REVIEW | Extracellular Matrix in Cardiovascular Pathophysiology

Targeted HFpEF therapy based on matchmaking of human and animal models

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INTRODUCTION

Heart failure (HF) with preserved ejection fraction (HFpEF) is a complex syndrome, with high morbidity and mortality. In the last decade, with a 1% incidence increase per year, HFpEF has become a health care problem of epidemic proportions (87). Despite many efforts, so far, no randomized controlled trial has shown improved survival. One likely explanation for the lack of success in HFpEF trials is the incompatibility of the classical one-size-fits-all trial design approach with the highly heterogeneous and comorbid HFpEF population. The heterogeneity of HFpEF is constantly referred to in negative terms, sometimes like a discouraging mantra, forgetting that this diversity may actually be key to solving the puzzle. Up to now there is no unanimity about which clinical phenotypes exist. Nevertheless, the importance of a phenotype-based classification was highlighted by several authors showing a divergence in prognosis and outcome (102, 107, 108). Although the recognition of clinical HFpEF phenotypes is important, an improved understanding of the pathophysiological processes involved is also required to pinpoint the heterogeneity in HFpEF phenotypes (51). From this, specific interventions can follow, targeting individuals on the basis of their biological phenotype.

This review addresses several important steps that are needed to accomplish successful therapeutic intervention aimed at specific underlying pathological processes, e.g., biological phenotypes. First, we will discuss the difficulties in identifying clinical and biological phenotypes. Second, we review how matching complex animal models with human biological phenotypes provides a unique opportunity to develop and test novel targeted treatments. Finally, we will discuss innovative trial designs that suit our pursuit of personalized targeted therapy.

PRECISION THERAPY STARTS WITH PHENOTYPING

Diagnostic Challenge

One of the main difficulties in clinical practice is the complexity of diagnosing HFpEF. Recently, serious efforts have been made to develop more concise and reliable diagnostic criteria for HFpEF (77, 84). The different guidelines and diagnostic algorithms agree that HF signs and/or symptoms are mandatory but not sufficient; increased natriuretic peptide levels and structural and functional cardiac abnormalities are also required (49, 84). Nevertheless, there is a lack of agreement about which cutoff values should be used for these parameters. Hemodynamic abnormalities, characterized by in-
increased left-sided filling pressures, play an important role in HFpEF. Cardiac structural and functional alterations are surrogate markers widely used to noninvasively estimate left-sided filling pressures. However, these estimates lack sensitivity (47). To complicate matters even further, in some patients these key features of HFpEF are not evident at rest and are only observed during exercise. Therefore, structural incorporation of an exercise evaluation should be considered for the diagnosis of HFpEF, particularly when the clinical diagnosis remains unclear (10, 72). Addition of exercise echocardiograph, i.e., exercise ratio of mitral peak velocity of early filling (E) to early diastolic mitral annular velocity (E’) (exercise E/E’), improves sensitivity, but the most accurate basis to diagnose HFpEF will likely be provided by the invasive assessment of hemodynamic responses to exercise (72, 121). Once this diagnosis has been secured, further cracking of the HFpEF code will be needed for effective treatment. Therefore, a better understanding of the underlying pathophysiology by means of translational research and improved diagnostic tools such as novel imaging techniques and biomarker panels are key.

Identifying Clinical Phenotypes

HFpEF is related to several comorbid conditions and has a broad spectrum of clinical phenotypes. The systematic assessment of comorbid conditions and clinical features has been demonstrated to be effective to determine prognosis and outcome of these patients (107). In the present literature, a wide variety of clinical phenotypes has been proposed by reviewing the most common comorbid conditions. Samson et al. (102) argued that the presence of aging, obesity, pulmonary hypertension, or coronary artery disease defines four specific phenotypes, with hypertension underpinning all of them. Moreover, a review by Shah et al. (108) proposed a phenotyping matrix distinguishing patients with HFpEF with a general lung congestion/metabolic risk phenotype, also called the “garden variety,” and several more specific phenotypes, characterized by atrial fibrillation (AF), arterial hypertension, renal insufficiency, chronotropic incompetence, coronary artery disease, chronic obstructive pulmonary disease (COPD), skeletal weakness, or pulmonary hypertension. Evidence supporting the existence of such phenogroups is scarce. The authors of only one study based their HFpEF phenogroups on clustering of different clinical features, by which 397 patients with HFpEF were classified in three categories with a matching differential diagnosis. Here, younger patients with mild diastolic dysfunction had the best prognosis and outcome followed by patients with a metabolic phenotype (obesity, diabetes, and obstructive sleep apnea). The worst outcome was seen in elderly patients with significant chronic kidney disease (CKD), electrophysiological and structural myocardial remodeling, pulmonary hypertension, and right ventricular dysfunction (107). Additionally, Obokata et al. (73) identified a distinct obese phenotype, with greater volume overload and more biventricular remodeling compared with nonobese patients with HFpEF. In patients with HFpEF with obesity, other factors contributed to high left-sided filling pressures, suggesting that pathophysiological mechanisms differ in patients with obesity compared with nonobese patients. Although these studies have in common that subgroups of patients with HFpEF may exist, there is no consensus on what these should be.

All in all, there is still a long way to go to elucidate which phenotypes are clinically relevant. We propose additional evaluation of the underlying pathophysiological processes to evaluate biological phenotypes to guide us toward a phenotype-specific HFpEF treatment.

