrosis [33–35].

### Biomarkers in HFpEF

The diagnosis of HFpEF remains challenging and so far largely relies on echocardiography and natriuretic peptide testing. Additional indicators of HFpEF including biomarkers that reflect left ventricular filling pressure (LVFP) would be of great clinical value. Biomarkers in this context embrace parameters measured in blood that may provide important information on the pathogenesis of HFpEF, but may also be a valuable clinical tool in the identification of patients at risk for HFpEF, in the diagnosis of HFpEF, in risk stratification, and in monitoring therapy. However, most of the known biomarkers are poorly validated for their use in clinical trials, and there is a need for new biochemical and imaging parameters enabling physicians to assign cardiac dysfunction to the symptoms of the patient [31]. Currently we are awaiting the results of a study which aims to identify new biomarkers of HFpEF progression and to find pathophysiological mechanisms to support explorations of new treatment regimens for HFpEF [36]. The result may not be a single biomarker, but rather a panel of biomarkers that identifies different pathophysiological changes, as has been used in previous studies [37]. Indeed, the Preserved and Reduced Ejection Fraction Epidemiological Regional Study (PREFERS) Stockholm HF study will enable the characterization and comparison of new onset HFpEF and HFrEF patients by using new cutting edge techniques such as advanced bioinformatics and spatial transcriptomics [36]. Our selection of biomarkers was based on our own investigations and research experience [27, 38, 39].

### Search strategy

We searched the electronic databases MEDLINE (1966 to January 2018), EMBASE and SCOPUS (1965 to January 2018). Additionally, abstracts

from national and international cardiovascular meetings were searched. When necessary, the relevant authors were contacted to obtain further data. The main data search terms were: heart failure with preserved ejection fraction, diastolic heart failure, heart failure with normal ejection fraction, biomarker(s), marker(s), microRNA, diagnosis and heart failure, comorbidities and heart failure.

### Natriuretic peptides as the gold standard

The biologically active BNP and its inactive amino-terminal fragment NT-proBNP are synthesized within the ventricular myocardium in response to myocyte stretch and/or pressure overload. The major physiological effects of BNP are natriuresis, vasodilation and inhibition of the renin-angiotensin-aldosterone system (RAAS) and the sympathetic nervous system [40, 41]. BNP is quickly degraded by endopeptidases and natriuretic peptide receptor C (NPR-C) and has a half-life of approximately 20 min. By contrast, NT-proBNP circulates as a biologically inert substance that is cleared passively primarily by the kidney, and accordingly has a longer half-life of approximately 1 to 2 h [40, 41].

Recent studies have shown that the plasma levels of BNP and NT-proBNP are powerful biomarkers for the diagnosis and prognosis of patients with HF. However, there are also limitations to natriuretic peptide use that need to be kept in mind when interpreting their results. Apart from advancing age and ventricular function, other factors influence the clinical interpretation of BNP and NT-proBNP, including obesity, renal dysfunction, atrial arrhythmias, and cardiotoxic agents as well as structural heart disease [42]. The diagnostic cut-off values to rule out HF suggested by the ESC, however, apply to both HFrEF and HFpEF. For BNP, a cut-off value of < 35 pg/ml has been suggested, while for NT-proBNP the corresponding value is < 125 pg/ml even though patients older than 75 years may benefit from using a threshold of < 400 pg/ml. Despite the fact that circulating levels of natriuretic peptides are elevated in patients with HFpEF as compared to subjects without HF, they are usually lower than levels seen in patients with HFrEF [43]. Sanders-van Wijk *et al.* [44] supported these findings with regard to differential expression of NT-proBNP in HFpEF compared with HFrEF. HFrEF patients exhibited higher NT-proBNP values than those with HFpEF (4202 pg/ml (interquartile range (IQR): 2239–7411, pg/ml) vs. 2142 pg/ml (IQR: 1473–4294 pg/ml) vs.  $p < 0.001$ ). Moreover, it was found that NT-proBNP had significantly lower prognostic value in HFpEF compared with HFrEF in the NT-proBNP-guided study arm only [44]. However, in another study the authors found that, upon reaching a certain concentration,

the prognostic value of BNP was not different in HFpEF vs. HFrEF [45, 46]. Because HFpEF patients usually have a smaller LV cavity and thicker LV walls, their end diastolic wall stress is much lower than in HFrEF, even in the setting of high diastolic pressures, thus producing a lower stimulus for BNP production [47]. In cases where BNP/NT-proBNP is required to be elevated for a definite diagnosis of HF, only the higher risk HFpEF patients will be identified, resulting in a reduced prevalence of HFpEF, especially when current guidelines [4] recommend the same cut-off points for NT-proBNP and BNP levels for all kinds of HF. The above considerations suggest that natriuretic peptides may be somewhat less useful as a diagnostic and prognostic tool in HFpEF as compared to HFrEF [48]. On the other hand, Jhund *et al.* [49] studied more than 4,000 patients from the I-Preserve trial aged  $\geq 60$  years in New York Heart Association (NYHA) class II–IV HF with LVEF  $\geq 45\%$ . They found that beyond NT-proBNP increases or decreases of 1000 pg/ml from baseline, there was little change in the risk of cardiovascular death or HF hospitalization. This study therefore suggests that the value of NT-proBNP, which can help the clinician to monitor prognosis, is higher than might be expected.

In the study of van Veldhuisen *et al.* [45] BNP levels were overall lower in patients with HFpEF than in those with HFrEF, but the associated risk for reaching the primary endpoint was at least similar for a given BNP level. These findings indicated, however, that for a given BNP level, which would suggest a similar severity of HF, the associated risk is the same for HFpEF and HFrEF, and this was true for patients with low, intermediate, and high BNP levels [45]. The applicability of only natriuretic peptides for the entire spectrum of HF is difficult, because there are still questions about the cut-off levels to recognize and monitor HFpEF. This might be important information for future HFpEF trials and strengthens the fact that the sole use of natriuretic peptides as the only HF markers may be insufficient [50].

The concentration of atrial natriuretic peptide (ANP) in the circulation is approximately 10- to 50-fold higher than that of BNP, and its production is increased in response to increased atrial wall stretch in HF [51]. However, reliable detection of circulating ANP concentrations can be challenging because its half-life is only 2–5 min [52]. The 126 amino acid prohormone of ANP, designated as proANP, has a longer half-life and makes serum measurement more feasible [42]. A novel immunoassay that detects the mid-regional sequence of proANP (MR-proANP) is now available, and has been evaluated as a diagnostic and prognostic test for HF patients [53]. Recently, Andersen *et al.* [54] found in subjects recovering from an acute

myocardial infarction with preserved LVEF a significant correlation between MR-proANP (but not NT-proBNP) and pulmonary capillary wedge pressure at rest ( $p = 0.002$ ) and at peak exercise ( $p = 0.002$ ). MR-proANP was measured at rest before the exercise.

In conclusion, when using natriuretic peptides for making a diagnosis of HFpEF, their plasma levels should not be used in isolation from the clinical context and echocardiography. Because natriuretic peptides are influenced by many factors other than diastolic function, their measurements may give false positive results for HF. On the other hand, the relatively low natriuretic peptide levels suggest lower diastolic wall stress in HFpEF when compared to HFrEF, which may give false negative results. In this context, besides natriuretic peptides, additional biomarkers are needed to characterize HFpEF in more detail.

