Chapter 9

Summary, General Discussion and Future Perspectives
HFpEF pathophysiology
In the current thesis we explored the pathophysiological mechanisms underlying heart failure with preserved ejection fraction (HFpEF) and tried to define future diagnostic and therapeutic options based on this knowledge.

In Chapter 2, a ZSF1-rat model for HFpEF is characterized. ZSF1-rats are hybrids based on a cross between a ZDF female and a SHHF male rat. This leads to a leptin resistant, spontaneously hypertensive rat that develops obesity and diabetes mellitus (DM). Interestingly, only obese rats developed the HFpEF-phenotype (further named ZSF1-HFpEF rats). Lean, hypertensive ZSF1-rats without leptin resistance did not develop a HFpEF-phenotype. This clearly indicates the importance of metabolic comorbidities in the development of HFpEF. Post-mortem analysis on ZSF1-HFpEF rats demonstrated increased passive stiffness ($F_{\text{passive}}$) of myocardial muscle strips and single cardiomyocytes. The increased $F_{\text{passive}}$ was predominantly attributable to titin hypophosphorylation on specific sites of the N2Bus and PEVK segments of titin. In contrast, there was no increased myocardial fibrosis or collagen deposition in ZSF1-HFpEF rats.

The study described in Chapter 3 explores how metabolic comorbidities lead to increased myocardial $F_{\text{passive}}$ via systemic, low-grade inflammation. In the myocardium of HFpEF patients and ZSF1-HFpEF rats, markers for inflammation and oxidative stress were measured. E-selectin and intercellular adhesion molecule (ICAM)-1 expression levels were upregulated in both human and rat HFpEF samples, indicating microvascular inflammation. Also, NADPH oxidase 2 expression as a marker of oxidative stress was raised in HFpEF. However, NADPH was only increased in macrophages and endothelial cells but not in cardiomyocytes. These findings indicate that the primum movens in HFpEF pathophysiology seems to be endothelial inflammation and activation and not a cardiomyocyte related problem. In HFpEF myocardium, endothelial nitric oxide synthase (eNOS) was shown to be uncoupled. eNOS-uncoupling was associated with reduced myocardial nitrite/nitrate concentration, cyclic guanosine monophosphate (cGMP) content, and protein kinase G (PKG) activity. In other words, a reduction of NO-dependent signalling from endothelial cells to cardiomyocytes was observed. This reduced
signalling can contribute to the high cardiomyocyte $F_{\text{passive}}$ and hypertrophy observed in HFPfEF.

Titin hypophosphorylation leading to increased cardiomyocyte $F_{\text{passive}}$ was reported in previous studies to be a key finding in HFPfEF. However, manipulation of titin phosphorylation state could not explain all the changes observed in cardiomyocyte based passive stiffness, as demonstrated in Chapter 7. Dephosphorylation of single cardiomyocytes from explanted donor hearts increased passive stiffness. However, $F_{\text{passive}}$ in aortic stenosis (AS) or dilated cardiomyopathy (DCM) cardiomyocytes still exceeded $F_{\text{passive}}$ in dephosphorylated donor cardiomyocytes. Incubation in an acidic environment and, more important, performing a prestretch, raised $F_{\text{passive}}$ in donor cardiomyocytes to values observed in diseased hearts. Interestingly, in vitro incubation with the small heat shock protein $\alpha$-B crystallin decreased $F_{\text{passive}}$ to donor baseline levels in donor as well as in diseased cardiomyocytes. The mechanism behind this is supposed to be aggregation of titin due to (myocardial) stretch, present in conditions such as heart failure. Stretch unfolds immunoglobulin-like (Ig) domain-containing regions of titin, which can aggregate, especially under acidic conditions. This aggregation is prevented by $\alpha$-B crystallin.

These findings raise the hypothesis that there is a continuum in HFPfEF pathophysiology. This pathophysiology might start with (metabolically induced) endothelial inflammation and activation and leads to decreased NO-bioavailability. Subsequently, the cardiomyocytes develop a relative hypophosphorylation of titin, which leads to increased passive stiffness. These processes probably set in rapidly. In a more advanced phase, where wall stress and cardiomyocyte stretch increase, titin aggregation can be expected to play a more prominent role in causing increased passive stiffness. In a last stage, the HFPfEF myocardium is characterized by increased collagen deposition and fibrosis, as reviewed in Chapter 4. These stages in HFPfEF pathophysiology are probably the basis for future diagnostic and therapeutic strategies, as will be discussed next.