Biological Phenotypes: Origin of the Disease

When we refer to biological phenotypes, we refer to the pathophysiological processes responsible for the structural and functional cardiovascular alterations seen in HFpEF. In the next sections, we will discuss the principal pathogenic mechanisms that are believed to be involved in the development of HFpEF. We will focus on inflammation, endothelial dysfunction, and impaired muscle relaxation due to changes outside (fibrosis) and within the cardiomyocyte (increased passive stiffness, impaired electrical-mechanical coupling, derangements in Ca2+ handling, and energy imbalance) (117).

Inflammation as a therapeutic target for HFpEF. Systemic low-grade inflammation, produced by the direct influence of diverse comorbid conditions, has been proposed to be the driving force in HFpEF development and is thought to induce endothelial dysfunction and microvascular disease (76, 117). Circulating levels of inflammatory markers are increased in patients with diastolic dysfunction and HFpEF (15, 61, 86, 103). Besides systemic inflammation, myocardial inflammation is also present, as shown by studies in human myocardial tissue with high numbers of CD3-, CD11-, and CD45-positive leukocytes (129). Franssen et al. (25) confirmed these findings and showed hallmarks of inflammation, upregulation of adhesion molecules (E-selectin and ICAM-1), increased inflammatory cell recruitment and activation, and increased oxidative stress in myocardial biopsies from patients with HFpEF. Since inflammation seems to be the initiator of a downward cycle of pathophysiological events culminating in HFpEF, it is a logical target for intervention. Still, we are only just starting to understand the functions of immune cells in the heart, and further translational studies are of pivotal importance to identify which immune and inflammatory processes in the heart are detrimental and which processes should remain untouched (38, 39, 58).

Currently, there are several promising interventions to target inflammation that show potential for patients with HFpEF, but none of them have made their way into clinical routine yet. First, IL-1 blockade was shown to improve diastolic function in animal models (116). In patients with rheumatoid arthritis, anakinra, an IL-1 blocker, displayed similar findings, improving diastolic function (40). Moreover, the Diastolic Heart Failure Anakinra Response Trial (D-HART), a pilot study that included only patients with HFpEF with high inflammation parameters [defined as plasma high-sensitivity C-reactive protein (CRP) levels $> 2$ mg/l], showed significantly reduced systemic inflammation and improved exercise capacity after 14 days of treatment with anakinra (123). Interestingly, reduction of CRP was correlated to improvement in exercise capacity. However, D-HART2, a phase 2 study, failed to show an increase in peak oxygen consumption or reduction in CRP after 24 wk of treatment (NCT02173548) (124). Nevertheless, CRP level reduction by itself could be beneficial, as recently demonstrated in the Canakinumab Anti-inflammatory Thrombosis Outcomes Study (CANTOS), in which patients with a history of myocardial infarction were treated with canakinumab, another IL-1 blocker (94). In this subanalysis, only patients who achieved a high-sensitivity CRP reduction...
with levels below 2 mg/l after 3 mo of treatment had a mortality rate reduction of 31%. These results link anti-inflammatory therapy with improvement of outcome, but only if inflammation was measurably diminished, which could be beneficial for (a subset of) HFpEF as well.

In addition to cholesterol-lowering effects, statins have demonstrated anti-inflammatory effects. Several trials have shown the beneficial effects of statins in reducing inflammation, which was associated with reduction in cardiovascular events, independent of its lipid-lowering effect (2, 93, 95). Furthermore, statins inhibit left ventricular (LV) remodeling and improve diastolic function by increasing coronary perfusion, restoring endothelial function (36, 48). Since diastolic dysfunction, hypertrophy, endothelial dysfunction, and fibrosis are hypothesized to be involved in the etiology of HFpEF, statins could be beneficial. Moreover, less hypertrophy, decreased resting tension, and increased PKG activity were observed in myocardial biopsies of patients with HFpEF treated with statins (76). A meta-analysis of 11 studies, including almost 18,000 patients with HFpEF, compared statin users with nonstatin users and showed that statin use was associated with a 40% lower risk of mortality compared with nonstatin users (54). All in all, prospective randomized controlled trials to evaluate the effect of statins are warranted.

Inhibition of inflammation in patients with HF has been tried before, with limited success and even detrimental effects in some patients (59). Evaluated candidate treatments come from the repurposing of drugs used in inflammatory disease such as rheumatoid arthritis. For the heart, it seems important to find a balanced inhibitor that does not totally block inflammatory responses but rather dampens them (38). Anakinra may be such an intervention. Other available ways to tackle inflammation are limited. One option could be phosphodiesterase (PDE) enzyme inhibitors, for which preclinical studies have shown attenuated systemic inflammation in animal models of inflammatory and neurological disorders, including Alzheimer’s disease, Parkinson’s disease, multiple sclerosis, and many others (101). In particular, PDE4 and PDE7A isofrom inhibitors are known for their potent anti-inflammatory effects (52, 104). PDE4 inhibitors are currently being tested in humans with psoriasis and psoriatic arthritis in phase 2 (NCT02576678) and phase 3 (NCT01212770) trials. However, the effect of PDE4 and PDE7A inhibition in HF is unknown. Furthermore, an important role of PDE4 in Ca$^{2+}$ management and in cardiomyocyte excitation-contraction coupling has been demonstrated in mice; therefore, inhibition of PDE4 could possibly provoke arrhythmogenic effects (50).

In conclusion, repurposing anakinra to treat HFpEF seems a promising strategy to dampen systemic inflammation and improve cardiac outcome in the subset of patients with HFpEF that would benefit most, namely, those with high CRP levels. Further understanding of the functions of cardiac immune cells and the contribution of local inflammation to HFpEF will be essential to develop new therapeutic strategies for cardiac inflammation inhibition.