## Biomarkers of fibrosis in HFpEF

### Soluble source of tumorigenicity 2 (sST2)

Source of tumorigenicity 2 (ST2) is a receptor belonging to the interleukin-1 receptor family that is expressed in cardiomyocytes and elsewhere [55]. The receptor has 2 isoforms: a transmembrane form and a soluble circulating form (sST2), which lacks the intracellular and transmembrane domains. The ligand for both isoforms is the cytokine interleukin-33 (IL-33), which, when bound to the transmembrane form of the receptor, exerts a potentially protective antihypertrophic, antifibrotic effect on the cardiomyocyte [56, 57]. sST2 binds and removes IL-33 from the circulation, thus potentially promoting cardiac hypertrophy, fibrosis, and ventricular dysfunction. sST2 has been found to have diagnostic and prognostic value in patients with destabilized HF, acute coronary syndrome [58] and advanced chronic HF, and was included in the 2013 ACCF/AHA guidelines for additive risk stratification of patients with acute and chronic HF [59]. However, the diagnostic utility of sST2 in patients with HFpEF has been less well studied. A *post-hoc* analysis of 200 patients with HFpEF provided the first evidence that sST2 levels could be of interest in patients with HFpEF [55]. In the subpopulation of individuals with normal systolic function and acute dyspnoea, only sST2 continued to predict mortality. The authors found an approximately 2-fold higher median sST2 concentration in those with an  $E/e'$  ratio  $> 15$  (suggestive of increased LV end-diastolic filling pressure) than in the remaining patients, but, in contrast, the median NT-proBNP was approximately 157-fold higher in those with an  $E/e'$  ratio  $> 15$  than in the others. The weak correlation of sST2 with echocardiographic parameters in this unselect-

ed dyspnoea population, compared with NT-proBNP, calls into question the clinical relevance of the stretch-mediated pathway of sST2 secretion. The results of studies assessing the significance of the concentration of sST2 in patients with HFpEF are likewise inconclusive. Santhanakrishnan *et al.* found that HFpEF patients had higher serum levels of sST2 compared with healthy subjects, but healthy subjects *per se* may not be the ideal comparator [60]. Besides, this difference did not remain statistically significant after adjustment for age, sex, and clinical covariates. Wang *et al.* [61] evaluated 107 hypertensive patients with LVEF > 50%. Among them, 68 presented with symptoms of HFpEF. The area under the receiver-operator curve (AUC) was 0.80 for sST2 as compared to 0.70 for NT-proBNP to correctly diagnose HFpEF. The sST2 concentration was significantly lower in patients with  $E/e' < 8$  compared with those with  $E/e' 8-15$  or  $E/e' > 15$ . Multivariate analysis demonstrated that  $sST2 > 13.5$  ng/ml was independently associated with the presence of HFpEF. Jhund *et al.* [62] studied 296 patients from the Prospective Comparison of Angiotensin Receptor-Nephrilysin Inhibitors (ARNI) with Angiotensin II receptor blocker (ARB) on Management of Heart Failure With Preserved Ejection Fraction (PARAMOUNT) trial with a measurement of sST2. Higher sST2 was associated with older age, male sex, atrial fibrillation, higher NYHA class, higher NT-proBNP and lower estimated glomerular filtration rate (eGFR). Increasing sST2 levels were associated with higher  $E/e'$  and larger left atrial size. Associations were similar after excluding patients with atrial fibrillation. In a multivariable model, male sex, increasing NYHA class and left atrial volume were independently associated with higher sST2 [62].

More clinical data on sST2 in HFpEF were provided by Friões *et al.* [63] In this study, the authors divided patients with acute HF according to their LVEF, and reported that NT-proBNP predicted all-cause mortality or HF readmission at 6 months regardless of LVEF. sST2 was found to be a significant predictor of prognosis in HFrEF, but not in HFpEF patients. By contrast, in another analysis the authors demonstrated comparable prognostic value of sST2 in both HFpEF and HFrEF patients [64]. Hypertension is the most prevalent co-morbidity in patients with HFpEF and might lead to activation of different pathophysiological mechanisms in the course of the disease. This has been recently investigated by Zile *et al.* [24] sST2 was measured in 70 coronary artery bypass grafting (CABG) patients stratified into three groups: control (no hypertension), hypertensive patients without HFpEF, and hypertensive patients with HFpEF. The authors observed that sST2 was higher in hypertensive patients without HFpEF com-

pared with the control subjects. Moreover, the sST2 levels were raised in hypertensive patients with HFpEF ( $105.5 \pm 31.4$  vs.  $82.0 \pm 35.7$  ng/ml;  $p < 0.05$ ) [24]. In the study of Parikh *et al.* [65] in 3915 older, community-dwelling subjects from the Cardiovascular Health Study without prevalent HF, sST2 level above the US Food and Drug Administration-approved cut-off value ( $> 35$  ng/ml) was significantly associated with incident HF (hazard ratio (HR) 1.20; 95% confidence interval (CI): 1.02–1.43) and cardiovascular death (HR = 1.21; 95% CI: 1.02–1.44), and greater sST2 was continuously associated with cardiovascular death (per 1-ln increment: HR = 1.24; 95% CI: 1.02–1.50). Addition of sST2 to existing risk models of HF and cardiovascular death modestly improved discrimination and reclassification into a higher risk [65].

Summarizing research on the importance of sST2 concentrations in HFpEF, the ambiguity of the results draws our attention, and available studies remain retrospective in nature and altogether rather inconclusive. One of the important points in this regard is the heterogeneity of inclusion criteria used across different studies, rendering even meta-analyses difficult. sST2 could be used as a biomarker to predict HFpEF. However, such an approach may still merit consideration in order to provide additional insight.

### Growth differentiation factor 15

Growth differentiation factor 15 (GDF-15) is a member of the transforming growth factor- $\beta$  cytokine superfamily and a marker of cell injury and inflammation. GDF-15 could be used as a novel biomarker to evaluate myocardial fibrosis. In the study by Zhou *et al.* [66] after linear correlation analysis, the expression GDF-15 was found to be positively related to the degree of cardiac fibrosis in patients with atrial fibrillation [66]. In patients with end-stage non-ischemic dilated cardiomyopathy, serum GDF-15 level correlated with the severity of myocardial fibrosis [67]. Expression of GDF-15 is strongly induced in cardiomyocytes in response to metabolic stress such as cardiac ischaemia (nitric oxide-dependent), tissue injury, or a pressure overload state (angiotensin 2-dependent) [42, 68, 69]. Serum GDF-15 levels positively correlate with LV mass in elderly individuals [70], and a high level of plasma GDF-15 is an independent predictor of LV hypertrophy in patients with hypertension [71]. It was recently found that circulating GDF-15 is a useful biomarker for differentiating hypertrophic cardiomyopathy from hypertensive LV hypertrophy [72]. Izumiya *et al.* [73] reported that serum GDF-15 levels were positively correlated with NYHA functional class ( $p \leq 0.0001$ ), plasma BNP levels ( $p \leq 0.0001$ ), and serum high sensitive troponin T levels ( $p = 0.001$ ) in

150 patients with LV diastolic dysfunction (LVDD) and with HFpEF (LVEF > 50%). Serum GDF-15 levels in the HFpEF group were significantly higher than those in the LVDD group ( $p < 0.0001$ ). In another small population study ( $n = 151$ ), the discriminatory capacity of GDF-15 (area under the receiver-operator curve (AUC) = 0.936) was similar to that of NT-proBNP (AUC = 0.934) for HFpEF versus controls [60]. Increasing levels of both biomarkers correlated with worsening diastolic indices. The ratio NT-proBNP : GDF-15 best distinguished HFpEF from HFrEF (AUC = 0.709) [60].