**HFPfEF diagnosis**
The diagnosis of HFPfEF remains challenging and requires signs or symptoms of congestion, preserved or mildly abnormal LV systolic function (EF>50%, end-diastolic volume index <97 mL/m$^2$) and diastolic LV dysfunction. Diastolic LV
dysfunction is defined as the inability of the ventricle to fill to a normal preload volume at low pressures. It can be diagnosed invasively by measurement of an increased pulmonary capillary wedge pressure (PCWP), LV end-diastolic pressure or prolonged LV isovolumic relaxation. Doppler echocardiography guides non-invasive diagnosis with an E:E’ >15 (ratio of early transmitral diastolic flow velocity to tissue Doppler early mitral annular diastolic velocity). When E:E’ is in the “grey zone” of 8–15, secondary evidence for diastolic dysfunction is needed. This evidence can be based on echocardiographic parameters (left atrial size, transmitral and pulmonary flow velocities, LV hypertrophy), the presence of atrial fibrillation or increased natriuretic peptides.

As reviewed in Chapter 8, the current diagnostic criteria for HFpEF have some limitations. First of all, many patients only develop symptoms during exercise, but the current diagnostic criteria use measurements at rest. Diastolic stress-testing will probably play an important role in the near future in HFpEF-diagnosis. Ideally echocardiography is a cornerstone in the diagnosis, since it is readily available and non-invasive. However, limitations of echocardiography in HFpEF diagnosis are recently recognized both at rest and during exercise. For example, even despite high values of invasively measured PCWP, E:E’ can sometimes be misleading. Even more important, within individual patients E:E’ does not reliably track changes in left-sided filling pressures (PCWP), making its usefulness debatable in HFpEF. Many protocols and parameters have been tested during exercise. A change in E:E’ was the most frequently used measurement, but currently there is insufficient evidence to use this or any other specific parameter or protocol for routine use when evaluating patients suspected of having HFpEF.

At the moment, exercise testing with measurement of invasive hemodynamics seems a promising approach in HFpEF diagnosis since already a limited workload induces a significant increase in PCWP (≥25mmHg) in HFpEF patients. Of interest is also the finding that invasive exercise hemodynamics unmasked diastolic dysfunction in symptomatic patients with a tentative diagnose of primary pulmonary hypertension and normal PCWP at rest. This reclassifies these pulmonary hypertension patients as HFpEF patients as discussed in Chapter 6. Also, since patients are usually under fluid restriction at the time of conventional testing, exercise hemodynamics may identify HFpEF, especially in obese patients with a dilated left atrium and
Presenting with pulmonary hypertension. These findings indicate the importance of exercise testing with invasive hemodynamic measurements in patients in whom HFpEF is suspected.

A second pitfall in the current diagnostic criteria for HFpEF is the use of natriuretic peptides as biomarkers. It is known that normal levels of natriuretic peptides do not rule out HFpEF. As discussed in Chapter 5, many recent studies focused on biomarkers reflecting the inflammatory and profibrotic changes in the myocardium and extracellular matrix. This is a potential advantage above echocardiography. Although none of these biomarkers has a place in clinical practice yet, MMP9 and TIMP1, for example, were shown to have a potential role in predicting the development of heart failure in patients with asymptomatic LV diastolic dysfunction. This might help to identify a population of patients at risk for HFpEF that would benefit most from cardiovascular risk reduction strategies.

Currently, especially ST2 and galectin-3 predict prognosis in HFpEF. Besides diagnosis and prognosis, biomarkers might identify patients that potentially benefit most from certain treatment options. For example, higher levels of soluble ST2 and galectin-3 correlated with more reduction in left atrial size in patients treated with LCZ696 (valsartan/sacubitril). The finding that biomarkers may identify patients that benefit more from specific therapies than other patients brings us to future therapeutic options, for which this “staging of HFpEF” will be of great help.