**Endothelial dysfunction as a therapeutic target for HFpEF.**

One of the prevailing hypotheses states that inflammation impairs signaling of endothelial nitric oxide (NO) to guanylate cyclase, cGMP, and PKG, causing endothelial dysfunction (25). Borlaug et al. (11) demonstrated that endothelial dysfunction was more prevalent in patients with HFpEF compared with hypertensive and healthy age-matched control subjects.

When addressing therapeutic options, PDE5 inhibitors could play an important role. PDE5 inhibition increases cellular cGMP levels, thereby promoting NO-dependent vasodilation, among others (30). Moreover, PDE5 inhibitors attenuate myocardial fibrosis by inhibiting transforming growth factor-β1, which stimulates cardiac fibroblast proliferation (105). In animal models, PDE5 inhibitors such as sildenafil have been effective in reducing LV remodeling, hypertrophy, and fibrosis (67, 111). However, in human studies, the use of PDE5 inhibitor showed disappointing results. Although a single-center trial showed improvements in hemodynamics after 12 wk of sildenafil treatment, larger randomized controlled trials, such as the Phosphodiesterase-5 Inhibition to Improve Clinical Status and Exercise Capacity in Diastolic Heart Failure (RELAX) trial, failed to observe clinical benefit of PDE5 inhibition in HFpEF (33, 89). Potential reasons for these disappointing results could be the inability of sildenafil to enhance cGMP levels sufficiently. However, maybe the NO-cGMP pathway is not a central mechanism of HFpEF, and therefore increasing cGMP levels in the cardiomyocyte is not sufficient to improve cardiac function.

Administration of soluble guanylate cyclase activators, which has been shown to improve quality of life and exercise capacity in patients with pulmonary arterial hypertension (PAH) (27), also looks promising for patients with HFpEF to provide a downstream correction of the low myocardial cGMP levels. Although the Soluble Guanylate Cyclase Stimulator in Heart Failure Patients with Preserved Ejection Fraction (SOCRATES-PRESERVED) study, a phase 2b study with vericiguat, did not show changes in NH$_2$-terminal pro-B-type natriuretic peptide and left atrial volume after 12 wk of treatment, encouraging results on quality-of-life scores warrant further studies, with longer followup and harder end points (81).

Preliminary data suggest a possible role for Na$^+$/glucose cotransporter 2 (SGLT2) inhibitors, a diabetic medication, in cardiovascular disease (96). These drugs were tested in patients with type 2 diabetes mellitus and, surprisingly, showed a marked reduction in cardiovascular mortality (23, 70, 136). However, which underlying pathophysiological mechanism is responsible for these positive effects remains unclear. The direct impact of SGLT2 inhibitors on the cardiovascular remains controversial since only the SGLT1 isoform, and not SGLT2, is expressed in the myocardium. It was suggested that cardiovascular risk reduction was obtained because of systemic improvements, such as lower glycemic index, lower body weight, increased diuresis, and increased blood pressure control, as reviewed by Kaplan et al. (43). These favorable effects on diabetes mellitus, hypertension, and obesity could indirectly benefit patients with HFpEF. In addition, SGLT2 inhibitors could affect the vasculature directly: in Zucker diabetic fatty rats, SGLT2 inhibitor treatment prevented oxidative stress and inflammation and improved endothelial function (110). However, in a small human study, 6 wk of SGLT2 inhibitor treatment did not result in an improvement of macrovascular or microvascular function (74). Further exploration of the direct and indirect effects of SGLT2 inhibitors in patients with and without diabetes is therefore warranted, and trials in HFpEF with and without diabetes mellitus are ongoing (NCT03057951 and NCT03030235).
Fibrosis as a therapeutic target for HFpEF. Extracellular matrix (ECM) deposition in the intercellular space between cardiomyocytes forms the cement that holds the heart together but, when excessively present, reduces tissue compliance and consequently diastolic ventricular filling. Extracellular elastin, collagen deposition, and subsequent chemical alterations (e.g., collagen cross-linking) produce myocardial interstitial and perivascular fibrosis (31). Human myocardial biopsy samples from patients with HFpEF showed myocardial fibrosis with an increased collagen volume fraction compared with healthy control subjects (9, 129). This was confirmed in patients with an ante mortem diagnosis of HFpEF expressing more myocardial fibrosis in autopsies compared with age-matched control subjects (63). However, it is not the quantity of collagen that seems to have functional implications, but, more, the amount of the stiffer collagen type I over the more compliant collagen type III (129). Whether HFpEF hearts also have more collagen type I remains unclear.

Preclinical research has already shown that the renin-angiotensin-aldosterone system is related to myocardial ECM remodeling and fibrosis (12, 57). However, the randomized trials performed using renin-angiotensin-aldosterone system inhibitors in humans, in contrast to the results obtained in patients with HF with reduced ejection fraction (HFREF), have shown only disappointing results in HFpEF (14, 60, 131). Recently, Zhi et al. (133) used mice on a hyperhomocysteinemia-inducing diet to induce myocardial fibrosis in the absence of pressure overload to demonstrate that the renin inhibitor aliskiren effectively reduces myocardial fibrosis. Perhaps this opens the door to new clinical trials evaluating aliskiren in patients with HFpEF with a fibrotic phenotype.