Sinning *et al.* [74] recently described GDF-15 as a useful marker to detect prevalent HF in addition to NT-proBNP and found elevated levels in HFrEF and HFpEF, whereas NT-proBNP was higher in HFrEF than in HFpEF. All biomarkers were useful to predict mortality in the general population. The index calculated from the equation ((C-reactive protein [CRP]+GDF-15+sST2)/NT-proBNP) was able to discriminate HFpEF from HFrEF [74]. The prognostic value of GDF-15 was also confirmed in recent studies. GDF-15 remained a significant independent predictor of the composite outcome even after adjusting for important clinical predictors including high-sensitivity troponin T and NT-proBNP (adjusted hazard ratio 1.76 per 1 Ln U, 95% CI: 1.39–2.21;  $p < 0.001$ ), regardless of HF group ( $p$ -value for interaction = 0.275) [75, 76]. The study by Kopytsya *et al.* [77] detected an association between high levels of GDF-15, determined within the first 24 h after acute coronary syndrome, and congestive HF progression after 12 months. Overall, GDF-15 could serve as a biomarker helpful in differentiating the type of HF – preserved vs. reduced EF – and additionally as an indicator of prognosis.

### Galectin-3

Myocardial fibrosis resulting in increased myocardial stiffness is a major component of HFpEF pathophysiology, and it is a manifestation of collagen synthesis (or decreased collagen degradation), inflammation, and oxidative stress [76, 78–80]. Galectin-3 is a macrophage product and member of the family of soluble  $\beta$ -galactoside-binding lectins. It can be found on a wide variety of cells and tissue surfaces. The function of galectin-3 is probably related to the inflammatory cascade following cardiac injury, as well as pathways regulating cardiac contractility [79]. Recent studies have confirmed that galectin-3 is linked to HF development and that it is implicated in a variety of processes [80] that are thought to play roles in the pathophysiology of HFpEF including myofibroblast proliferation, fibrogenesis, tissue repair, inflammation, and ventricular remodelling [81–83]. In the study of Wu *et al.* [84], both plasma and myocar-

dial galectin-3 levels correlated with the severity of diastolic dysfunction. To date, only a few clinical studies have described the clinical value of galectin-3 in patients with HFpEF. The first study to identify galectin-3 as a predictive marker for HF was a substudy from the Pro-BNP Investigation of Dyspnea in the Emergency Department (PRIDE) study [85]. In a subanalysis of about 200 patients diagnosed with acute decompensation of HF, galectin-3 levels were correlated with echocardiographic measures of higher filling pressure (higher  $E/e'$  ratio), abnormalities of diastolic relaxation (lower  $e'$  velocity), and valvular regurgitation. Additionally, the highest levels of galectin-3 were associated with a higher risk of 4-year mortality, independent of LV dimensions and function [86]. In the Advising and Counseling in Heart Failure (COACH) trial, galectin-3 was found to be an independent predictor of all-cause mortality or HF hospitalization during 18 months of follow-up in 592 patients with NYHA class II–IV HF (hazard ratio (HR) 1.38, 95% confidence interval (CI): 1.07–1.78,  $p = 0.015$ ; adjusted for age, gender, BNP, eGFR, and diabetes) [87]. De Boer *et al.* [87] observed that increasing quartiles of baseline galectin-3 levels were associated with a greater risk for all-cause mortality or HF hospitalization in patients with HFpEF (defined as LVEF > 40%) than in patients with HFrEF ( $n = 485$ ) [87]. Similar results were obtained in another study in which galectin-3 above the median of 13.8 ng/ml independently predicted all-cause mortality of 419 patients hospitalized with HF and an LVEF > 45% [88].

Currently, there are few data regarding the relationship between galectin-3 levels and the response to specific pharmacological or non-pharmacological therapeutic approaches in HFpEF. This is in contrast to data available in cohorts of patients with HFrEF, where there is some evidence that baseline galectin-3 levels are predictive for the response to a specific drug therapy [89, 90]. For example, in the Aldosterone Receptor Blockade in Diastolic Heart Failure (Aldo-DHF) trial [91], the authors determined the association between galectin-3 levels and patient characteristics in HFpEF and assessed the association between galectin-3 and clinical outcomes. The study was performed in patients in NYHA classes II–III, LVEF  $\geq 50\%$ , grade  $\geq I$  diastolic dysfunction, and peak oxygen consumption ( $\text{peakVO}_2$ )  $\leq 25$  ml/kg/min. Following multivariable adjustment, baseline galectin-3 inversely correlated with peak  $\text{VO}_2$ , 6 min walk distance, and Short Form 36 (SF-36) physical functioning and with NYHA class. At 6 and 12 weeks, increasing galectin-3 levels were associated with all-cause death or hospitalization independent of treatment arm (HR = 3.319, 95% CI: 1.214–9.07,  $p = 0.019$ ) and NT-proBNP (HR = 3.127, 95% CI: 1.144–8.549,  $p = 0.026$ ) [91].

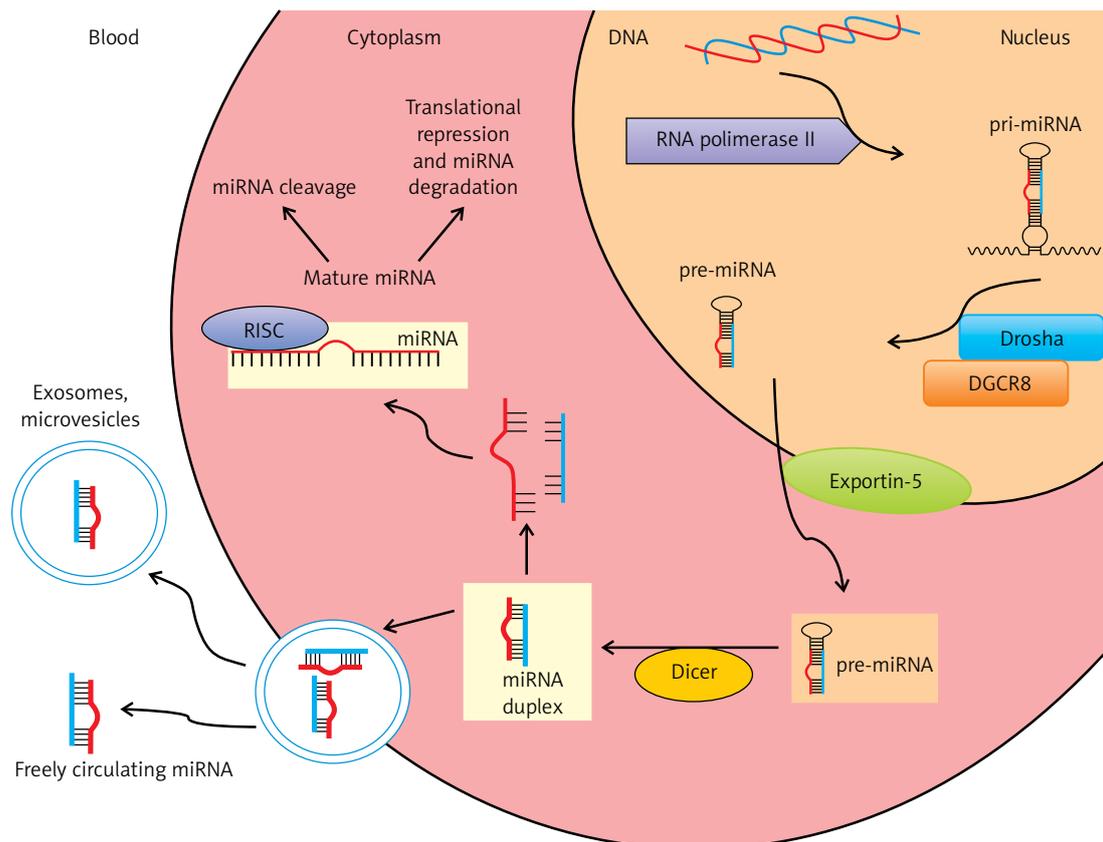
AbouEzzeddine *et al.* [92] observed that in overt HFpEF galectin-3 was related to severity of renal dysfunction and, accounting for this, it was not independently associated with the severity of pathophysiological derangements. These findings underscore the need to adjust for renal function when interpreting galectin-3 levels, and call into question the value of galectin-3 in quantifying disease severity in overt HFpEF [92]. Altogether, galectin-3, like sST2, is an interesting candidate biomarker that may find a role in HFpEF. However, in the absence of clinical studies and clinical algorithms that establish the value of specific cut-offs, their clinical importance remains elusive.