HFpEF treatment
In contrast to heart failure with reduced ejection fraction (HFrEF), the current guidelines on heart failure from the Heart Failure Association (HFA) of the European Society of Cardiology (ESC) lack evidence based treatment options for HFpEF and advice the clinician to reduce congestion with diuretics and to treat comorbidities. As reviewed in Chapter 8, no treatment strategy studied to date in large HFpEF trials has proven to improve disease progression and survival, including betablockers, angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, and digoxin. A subanalysis of the TOPCAT trial (which had a neutral outcome) suggested that spironolactone may be more beneficial in older patients with more advanced HFpEF, comorbidities and a higher BNP than in younger patients with fewer comorbidities, less
severe HFpEF and a lower BNP. This again that a staging strategy in HFpEF may be helpful.

Based on the expanding knowledge about HFpEF pathophysiology, newer treatment options aiming to restore the soluble guanylate cyclase (sGC)-cGMP-PKG pathway were more recently explored and they are reviewed in **Chapter 8**. Unfortunately, chronic treatment with the phosphodiesterase-5 inhibitor sildenafil did not improve exercise capacity and clinical status in the RELAX trial. Also, sildenafil failed to increase plasma cGMP concentrations or yield hemodynamic benefits. In this study, plasma levels of N-terminal pro-BNP (NT-proBNP) and prevalence of atrial fibrillation were high. This indicates that patients were at an advanced stage of HFpEF and therefore less likely to benefit from a limited strategy involving only inhibition of cGMP breakdown. Direct stimulation of sGC with oral vericiguat is currently under investigation in HFpEF (NCT01951638).

The first-in-class angiotensin receptor inhibitor nepriylisin inhibitor (ARNI) is a combination of the angiotensin receptor blocker valsartan and the nepriylisin inhibitor sacubitril. Nepriylisin inhibition leads to increased stimulation of particulate GC via natriuretic peptides and hence to upregulation of cGMP/PKG. In HFrEF, valsartan/sacubitril was superior to enalapril in reducing the risks of death and of hospitalization for heart failure. In HFpEF, valsartan/sacubitril reduced NT-proBNP to a greater extent than did valsartan at 12 weeks and was well tolerated in a Phase II-study (PARAMOUNT). A PARAMOUNT-substudy demonstrated that biomarkers that reflect collagen homeostasis, such as soluble ST2 and galectin 3, correlated with the presence and severity of HFpEF. Furthermore, higher levels of soluble ST2 and galectin 3 predicted a more pronounced decrease in left atrial volumes after treatment with valsartan/sacubitril, suggesting that patients with more advanced HFpEF might benefit most from this new drug.

Although these recent studies have some promising results, the phenotypic diversity of HFpEF patients is increasingly recognized and probably personalized therapeutic strategies with combinations of treatment modalities are needed. An approach that requires further research would be to classify patients into different stages of HFpEF based on disease severity. Subsequently, various treatment strategies can be studied according to their HFpEF-stage. For example, anti-inflammatory treatments aimed at restoring the NO-sGC-cGMP-PKG-pathway might be more effective in early stages. These NO-sGC-cGMP-
PKG restoring therapies will probably be less successful when myocardial fibrosis or atrial fibrillation suggest a more advanced HFrEF, as suggested by the negative results of the RELAX-trial. Later stages might benefit more from therapeutic efforts to raise concentrations of α-B crystallin, which is able to decrease cardiomyocyte stiffness in vitro, as demonstrated in Chapter 7. This might be achieved through direct administration of α-B crystallin, through administration of α-B crystallin analogues or through administration of heat shock protein inducing drugs such as geranylgeranylatedone (GGA) or NYK9354. The most advanced stages of HFrEF are probably characterized by marked remodeling of the extracellular matrix. In an advanced stage patients might benefit more from treatment with valsartan/sacubitril or spironolactone, as suggested by the PARAMOUNT and TOPCAT substudies, respectively.

CONCLUSION

The pathophysiological mechanisms in HFrEF are gradually becoming unraveled and non-cardiac comorbidities drive a systemic inflammatory state that also affects the coronary microvasculature. This coronary microvascular inflammation induces oxidative stress and leads to reduced NO-dependent signaling to cardiomyocytes with a subsequent PKG-deficit and increased passive stiffness and diastolic dysfunction. In probably more advanced stages, titin aggregation and myocardial fibrosis develop, further aggravating passive stiffness. Future therapeutic options will need to target the different aspects contributing to increased myocardial stiffness. Personalized treatment strategies are required and in order to make this feasible, a classification system for HFrEF is needed. Whether optimal classification is based on phenotypic characteristics, invasive hemodynamic findings, biomarkers or a combined approach, will be topic of future studies.

REFERENCES