Another drug assigned antifibrotic properties is the mineralocorticoid receptor antagonist (MRA). The role of the mineralocorticoid receptor has been extensively studied in animal models (130), which show that activation of the mineralocorticoid receptor promotes inflammation and fibrosis of the myocardium, with negative effects on cardiac structure and function. Moreover, inhibition prevents these effects. In patients with HFrEF, MRA treatment reduces hospitalization and mortality rates (83). However, the results in patients with HFpEF were not that straightforward. MRA therapy was associated with improvement of diastolic function and reduction of fibrosis not only in patients with HFpEF but also in patients with asymptomatic diastolic dysfunction (75). However, the Treatment of Preserved Cardiac Function Heart Failure with an Aldosterone Antagonist (TOPCAT) study, a large randomized controlled trial, was negative for its primary composite end point but did show a decrease in HF hospitalizations (82). In this study, there were regional differences between the Americas and Russia/Georgia, questioning the validity of conclusions based on outcomes in the combined populations, and it is open for debate whether the correct patients were enrolled or even received the drug in the latter region (78). Indeed, in the Americas, spironolactone was evidently superior compared with placebo. Therefore, we believe that the use of MRA is still advocated, especially in appropriately selected patients with HFpEF. Currently, the ongoing phase 4 Spironolactone Initiation Registry Randomized Interventional Trial in Heart Failure with Preserved Ejection Fraction (SPIRRIT-HFpEF), a multicenter randomized controlled trial (NCT02901184), will test the effect of spironolactone on mortality and HF hospitalization in the HFpEF population with emphasis on patient characteristics and selection. In this trial, regional enrollment caps are introduced, with the aim of avoiding the extreme geographical patient heterogeneity.

Cardiomyocyte stiffness as a therapeutic target for HFpEF. Besides fibrosis, reduced ventricular compliance in HFpEF has also been attributed to an increased myocardial passive stiffness and resting tension of cardiomyocytes. The giant sarcomeric protein titin is considered to be primarily responsible for the changes in cardiomyocyte stiffness (32, 34). The titin protein is encoded by a single gene, and alternative splicing gives rise to two isoforms in the adult heart, the stiffer N2B and more compliant N2BA isoforms. Titin stiffness is regulated by isoform switching and through phosphorylation. Using a mouse model of diastolic dysfunction, it was recently shown that by knocking out RNA-binding motif-20 (RBM20), alternative splicing of titin was reduced resulting in a higher N2BA-to-N2B ratio, a reduction in myocardial passive stiffness, and consequently improved myocardial function and compliance (62).

Accumulating evidence indicates that titin stiffness is primarily regulated by changes in its phosphorylation state (8, 68, 69, 135). In particular, hypophosphorylation of PKG-dependent sites in titin seems to be responsible for the increased myocardial passive stiffness observed in patients with HFpEF and in rodent models of HFpEF, such as the obese Zucker spontaneous fatty (ZSF1) rat (35). Studies with cardiomyocytes isolated from obese ZSF1 rats and patients with HFpEF demonstrated that the administration of PKA or PKG reversed titin hypophosphorylation and reduced myocyte passive resting tension (8, 9, 35).

Reversion of titin-related myocardial stiffness has also been an object of interest in different clinical trials, and so far, strategies targeting titin hypophosphorylation in patients with HFpEF have failed. By increasing cGMP and PKG activity, sildenafil, PDE-5 inhibitors, and nitrates were supposed to show beneficial effects by reverting titin-related myocardial alterations, but, unfortunately, this was not the case (88, 89).

Ca^{2+} handling as a therapeutic target for HFpEF. Next to changes in passive stiffness, derangements in active relaxation will also contribute to diastolic dysfunction. In mouse models of HFpEF, cardiac levels of sarco(endo)plasmic reticulum Ca^{2+}-ATPase and phosphorylation of phospholamban were reduced (113). In patients with hypertension and aortic stenosis, a heart rate-dependent impairment in relaxation was observed, which was attributed to a reduction in sarcolemmal Ca^{2+} removal (106). In a subsequent study of patients with HFpEF with hypertension, the same researchers showed that impaired relaxation was associated with increased resting Ca^{2+} levels, especially at higher heart rates (99). However, the mechanism underlying the changes in Ca^{2+} handling remained elusive, as expression levels of proteins involved were not changed and evidence for involvement of the Na^{+}/Ca^{2+} exchanger was not found (99). This seems to contrast with a preclinical study using the Dahl salt-sensitive rat model of HFpEF, in which blockade of the Na^{+}/Ca^{2+} exchanger entry mode proved to be beneficial (42).

Energy metabolism as a therapeutic target for HFpEF. Cardiac relaxation is an active process that requires chemical energy in the form of ATP to be completed. In HFpEF, microvascular dysfunction and ECM expansion may hinder oxygen supply and diffusion (100), resulting in reduced myo-
cardial energy production and, consequently, diastolic dysfunction and diminished exercise capacity. Previously, we showed that proinflammatory signaling as such also impairs cardiac mitochondrial energy metabolism (92, 109). Recent preclinical studies have pointed out that Ca$^{2+}$ is an important determinant of mitochondrial function (6), thereby providing a link between alterations in Ca$^{2+}$ fluxes, as observed in HFpEF, and altered mitochondrial function. Cardiomyocyte dysfunction and energy imbalance have been demonstrated in animal models and humans with HFpEF (17, 76). Using cardiac spectroscopy, a reduced phosphocreatine-to-ATP ratio has been demonstrated in patients with HFpEF during exercise (79).