### Circulating microRNAs

MicroRNAs (miRNAs) are small (17–25 nucleotides) non-coding RNAs that play a role in gene expression by binding to the 3'-untranslated regions of target messenger RNAs (mRNAs). miRNAs initiate either translational repression or degradation of mRNAs, thereby regulating gene expression at the

post-translational stage (Figure 1) [93–95]. They are found in humans, animals, plants, and viruses [96]. Due to the ability of a single miRNA to target hundreds of mRNAs and their involvement in biological processes including stem cell differentiation, phase change, signalling, and response to stress, incorrect miRNA expression may be associated with the development of HF and is being viewed as a potential biomarker of this disease [97].

Identification of aberrant miRNA expression in the plasma could be useful in early and differential diagnosis of selected diseases. In this context, the recent detection of miRNAs in various body fluids (e.g. blood, saliva, serum, milk) has led researchers to consider them as intriguing candidate biomarkers for various diseases [97]. MicroRNAs offer many attractive features as biomarkers. They are stable in the circulation, their sequences are evolutionarily conserved, their expression is often tissue or pathology specific, and their detection is based on sequence-specific amplification – requisite features for valuable biomarkers [98]. The diagnostic potential of miRNA detection in human



**Figure 1.** MicroRNA biogenesis pathway. In the nucleus, miRNAs are transcribed by RNA polymerase II to generate long primary transcripts (pri-miRNAs) which may contain more than one miRNA. Pri-miRNAs are subsequently processed by the endonuclease Drosha and its binding partner DGCR8, forming hairpin-like precursor miRNAs (pre-miRNAs). Pre-miRNAs are exported into the cytoplasm by exportin-5. Pre-miRNAs are cleaved by the RNase III enzyme (Dicer) to mature miRNAs. The mature miRNAs are incorporated into the RNA-induced silencing complex (RISC), in this form leading to degradation of target miRNAs and/or inhibition of translation. Mature miRNAs can be released into the circulation, incorporated into vesicles such as multivesicular bodies (MVB), exosomes, microvesicles or as freely circulating miRNAs

plasma for cardiovascular disorders is beginning to be explored. Recent studies have shown that miRNAs are also associated with cardiac disease and HF, and their expression in myocardial tissue was more sensitive than mRNAs for the prediction of functional status in HF patients [99]. Thus far, distinctive patterns of circulating miRNAs have been found for myocardial infarction, HF, atherosclerosis, type 2 diabetes, and hypertension. Although these reports indicate that plasma levels of specific miRNAs correspond to different forms of cardiovascular disease, they do not currently determine whether plasma miRNA levels correspond to a deterioration or improvement in heart disease in humans [100].

It was recently reported that miRNAs, which can be reliably isolated from human serum and are stable in the circulation, can be used as a diagnostic tool for early HF detection [101]. One study has shown that miR-1246 and miR-124-5p in the buffy coat might be used as biomarkers for the assessment of diastolic dysfunction and dilated cardiomyopathy [102]. The authors suggest that in patients with diastolic dysfunction with or without systolic dysfunction, there is striking dysregulation of plasma levels of miR-454, miR-142-3p and miR-500. Conversely, miR-1246 was steadily upregulated in all three groups (DD – isolated diastolic dysfunction; DCM – stable compensated dilated cardiomyopathy; DCM-CHF – decompensated congestive heart failure secondary to dilated cardiomyopathy), while miR-124-5p was significantly up-regulated in the DCM group. In patients with isolated diastolic dysfunction, the expression of miR-1246 levels demonstrated highly specific diagnostic accuracy, while miR-124-5p showed highly specific diagnostic accuracy for DCM prediction. These findings point to the possibility of a functional correlation between dysregulation of these miRNAs and target gene alteration in the distant organ. Nevertheless, this study was limited by the small number of subjects and requires validation in a larger population (Table II) [102].

Interestingly, Wong *et al.* [103] found that expression of four miRNAs (miRNA-125a-5p, -190a, -550a-5p and -638) could differentiate HFrEF from HFpEF. Furthermore, miRNA-183-3p, -190a, -193-3p, -193-5p, and -545-5p distinguished HFpEF from non-HF controls. These authors also found that miRNA-183-3p, -190a, -193b-3p, -193b-5p, -211-5p, -494, -671-5p, and -1233 could differentiate HF from non-HF. In addition, the AUC values of individual miRNAs had a lower discriminative power in HFpEF vs. HFrEF than NT-proBNP, but the miRNA panel in combination with NT-proBNP achieved maximal diagnostic accuracy (AUC 1.0, Table II). Thus, combining assessment of individual miRNA with NT-proBNP or employing a unique panel of multiple miRNA biomarkers can signifi-

cantly improve the specificity and sensitivity of diagnostic assays and accelerate HF diagnosis.

In order to identify a novel miRNA signature helping to differentiate HFpEF from HFrEF, Watson *et al.* [104] performed miRNA profiling from the plasma RNA pool of 15 HFpEF patients, 15 HFrEF patients and 15 healthy controls. Five miRNA candidates – miR-30c, miR-146a, miR-221, miR-328, and miR-375 – showing potential as HF biomarkers that could differentiate HFpEF and HFrEF were selected and further verified and validated in an independent cohort of 225 patients ( $n = 75$  per group). In this study, all five miRNAs were reduced in HF; however, miRNA-375 was only reduced in HFrEF. miRNA-328 and miRNA-375 levels were significantly different between HFpEF and HFrEF when comparing average circulating levels (Table II) [104]. Circulating miRNA-328 plays an important role in cardiac hypertrophy through mechanisms involving SERCA2a [105] and could be a potential indicator of acute myocardial infarction, and increased plasma levels of miRNA are strongly associated with increased risk of mortality and development of HF [106]. Meanwhile, miR-375, which was the only down-regulated miRNA in HFpEF patients, has been linked to diabetes [107]. Similarly to the earlier study, the authors performed AUC analyses to distinguish HFpEF from HFrEF and reported equally predictive values of each of the single miRNAs compared with the use of two or more of miRNA-146a, -221, -328 and BNP. Importantly, the combination of miRNA-375 with BNP significantly improved the predictive power to differentiate HFpEF from HFrEF as compared with BNP alone in the AUC model. Again, the highest intergroup distinction was achieved by combining the miRNA panel with BNP (AUC = 0.854).

Although the recent results in miRNA-based diagnostics of HFpEF and HFrEF are highly promising, there are some limitations to clinical implementation of miRNA signature detection. The first is the lack of a strong biological correlation between circulatory miRNA levels and the relevant organ/tissue expression in HF. The next limitation concerns the fact that circulating NT-proBNP level was far superior to signature miRNAs in predicting HF. The two recent studies (see above) suggested that combining miRNA levels with NT-proBNP might add diagnostic value to differentiate HFpEF from HFrEF [108].

There is a growing body of evidence that miRNAs play a major role in the transcriptional and translational changes in gene expression with respect to cardiac hypertrophy and fibrosis in humans and mouse models of HF [109, 110]. Dysregulation of specific miRNAs could alter the cellular responses of cardiomyocytes and non-cardiomyocytes to specific signalling upon patholog-

**Table II.** MicroRNAs in the diagnosis and differentiation of heart failure with preserved ejection fraction and heart failure with reduced ejection fraction

Study	Material	Groups/size	miRNA	Effect	Detection method
Nair <i>et al.</i> [102]	Human whole blood and plasma	DD (n = 8) Stable DCM (n = 10) DCM-CHF (n = 13) HC (n = 8)	miRNA-124-5p miRNA-142-3p miRNA-454 miRNA-500 miRNA-1246	Patients with DD with or without SD showed striking dysregulations of plasma levels of miRNA-454, miRNA-142-3p, and miRNA-500 with significant down-regulation. miRNA-124-5p was steadily up-regulated in DD, DCM and DCM-CHF groups. miRNA-124-5p was significantly up-regulated in the DCM group. In patients with DD, expression of miRNA-1246 levels showed highly specific diagnostic accuracy, while miRNA-124-5p showed highly specific diagnostic accuracy for DCM prediction	qRT-PCR
Wong <i>et al.</i> [103]	Human whole blood and plasma	HFPEF (n = 30) HFREF (n = 30) HC (n = 28)	miRNA-125a-5p miRNA-183-3p miRNA-190a miRNA-193b-3p miRNA-193b-5p miRNA-211-5p miRNA-494 miRNA-545-5p miRNA-550a-5p miRNA-638 miRNA-671-5p miRNA-1233	Expression of miRNA-125a-5p, -190a, -550a-5p and -638 could significantly differentiate HFREF from the HFPEF group. miRNA-183-3p, -190a, -193-3p, -193-5p, and -545-5p distinguished HFPEF from non-HF controls. miRNA-183-3p, -190a, -193b-3p, -193b-5p, -211-5p, -494, -671-5p and -1233 could differentiate HF from non-HF controls. AUC values of individual miRNAs had a lower discriminative power in HFPEF vs HFREF than NT-proBNP, but the miRNA panel in combination with NT-proBNP achieved maximal diagnostic accuracy (AUC 1.0)	qRT-PCR
Watson <i>et al.</i> [104]	Human serum	HFPEF (n = 90) HFREF (n = 90) HC (n = 90)	miRNA-30c miRNA-146a miRNA-221 miRNA-328 miRNA-375	miRNA-30c, -146a, -221, -328, and -375 were reduced in HF; miRNA-375 was only reduced in HFREF. miRNA-328 and miRNA-375 levels were significantly different between HFPEF and HFREF when comparing average circulating levels. Combination of BNP with miRNA-30c, -221, -328, and -375 generated an AUC of 0.854 for distinguishing HFPEF from HFREF	qRT-PCR
Marketou <i>et al.</i> [115]	Human whole blood	HFPEF (n = 39) HC (n = 29)	miRNA-21 miRNA-133	miRNA-21 levels were found to be higher (4.6 ± 0.45 vs. 2.05 ± 0.31, p < 0.05) in patients with HFPEF compared to HC. miRNA-133 levels were found to be lower (11.5 ± 6.9 vs. 37.03 ± 8.18, p < 0.05) in patients with HFPEF compared to HC. miRNA-21 levels showed strong negative correlation with E/E' ratio (r = -0.42, p < 0.001) while miRNA-133 levels showed strong positive correlation with E/E' ratio (r = 0.41, p < 0.001)	qRT-PCR
Dong <i>et al.</i> [118]	Rat tissue	HFPEF vs. HC	miRNA-21	miR-21 expression was significantly higher in HFPEF rats compared to HC. miR-21 promotes the development of HFPEF by up-regulating anti-apoptosis gene Bcl-2 and thereby inhibiting apoptosis of fibroblasts	qRT-PCR

HFPEF – heart failure with preserved ejection fraction, HFREF – heart failure with reduced ejection fraction, HC – healthy control, miRNA – microRNAs, HF – heart failure, qRT-PCR – quantitative real-time polymerase chain reaction, SD – systolic dysfunction, DD – isolated diastolic dysfunction, DCM – stable compensated dilated cardiomyopathy, DCM-CHF – decompensated congestive heart failure secondary to dilated cardiomyopathy, AUC – area under the operating receiver curve, NT-proBNP – N-terminal of the prohormone brain natriuretic peptide, BNP – brain natriuretic peptide.

ical haemodynamic overload, leading to cardiac hypertrophy and HF [111]. Recent studies have revealed that miRNA-21 and miRNA-133 play a role in heart hypertrophy and fibrosis. They have also been shown to regulate proliferation and phenotypic switch of vascular smooth muscle cells. More recent studies validate these findings of miR-21 promoting cardiac remodelling and fibrosis [112, 113]. Also, it was demonstrated that miRNA-133, which controls cardiomyocyte identity, can restore heart function in rodents and might play an important role in heart regeneration and repair [114]. However, there are limited data regarding its role in HFpEF. Marketou *et al.* [115] analysed miRNA of the peripheral blood mononuclear cells in 39 patients with signs and symptoms of HF who had an LVEF > 50% and evidence of LV diastolic dysfunction and 29 healthy volunteers for comparison. Quantitative real-time reverse transcription PCR revealed that miRNA-21 levels were higher in peripheral blood mononuclear cells (PBMCs) of HFpEF patients ( $4.6 \pm 0.45$  vs.  $2.05 \pm 0.31$ ,  $p < 0.05$ ), while miRNA-133 levels were lower in PBMCs of those patients ( $11.5 \pm 6.9$  vs.  $37.03 \pm 8.18$ ,  $p < 0.05$ ) compared to healthy controls (Table II) [115].