Several pharmacological approaches aimed at the modulation of cardiac energy metabolism to alleviate myoccardial dysfunction are being pursued. Approaches that are being pursued clinically (NCT03133793) are the administration to patients with HFpEF of d-ribose, a precursor of ATP synthesis, and ubiquinol (coenzyme Q10), a critical component of the mitochondrial respiratory chain (80). Another approach is the use of inhibitors of fatty acid oxidation, such as trimetazidine (118, 126, 132). Trimetazidine inhibits fatty acid oxidation at the level of long-chain 3-ketoacyl-CoA thiolase. The rationale behind this intervention is to shift cardiac metabolism more toward the oxidation of glucose, which is more energy efficient (less oxygen needed to produce chemical energy, reflected by a higher ATP-to-O ratio). In a small placebo-controlled trial, trimetazidine was found to increase the phosphocreatine-to-ATP ratio and to improve LV function in patients with HFpEF (24). It should be noted that the outcome of such metabolic interventions may depend on the clinical HFpEF phenotype. In the presence of insulin resistance and diabetes, the ability of the cardiac muscle to use glucose is limited, and the heart predominantly relies on the use of fatty acids. It is conceivable that inhibiting fatty acid oxidation under these conditions may actually have adverse effects.

Taken together, comorbidities such as hypertension, diabetes, and obesity differentially affect cardiac metabolism, and novel matchmaking trials could be designed taking into account the specific cardiac metabolic needs and therapeutic windows in specific HFpEF phenogroups. For example, metabolomics and more specific measures such as the cardiac phosphocreatine-to-ATP ratio could be used to link animal models mimicking metabolic syndrome (e.g., ZSF1 rats) to patients with HFpEF. The potential beneficial effects of metabolism-modifying drugs, still to be identified, in such animal models could be translated to patients with a comparable phenotype.

**NOVEL STRATEGIES FOR TARGETED THERAPY: A MATCHMAKING PROCESS**

Translational research is essential in the long and arduous path to a better understanding of the development of HFpEF and search for an effective treatment. The development of complex animal models, with the potential to be matched to specific HFpEF phenotypes, is necessary to design targeted therapy and to successfully translate effective animal model-based interventions into humans (Fig. 1) (98). Table 1 shows an overview of currently available animal models of HFpEF. Of note, the closest that most of these models get to clinical HFpEF is diastolic dysfunction in the presence of one or multiple comorbidities. Still, the pathophysiological processes that accompany clinical HFpEF and its comorbidities are recapitulated in these animal models, rendering them suitable for proof-of-concept studies that aim to tackle these processes to reverse cardiac (diastolic) dysfunction. In the sections below, we will describe the most common clinical phenotypes (metabolic syndrome, AF, COPD, renal insufficiency, and pulmonary hypertension) and their link to individual biological processes. We will end with a discussion on the extent to which an experimental model should recapitulate clinical phenotypes and how to deal with the complexity of such models.

**Metabolic Syndrome**

Most rat and mouse models of diastolic dysfunction show cardiac manifestations of the pathophysiological processes underlying HFpEF. In line with the overrepresentation of patients with metabolic syndrome in HFpEF cohorts, many of the present animal models of HFpEF are based on the induction of metabolic phenotypes including obesity, insulin resistance, or diabetes, whether or not in combination with pressure overload (Table 1). These models all present with diastolic dysfunction, and most of them also show cardiac endothelial dysfunction, cardiac inflammation by immune cell infiltration, fibrosis, and cardiomyocyte stiffness (Table 1) and therefore support the HFpEF hypothesis in which these four pathphysiological processes form the stepping stone toward HF. One of the most complete HFpEF models in that regard, matching the human garden-variety phenotype of HFpEF, is the obese ZSF1 rat model, which recapitulates all pathophysiological hallmarks of human HFpEF and additionally develops clinical features involving renal insufficiency and overt HF with pulmonary congestion, dying preliminarily of kidney failure. In addition, our recently developed mouse model, which combines high-fat diet (HFD) and angiotensin II infusion, largely recapitulates the pathophysiological events leading to diastolic dysfunction and HFpEF, including not only inflammation but also fibrosis and hypertrophy (M. Rech, K. Wouters, R. P. M. Moonen, S. Hermans-Beijnsberger, E. Wijnands, S. Vinckier, R. Van Leeuwen, W. Verhesen, C. Munts, J. Lecomte, E. A. Biessen, B. F. Coolen, G. J. Strijkers, M. E. Kooi, J. Van den Bossche, M. P. J. de Winther, S. Heymans, M. van Bilsen, and B. Schroen, unpublished observations).

We should realize that the end stage of clinical HFpEF, with all its signs and symptoms, will not always be reached in the available animal models, which is inherent to the physiology of the murine cardiac and circulatory system. Still, the possibility in murine models of specifically targeting candidate genes implicated in the pathophysiological processes underlying HFpEF, by genetic or molecular manipulation, offers a powerful tool to unravel the molecular pathogenesis of HFpEF. Moreover, these models will enable a better understanding of the way and extent to which pathophysiological pathways, alone or in combination, lead to cardiomyocyte stiffness, fibrosis, diastolic dysfunction, and HFpEF. Additionally, such models will be highly suitable for the assessment of novel therapeutic interventions.

Still, the list of models is incomplete and mainly representative of the subgroup of patients with HFpEF with hypertension, obesity, and diabetes. For instance, the factor “aging,” which in humans goes hand in hand with presence of metabolic syndrome and HFpEF, is mostly not included in metabolic...
models of HFpEF, but this would be interesting. Several studies have found indications for cardiac (diastolic) dysfunction in physiologically aged mice (18, 21, 119). In addition, spontaneous senescence-prone mouse (SAMP) strains show interesting features of diastolic dysfunction associated with cardiac hypertrophy and fibrosis (90). However, such models hardly represent the aged, HFpEF-prone human population, with its high prevalence of comorbidities.