In addition to developing a panel of miRNAs to function as biomarkers for diagnostic and prognostic purposes, miRNAs may be used in developing therapeutics. MicroRNA-208a is essential for cardiac hypertrophy and fibrosis. In the study of Wang *et al.*, miRNA-208a increased endoglin expression to induce myocardial fibrosis in volume-overloaded HF [116]. A study by Montgomery *et al.* [117] using an animal model showed that delivery of an anti-miRNA may inhibit miRNA-208a. Subcutaneous administration of anti-miRNA-208a during hypertension-induced HF in Dahl hypertensive rats prevented cardiac remodelling and improved function and survival [117]. The silencing of miRNA-208a by anti-miRNA-208a was noted to induce significant changes in cardiac gene expression, as might be expected, given that miRNAs play a large role in gene expression. In another study, Dong *et al.* [118] observed that after injection of an miR-21 antagonist, cardiac atrophy and cardiac fibrosis were conspicuously ameliorated in HFpEF rats. The authors suggested that miRNA-21 promoted the development of HFpEF by up-regulating the expression of the anti-apoptotic gene Bcl-2, thereby suppressing the apoptosis of cardiac fibrosis (Table II). Both *in vivo* and *in vitro*, inhibition of miRNA-21 expression resulted in reduced Bcl-2 expression while over-expression of miRNA-21 led to elevation of Bcl-2 expression.

The use of antisense oligonucleotides to silence miRNAs could pave the way to establishing a novel avenue of target-driven therapeutics. In recent years, several opportunities to alter levels

of circulating miRNAs by systemically administering agents such as mimimics or antagomirs have been identified. This therapeutic approach has been reported to significantly reduce hypertrophy and cardiac fibrosis and to improve LV function in animal models. It represents a promising approach to complement existing therapeutic options in the treatment of HF. However, the results of *in-vitro* and *in-vivo* experiments have not yet led to application in clinical studies. Successful implementation of these insights in clinical trials represents the next step to bring this idea to fruition [119].

### Abnormal levels of laboratory markers and co-morbidities as a potential prognostic tool and therapeutic target in HFpEF

Besides interventions at the level of the heart itself targeting fibrosis and myocardial stiffness, therapeutic considerations in the management of HFpEF largely rely on the management of its co-morbidities. Therefore, the identification of co-morbidities of HFpEF using diagnostic biomarkers is a very promising approach. However, cut-off values may differ from those in healthy subjects or patients with different illnesses. The list of co-morbidities in this regard is very long, but a few examples are discussed below.

Anaemia, for example, is a common comorbidity of HF, and has been associated with poor functional status, longer hospitalization duration, early re-hospitalization, and death. The incremental risks of death and lengthened hospital stay associated with anaemia are more pronounced in acute decompensated HF patients with HFpEF than those with HFrEF [120].

Martens *et al.* [121] evaluated 1197 patients (71% male) including 897 HFrEF, 229 HFmrEF and 72 HFpEF patients. The overall prevalence of ID was 53% (50% in HFrEF; 61% in HFmrEF; 64% in HFpEF) and 36% for anaemia. ID was associated with a lower  $VO_2$ max in patients with all types of heart failure ( $p < 0.001$  in all). Iron status was more closely related to a poor  $VO_2$ max than anaemia status ( $p < 0.001$ ). Furthermore, poor clinical outcome was more strongly associated with iron status than anaemia status. Patients with progression of ID exhibited a significantly higher risk of heart failure hospitalisation and all-cause mortality (HR = 1.4; 95% CI: 1.01–1.94;  $p = 0.046$ ) than patients without progression [121].

Vullaganti *et al.* [122] studied 56 patients who had anaemia defined as haemoglobin  $\leq 12$  g/dl (mean:  $10.4 \pm 1.0$  g/dl) with HFpEF defined as having an NHANES-CHF (National Health And Nutrition Examination Survey: Congestive Heart Failure) criteria score of  $\geq 3$  and an LVEF > 40% (mean:  $63 \pm 15\%$ ). Patients were randomly allocated to receive either epoetin alfa with ferrous

gluconate or ferrous gluconate only. In this analysis, a responder was defined as a patient with an increase of 1 g/dl in the first 4 weeks of the trial. Unfortunately, a significant haemoglobin response to anaemia treatment with epoetin alfa and oral iron did not lead to differences in LV remodelling, functional status, or quality of life. Given the results of this small trial, it appears as though using objective improvements in anaemia as a marker in older adult subjects with HFpEF does not have significant clinical utility [122]. Anaemia can be associated with iron deficiency (ID), but the two do not have to be present at the same time. Patients with HF and iron deficiency have shown symptomatic improvements from intravenous iron administration, and some evidence suggests that these improvements occur irrespective of the presence of anaemia [123]. Functional ID is an independent predictor of poor outcome in advanced HFrEF, but its role in HFpEF remains less clear [124]. A study by Kasner *et al.* [124] included 26 patients with HFpEF who showed an increase in LV stiffness as assessed using pressure-volume loop analysis obtained by conductance catheterization. HFpEF patients with serum ferritin < 100 µg/l or ferritin of 100–299 µg/l in combination with transferrin saturation < 20% were defined as having ID [124]. According to the linear regression analysis, LV stiffness was correlated with peak oxygen uptake ( $r = -0.636$ ,  $p < 0.001$ ) but not with ferritin level or transferrin saturation. No relation was found between ID and exercise capacity. The association of LV stiffness with exercise performance was independent from the level of ID. The authors concluded that in non-anaemic HFpEF patients, cardiac dysfunction and impaired exercise capacity occur independently of ID [124]. There is still a lack of large observational studies assessing the significance of anaemia and ID in HFpEF. However, a prospective trial of intravenous ferric carboxymaltose vs. placebo is underway in patients with HFpEF in order to improve exercise capacity in these patients.

Hyponatraemia has been shown to be a relevant prognostic factor in HFpEF. Serum sodium (sNa) cut-off, however, has not been defined in HFpEF, and it remains unclear whether alternative cut-off values should be used [125]. Kusaka *et al.* [125] assessed cardiac function using echocardiography and measured sNa in HFpEF patients in NYHA class II ( $n = 321$ ) or III ( $n = 84$ ) in a compensated condition after implementing medical therapy for HF. During a mean follow-up of 27 months, 73 patients developed HF-related events. In multivariate Cox hazard analysis including established predictors in HF, sNa level as a continuous variable was identified as an independent predictor for HF-related events in HFpEF (per 1.0 mmol/l: HR = 0.93; 95% CI: 0.87–0.98;

$p < 0.01$ ) [125]. Kaplan-Meier analysis demonstrated significantly higher probability of HF-related events in the lower sNa group (sNa < 140 mmol/l) than in the higher sNa group (sNa ≥ 140 mmol/l;  $p < 0.001$ ). Further, the low-normal sNa group (135 mmol/l < sNa < 140 mmol/l) was significantly associated with HF-related events compared with the higher sNa group ( $p < 0.001$ ) [125]. In The Korea Acute Heart Failure (KorAHF) registry of 5625 patients, hyponatraemia was defined as a serum sodium level < 135 mmol/l at hospital admission [126]. HFpEF and HFrEF in this study were defined as LVEF ≥ 50% and LVEF ≤ 40%, respectively. Hyponatraemic patients had higher in-hospital mortality or urgent heart transplantation in all (11.3% vs. 4.5%,  $p < 0.001$ ), in HFrEF (13.1% vs. 4.9%,  $p < 0.001$ ), and in HFpEF (6.0% vs. 1.9%,  $p < 0.001$ ) [126]. However, the long-term prognostic value of hyponatraemia was limited to patients with HFrEF, and did not apply to those with HFpEF. Both serum levels of sodium – low but still normal (135–140 mmol/l) and lower than normal (< 135 mmol/l) – could provide important prognostic information in patients with HFpEF.

The pathophysiological mechanisms underlying the associations between co-morbidities such as obesity, diabetes mellitus, lung diseases, chronic kidney disease, liver diseases and HFpEF remain unclear. They are partially associated with systemic inflammation, and Paulus *et al.* [127] hypothesized that chronic inflammation leads to endothelial dysfunction, myocardial hypertrophy, fibrosis, and diastolic dysfunction [127]. Other proposed pathophysiological mechanisms include abnormal haemodynamics, metabolic dysregulation, and neurohumoral activation [127]. Indeed, more than 80% of patients with HFpEF are overweight or obese, and in the TOPCAT and RELAX trials, median/mean BMI was 31 kg/m<sup>2</sup> and over 35 kg/m<sup>2</sup>, respectively [15, 128]. Obokata *et al.* [129] described a distinct obese HFpEF phenotype, characterized by greater concentric LV remodelling, higher LV filling pressures at rest and with exercise, greater plasma volume overload (yet lower NT-proBNP levels), a larger increase in pulmonary arterial pressures with exercise, larger right ventricular size, and more significant exercise intolerance compared with non-obese HFpEF [129]. In addition to inflammation, the obese insulin-resistant subjects demonstrated an increase in extracellular matrix markers such as collagen VI and matrix metalloproteinase-7 when compared with obese insulin-sensitive subjects [130]. In a large cohort of obese patients without previous HF, Sundström *et al.* [131] recently demonstrated a graded association between increasing weight loss and decreasing risk of incident HF, suggesting that comorbid intervention may be beneficial before HFpEF ensues [131]. Confirming this notion,

weight loss following bariatric surgery was associated with reduced LV mass and mass-volume ratio, and improved diastolic function [132].

It is difficult to discuss co-morbidities of HF without mentioning diabetes. Based on an analysis that included 3,385 patients (mean age: 69 ±9.6 years; 49% male; 89% white) with HFpEF from the Treatment of Preserved Cardiac Function Heart Failure With an Aldosterone Antagonist Trial (TOPCAT), Sandesara *et al.* [133] concluded that diabetes is associated with adverse cardiovascular outcomes in HFpEF, and the inherent risk of adverse outcomes in HFpEF patients with diabetes varies according to the presence of microvascular complications [133]. The appropriate treatment of diabetes is also important in the context of HFpEF prognosis. The activity of the sodium-hydrogen exchanger is markedly increased in patients with HF and may be responsible for resistance to both diuretics and endogenous natriuretic peptides. In addition, in the heart, empagliflozin appears to inhibit sodium-hydrogen exchange, which may in turn lead to a reduction in cardiac injury, hypertrophy, fibrosis, remodelling, and systolic dysfunction. The benefits of such sodium-glucose co-transporter 2 (SGLT2) inhibitors in HF may be mediated by the inhibition of sodium-hydrogen exchange rather than the effect on glucose reabsorption. Empagliflozin was associated with a 35% reduction in the risk of HF hospitalization and a 39% reduction in the risk of HF death or hospitalization compared with placebo in patients with type 2 diabetes mellitus and cardiovascular disease in the EMPA-REG OUTCOME (Empagliflozin Cardiovascular Outcome Event Trial in Type 2 Diabetes Mellitus Patients) trial, with benefits seen in patients with and without HF at baseline [134]. HF outcomes relative to LVEF were not assessed, although HF outcome rates in those with baseline HF were similar to those in HFpEF trials, leading to suggestions that empagliflozin may have been beneficial in patients with HFpEF. The mechanisms by which empagliflozin may positively affect HF are unclear, but the improvement in HF outcomes was independent of glycaemic control. Hypotheses include a blood pressure-lowering effect, sodium and fluid loss, beneficial effects on the renin angiotensin system, weight loss, maintenance of renal function, decreased atrial stiffness, and decreased inflammation [135].

Approximately 30–40% of patients with HFpEF have chronic obstructive pulmonary disease [136]. In a large, community-based sample, Lam *et al.* [136] found that airflow obstruction was the most prominent non-cardiac predictor of incident HFpEF, and Barr *et al.* [137] showed that emphysema and airflow limitation are linearly related to impaired LV filling. Indeed, HFpEF has several pathophysiological aspects, including stiffness

and/or congestion of multiple organs. Yoshihisa *et al.* [138] investigated the impact of non-alcoholic fatty liver disease fibrosis score (NFS; based on aspartate aminotransferase to alanine aminotransferase ratio, platelet count, and albumin) on prognosis in a prospective study based on 492 HFpEF patients, with consideration of the peripheral collagen markers. The NFS, a novel indicator of liver fibrosis, correlated with circulating systemic markers of fibrosis and congestion such as procollagen type III peptide, type IV collagen 7S, and hyaluronic acid and BNP and was independently associated with higher all-cause mortality in HFpEF patients. The NFS can be calculated in a simple manner and may be a useful tool to assess liver stiffness and prognosis in HFpEF patients [138].

Approximately 25–50% of patients with HFpEF have chronic kidney disease with an estimated glomerular filtration rate (GFR) < 60 ml/min/1.73 m<sup>2</sup>, the prevalence of which increases with age [139]. Renal impairment causes metabolic and systemic derangements in circulating factors, causing an activated systemic inflammatory state and endothelial dysfunction, which may lead to cardiomyocyte stiffening, hypertrophy, and interstitial fibrosis via cross-talk between the endothelium and cardiomyocyte compartments [140]. Patients with CKD and hypertension are prone to develop HFpEF, and myocardial fibrosis has been suggested as a major determinant of disturbances in diastolic function in these patients. In rat models, CKD was significantly correlated with interstitial myocardial fibrosis, suggesting a mechanism of action for abnormal cardiac mechanics in these patients [141]. Chronic kidney disease-associated mortality is worse in patients with HFpEF compared with patients with HFrEF even after controlling for covariates [142]. Go *et al.* demonstrated a stepwise increase in mortality with decreasing eGFR in HFpEF [143]. The correct assessment of GFR remains challenging. Cystatin C, for example, is a marker of renal function that also predicts cardiovascular outcomes [144]. In patients with HFpEF of hypertensive origin, cystatin C has been shown to be increased and associated with diastolic dysfunction and alterations in collagen metabolism independently of eGFR. An excess of cystatin C might contribute to diastolic dysfunction in HFpEF by facilitating myocardial fibrosis [144]. In a study of Li *et al.* [145] there was a positive correlation between serum cystatin C levels and interventricular septal thickness, posterior wall thickness, and LV weight index, and the serum level of cystatin C was an independent marker for hypertensive LV hypertrophy. Moran *et al.* [146] examined 4453 subjects aged 65 years or older without HF at baseline from the Cardiovascular Health Study. They compared the asso-