Whether inflammation and endothelial dysfunction, which in the HFpEF paradigm would precede hypertrophy and fibrosis, are implicated in this phenotype is currently unknown. Exposure of these mice to a metabolic syndrome environment, e.g., by exposing them to low-dose angiotensin II in combination with HFD, could render this a promising HFpEF model.

Atrial Fibrillation

A large proportion of the population of patients with HFpEF have AF, often coinciding with hypertension, diabetes, and obesity (108). Interestingly, HFpEF and AF seem to share both
Table 1. Murine models suitable for the examination of pathophysiological processes underlying HFpEF

| Model | Pathophysiological Process | Inflammation | Endothelial dysfunction | Fibrosis | Cardiomyocyte Remodeling* | (Pre) Diabetes Mellitus | Obesity | Hypertension | Atrial Fibrillation | Renal Insufficiency | Diastolic Dysfunction | Left Ventricular Hypertrophy | Left Atrial Enlargement | Pulmonary Edema† | Matchmaking with Human HFpEF | Reference(s) |
|-------|-----------------------------|--------------|------------------------|---------|--------------------------|------------------------|---------|-------------|------------------|------------------|----------------------|-----------------------------|--------------------------|--------------|----------------|----------------|------------------|
| Obese Zucker spontaneous fatty rat | Yes | Yes | Yes (mild) | Yes | Yes | Yes | Yes | No | No | Yes | Yes | No | Yes | Yes | No | No | Yes | Yes | Yes | Yes | Metabolic syndrome | 35 |
| ob/db + ANG II mouse | No | NA | Yes | Yes | Yes | Yes | Yes | No | No | Yes | Yes | No | Yes | Yes | No | No | Yes | Yes | Yes | Yes | Metabolic syndrome | Unpublished observations |
| HFD + ANG II mouse | Yes | NA | Yes | Yes | Yes | Yes | Yes | No | No | Yes | Yes | Yes | Yes | Yes | No | No | Yes | Yes | Yes | Yes | Metabolic syndrome | 65 |
| Female Dahl/salt-sensitive/obese rats | Yes | NA | Yes | Yes | Yes | Yes | No | No | No | Yes | Yes | No | No | No | No | No | Yes | Yes | Yes | Yes | Metabolic syndrome |
| Metabolic models | | | | | | | | | | | | | | | | | | | | |
| HFD mouse/rat | No | Yes | No | No | Yes | Yes | No | No | No | Yes | No | No | No | No | Yes | Yes | No | No | Yes | Obesity and diabetes | 122 |
| db/db Mouse | NA | Yes (males) | Yes | Yes | Yes | No | Yes | No | No | Yes | No | No | Yes | No | No | Yes | Yes | No | Yes | Obesity and diabetes | 3 |
| ob/ob Mouse | Yes | Yes | Yes | No | Yes | Yes | No | No | No | Yes | No | No | Yes | No | No | Yes | Yes | No | Yes | Obesity and diabetes | 53 |
| Zucker diabetic fatty rat | Yes | Yes | Yes | No | Yes | Yes | No | No | No | Yes | No | No | Yes | No | No | Yes | Yes | No | Yes | Diabetes type 1 | 127 |
| Pressure overload models | | | | | | | | | | | | | | | | | | | | |
| ANG II mouse | Yes | Yes | Yes | Yes | No | No | Yes | No | No | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No | Yes | Yes | Hypertension | 5, 13, 66, 91, 128 |
| TAC mouse/rat | Yes | Yes | Yes | Yes | No | No | No | No | No | Yes | Yes | Yes | Yes | Yes | Yes | No | Yes | Yes | Hypertension | 57 |
| Aldosterone mouse/rat | Yes | Yes | Yes | Yes | No | No | Yes | No | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No | Yes | Yes | Hypertension | 45 |
| Dahl salt-sensitive rat | Yes | Yes | Yes | Yes | No | No | Yes | No | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No | Yes | Yes | Hypertension | 22 |
| DOCA-salt rat | Yes | Yes | Yes | Yes | No | No | Yes | No | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No | Yes | Yes | Hypertension | 4, 41 |
| TAC + DOCA mouse | Yes | NA | Yes | Yes | No | No | Yes | No | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No | Yes | Yes | Hypertension | 62, 64 |
| Models of additional phenotypes | | | | | | | | | | | | | | | | | | | | |
| Cigarette smoke-exposed mouse | Yes | Yes | Yes | Yes | No | No | No | No | No | Yes | No | No | Yes | No | No | Yes | No | No | No | Chronic obstructive pulmonary disease | 112, 133 |
| Subtotal nephrectomized rat | Yes | NA | Yes | Yes | No | No | Yes | No | No | Yes | Yes | No | No | Yes | No | No | Yes | No | No | Renal insufficiency | 56, 85 |
| Monocrotaline rat | Yes | Yes | Yes | Yes | No | No | Yes | No | No | Yes | Yes | No | No | Yes | No | No | Yes | No | Yes | Pulmonary arterial hypertension | 26, 37, 46 |
| SuxHA rat | No | Yes | Yes | Yes | No | No | Yes | No | Yes | No | Yes | Yes | No | No | Yes | NA | Yes | No | PAH | 29, 71, 114 |

Shown is an overview of proposed animal models for heart failure with preserved ejection fraction (HFpEF) for studying the different pathophysiological mechanisms that are hypothesized to underlie HFpEF. HFD, high-fat diet; DOCA, deoxycorticosterone acetate; TAC, transverse aortic constriction; SuHx, Sugen-hypoxia rat with combined VEGF receptor 1 and 2 blocker SU5416; PAH, pulmonary arterial hypertension; NA, not applicable. *Cardiomyocyte hypertrophy/metabolic remodeling/passive stiffness by titin involvement and/or derangements in active relaxation. †Increased lung weight and/or pulmonary congestion.
comorbidities and underlying pathophysiological features, including inflammation, endothelial dysfunction, capillary rarefaction, and fibrosis (125). Unfortunately, the majority of murine AF models are genetic and not the result of exposure to a comorbidity (97), making it difficult to study the cause-effect relationship between comorbidities, pathophysiology, and outcome in these inherently pathway-focused models. Although transverse aortic constriction induces atrial remodeling, this apparently is not sufficient to increase AF susceptibility (20). HFD feeding, on the other hand, enhanced vulnerability to AF in mice (44). In this model, systemic administration of the anti-inflammatory cytokine IL-10 attenuated HFD-induced atrial remodeling and AF. These data point out that screening for AF in our “regular” murine models of HFpEF, and its potential reversibility by genetic or molecular interventions, is advisable. Whether, and how, we need to (further) enhance AF inducibility in the more complex models of HFpEF involving metabolic syndrome comorbidities can be debated.

**Chronic Obstructive Pulmonary Disease**

A specific subgroup of patients with HFpEF is formed by patients with COPD, often coinciding with hypertension. Interestingly, cardiac dysfunction is regarded by the pulmonologist as one of the comorbidities of COPD, rather than the other way around as the cardiologist would see it, showing the difference in perspective between these involved disciplines. Reflective of this difference in perspective is the limited interest in cardiac consequences of COPD in animal models. Elastase treatment and cigarette smoke exposure are being used to induce lung emphysema and COPD, but the number of studies that have addressed the cardiac effects of these interventions is very limited. In a mouse model of chronic cigarette smoke exposure, hypertension, endothelial dysfunction, cardiac hypertrophy, and an impaired LV pressure-volume relationship were observed (112). Inflammation is a primary cause of end organ damage upon COPD and a primary target in intervention studies (28). It will be interesting to examine whether therapeutic inhibition of inflammation upon establishment of cardiac dysfunction in the rather straightforward COPD animal models would reverse not only lung but also cardiac issues.

**Renal Insufficiency**

CKD is highly prevalent among patients with HFpEF (1). In rat models, this condition is often mimicked by subtotal nephrectomy. In a recent study (85), it was shown that nephrectomy in rats resulted in hypertension, cardiac hypertrophy, and fibrosis in combination with diastolic dysfunction and preserved ejection fraction. Diastolic dysfunction was attributed to impaired relaxation due to a slowing down of the Ca²⁺ re-uptake. Interestingly, an inhibitor of the Na⁺/Ca²⁺ exchanger was found to reduce diastolic dysfunction in this setting. Whether CKD promotes the development of HFpEF via the accumulation of uremic toxins, endothelial dysfunction, or other processes remains to be determined (115).

**Pulmonary Hypertension**

There are many mouse and rat models of PAH, but most of them do not lead to right HF (16, 29). The two most commonly used models that do cause cardiac issues are shown in Table 1. The monocrotaline rat model represents a complex multiorgan disease process that culminates in PAH and right HF, in the presence of cardiac capillary rarefaction, inflammation, and cardiomyocyte hypertrophy (26, 37). The Sugen-hypoxia (SuHx) rat model combines chronic hypoxia with VEGF receptor 1 and 2 blockade and involves cardiac endothelial dysfunction, fibrosis, and cardiomyocyte hypertrophy but no signs of immune cell infiltration (29, 114). Both models develop severe diastolic dysfunction (46, 71) and seem suitable for the study of pulmonary mechanisms involved in the development of diastolic dysfunction and right HF. Present clinical approaches to tackle PAH aim at increasing pulmonary vaso-dilation, and emerging therapies target inflammation and intracellular signaling pathways potentially operational in pulmonary arterial endothelial cells, smooth muscle cells, and fibroblasts (16). Mouse and rat models of PAH promote the idea that both the lungs and immune system are potentially important players in pulmonary vascular remodeling, but whether inflammation is a cause or consequence of pulmonary vascular remodeling continues to be debated (29).

**Simple or Complex Animal Models to Study HFpEF?**

Although HFrEF generally starts within the heart, HFpEF is a syndrome that is thought to start in the periphery and culminates at the heart. This asks for different animal models compared with HFrEF. Given the heterogeneity seen in patients with HFpEF, any animal model only represents a certain subgroup, and the “ideal” animal model of HFpEF does not exist. Still, given that comorbidities are central in the HFpEF paradigm, they should be central in animal models of HFpEF as well. Comorbidities of metabolic syndrome are most common in patients with HFpEF, and many animal models that mimic metabolic syndrome exist. Table 1 shows that the ZSF1 rat model is a very inclusive model that combines common comorbidities of metabolic syndrome, including obesity, diabetes, and hypertension, and recapitulates many, if not all, of the features of the clinical metabolic HFpEF phenotype as defined by Shah et al. (108), including systemic inflammation, diastolic dysfunction, and exercise intolerance. This rat model seems highly suitable for the preclinical investigation of novel therapeutic interventions directed toward inflammation, endothelial dysfunction, fibrosis, cardiomyocyte metabolism, and/or titin-associated cardiomyocyte stiffness. Unfortunately, inclusion of the factor of aging is not feasible in this model because the rats die preliminarily of kidney failure.