ciation of cystatin C with risk of incident HFpEF and HFrEF. During 8 years of follow-up, increased risk of HFpEF was apparent only in the highest cystatin C quartile (HR = 2.25; 95% CI: 1.33–3.80), while a linear trend was present for HFrEF [146]. In the study of Bielecka-Dabrowa *et al.*, worse kidney function assessed with both eGFR and cystatin C was independently associated with worse course of HF with preserved and moderately reduced EF [147]. In a prospective analysis of a cohort of 299 patients with HFpEF, Unger *et al.* [148] found that chronic kidney disease and reduced GFR were both associated with worse cardiac mechanical indices including left atrial reservoir strain, LV longitudinal strain, and right ventricular free wall strain, even after adjusting for potential confounders, including co-morbidities, LVEF, and volume status. Reduced GFR was also associated with worse outcomes (adjusted HR = 1.28, 95% CI: 1.01–1.61 per 1 – SD decrease in GFR;  $p = 0.039$ ) [148]. Gori *et al.* studied 217 participants with HFpEF from the PARAMOUNT trial who had echocardiography and measures of kidney function assessed [149]. Renal dysfunction was associated with abnormal LV geometry (defined as concentric hypertrophy, or eccentric hypertrophy, or concentric remodelling), lower mid-wall fractional shortening (MWFS), and higher NT-proBNP values [149]. In these studies in HFpEF patients, chronic kidney disease was independently associated with worse cardiac mechanics, which may explain why HFpEF patients with kidney disease have worse outcomes [148, 149]. Thus, the therapeutic strategies aimed at reducing cardiac fibrosis may provide a particular cardioprotective benefit in patients with chronic kidney disease. There is evidence that long-term treatment with different loop diuretics may have a variable impact on myocardial fibrosis in chronic HF patients [150]. In fact, although torasemide treated patients exhibited a reduction in myocar-

dial collagen accumulation and a diminution of collagen type I synthesis, furosemide-treated patients did not [150]. Non-cardiac factors influencing prognosis in HFpEF are presented in Figure 2.

### Conclusions

The diagnosis of HFpEF remains challenging, especially when the patient presents in an outpatient setting without obvious signs of volume overload. In this context, biomarkers, with their objectivity, reproducibility and accessibility, would be excellent additions to imaging studies in HFpEF diagnosis and useful to tailor therapies to an individual's unique risk profile [39, 151–155]. Currently, the only guideline-recommended biomarkers in this regard are NT-BNP and NT-proBNP. Indeed, studies suggest that natriuretic peptides have the potential to improve even the diagnosis of early HFpEF, but they still have significant limitations. There remain doubts whether the cut-off points of NT-proBNP and BNP in diagnosis and monitoring should be the same across the entire HF spectrum. Based on evidence from endomyocardial biopsies, some of the specific cardiac structural phenotypes to be targeted in HFpEF may be represented by myocyte hypertrophy, interstitial fibrosis, and myocardial inflammation. Hence biomarkers reflecting these mechanisms may be useful in HFpEF.

The new therapies should be targeted towards specific HF phenotypes, instead of the 'one-size-fits-all' approach, which has not been successful in clinical HFpEF. Unless the structural and biological determinants of the failing heart are deeply understood, it will be impossible to differentiate HFpEF patients appropriately, identify subtle myocardial abnormalities, and finally reverse abnormal cardiac function. The identification of specific structural cardiac HFpEF phenotypes may represent one way to differentiate subgroups of HFpEF patients with specific therapeutic targets. The use of selec-

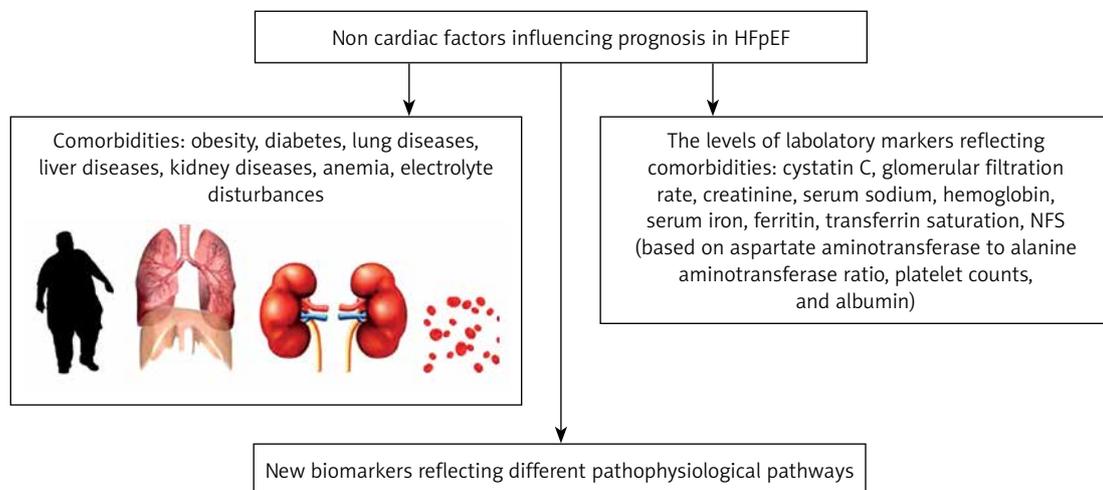


Figure 2. Non-cardiac factors influencing prognosis in HFpEF

tive biomarkers in HFpEF may help to distinguish pathophysiological mechanisms of HFpEF [20].

Concentrating on biomarkers of fibrosis and inflammation, sST2, GDF-15, galectin-3 and other new cardiac markers associated with diastolic dysfunction and disturbed left ventricular filling have emerged, but still evidence from large cohorts of patients with HFpEF is lacking. In particular, there are no clinical studies to suggest cut-off values that can help in clinical decision making for these markers. Furthermore, the field of miRNAs is a very promising area of research, and further exploration of miRNAs may offer diagnostic and prognostic applications and insights into pathology pointing to new phenotype-specific therapeutic targets [156]. This is especially interesting considering the fact that their molecular construction and integration in cellular and intercellular transport mechanisms define miRNAs as ideal circulating biomarkers. Nevertheless, looking at the large number of studies, only a few of them have confirmed previous findings with identical results, and still different miRNAs are being identified as linked to HF, presumably reflecting the complex interactions of miRNAs and their target sites. An important aspect when performing miRNA analyses is the comparability and standardization of analytical methods and the need for well-defined study samples [157, 158]. In our opinion, biomarkers of fibrosis or inflammation cannot replace natriuretic peptides for the diagnosis of HFpEF, but they may provide additive value, especially for assessing aggravation and prognosis in these patients. In our view, results from large cohorts and meta-analyses are required to establish the role of combinations of biomarkers as clinically applicable diagnostic and/or prognostic tools in HFpEF. Characterization of the biological phenotypes, understanding the biological differences between patients with typical comorbidities but without HF and those with clinically similar profiles but with HF, and identification of interventions that disrupt or reverse this pathobiology, may pave the way for patients to be targeted before the HFpEF syndrome ensues.

In describing the selected biomarkers of fibrosis and inflammation sST2, GDF-15, galectin-3, and miRNAs associated with diastolic dysfunction and disturbed left ventricular filling, we note that there is still a lack of evidence from large cohorts of patients with HFpEF. In this review we excluded markers of interstitial matrix turnover, because we presented them in a previous paper to which the reader is referred [39]. There have not been sufficient studies assessing the utility of biomarkers of fibrosis or inflammation in HFpEF to specify their role in only one phenotype. It is worth noting that the concentrations of biomarkers indicat-

ing the diagnosis of HFpEF vary in patients with acute versus chronic compensated HF [149]. For example, threshold values of BNP and NT-proBNP validated for diagnosis of undifferentiated acutely decompensated HF remain useful in patients with HFpEF, with minor loss of diagnostic performance. In stable treated HF, plasma natriuretic peptide concentrations often fall below cut-off values used for diagnosis of acute decompensated HF in the emergency department; in HFpEF, levels average approximately half of those in HFpEF [149].

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