However, rats are not that suitable for higher-throughput genetic and molecular studies, and mice offer the ease of a quicker, cheaper, and genetically manipulatable model. We and others have recently investigated the potential of combining angiotensin II infusion and HFD in mice and found this combination to induce inflammation, fibrosis, metabolic remodeling, cardiomyocyte hypertrophy, and diastolic dysfunction (M. Rech, K. Wouters, R. P. M. Moonen, S. Hermans-Beijnsberger, E. Wijnands, S. Vinckier, R. Van Leeuwen, W. Verhese, C. Munts, J. Lecomte, E. A. Biessen, B. F. Coolen, G. J. Strijkers, M. E. Kooi, J. Van den Bossche, M. P. J. de Winther, S. Heymans, M. van Bilsen, and B. Schroen, unpublished observations). Although this model is rather complex, it gives the opportunity to study genetic manipulations in a relevant setting of metabolic syndrome and diastolic dysfunc-
tion. Additionally, the factor of aging can be implemented in this mouse model.

As emphasized above, in the HFpEF field there are many unanswered questions that require specific hypotheses. Models that induce HFpEF by the most common comorbidities are well associated with human HFpEF (Table 1). An open question is whether diagnosis of HFpEF, by showing exercise intolerance and/or postmortem pulmonary congestion, in these models is strictly necessary, although highly desirable. When evaluating therapeutic potential of an intervention, surrogate end points, such as the attenuation of specific pathophysiological processes in conjunction with improved cardiac function, should be accepted as preliminary evidence in experimental models. This is a common strategy in clinical trials, where often surrogate end points are accepted as a preliminary proof of effectiveness of therapy. Further evidence has to be generated via the common pipeline by studies in larger-animal models, although, admittedly, these models are still in a developing phase (17).

Another open question is whether simpler models of diastolic dysfunction, such as angiotensin II infusion by itself, are sufficient 1) to enhance understanding of development of HFpEF and 2) to study therapeutic interventions. An intuitive answer is that such simple models will not likely further our understanding of the complexity of HFpEF pathophysiology per se but will help to test specific candidate therapeutic interventions. The angiotensin II model is highly suitable for the investigation of interventions that target inflammation, interstitial fibrosis, and cardiomyocyte hypertrophy. Whether titin-associated stiffness is implicated in this model is yet unknown. The HFD rat model, on the other hand, is highly suitable for the study of interventions aimed at improving microvascular endothelial functions. Recent studies from our group showed that a relatively short period of HFD feeding of rats already compromises cardiac microvascular function, as reflected by a diminished cardiac perfusion reserve capacity as assessed by contrast-enhanced MRI (122).

DESIGN OF FUTURE TRIALS

In cardiovascular research, conventional large-scale randomized controlled trials are still the rule. Although successful in HFrEF, the complexity of HFpEF requires us to leave this beaten path. In an era in which we advocate personalized medicine, we should look for new ways for drug development to evolve so that treatment advances are matched to specific patients. Other fields, such as oncology, have already incorporated innovative trial designs such as the umbrella trial or basket trial design (7). An umbrella trial tests a variety of treatments in parallel. For instance, in one single type of cancer with different underlying genetic mutations, treatment is targeted at the specific genetic mutation: mutation A receives treatment A; mutation B receives treatment B, etc. The downside is the large number of patients needed to enroll enough patients in each treatment arm. However, for a common disease such as HFpEF this should not be a problem. Moreover, an umbrella approach fits the general consensus that a personalized treatment is needed to successfully treat a heterogeneous disease such as HFpEF.

The basket trial design is centered on a target or specific mutation and not on the disease itself. In oncology, this means that patients with different types of cancer but the same genetic mutations are included in the trials; for example, patients with lung, colorectal, ovarian, and breast cancer but with the same genetic alteration receive the same treatment (19, 55). For HFpEF, this method would mean focusing on underlying pathophysiology. A possible treatment target could be endothelial dysfunction, which is highly present not only in HFpEF but also in diabetes and in patients with myocardial infarction and nonobstructive coronary artery disease. All patients with severe endothelial dysfunction would be included, regardless of their underlying disease. However, a major drawback is that this approach does not take into account that different diseases may respond differently despite targeting the same pathway and, moreover, relevant end points could be different for the various diseases. Still, commonly used end points overarching the exemplified diseases are quality of life, exercise capacity, and improvement of systolic and/or diastolic heart function.

A common feature, especially in umbrella trials, is biomarker enrichment, where treatment is only evaluated if a certain biomarker is positive. This could be not only a serum biomarker (e.g., for inflammation) but also a cardiovascular imaging-based marker (e.g., endothelial dysfunction). Enrichment designs increase the likelihood for a positive response to investigational treatments. However, good, relevant preclinical models are required not only to identify relevant (bio)markers but also for the biological knowledge of the treatment mechanisms of action.

These innovative clinical trial designs have the potential to improve drug development so that the right therapies can be delivered to the right patients.

CONCLUSIONS

The lack of treatment in HFpEF relies on multiple factors. First and foremost, the classical one-size-fits-all clinical trial approach performed successfully in HFrEF was proven unsuccessful in a heterogeneous syndrome such as HFpEF. A personalized, phenotype-specific therapy is probably more fruitful. Selecting adequate patients for each intervention requires profound and systematic phenotyping. Including underlying pathophysiological processes in this selection, to distinguish biological phenotypes, will provide essential additional information. Targeting patients on the basis of their biological phenotype is an innovative approach. Animal models are needed to improve understanding of the way and extent to which pathophysiological pathways contribute to HFpEF and for the assessment of novel therapeutic interventions. Matching animal models with human HFpEF phenotypes based on clinical and pathophysiological properties is a crucial step to design targeted therapy and to successfully translate effective animal model-based interventions into novel human clinical trials.

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