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FROM SYSTEMIC INFLAMMATION TO MYOCARDIAL FIBROSIS: THE HFPEF PARADIGM REVISITED.

Walter J. Paulus, M.D., Ph.D., **Michael R. Zile, M.D.**

Amsterdam University Medical Centers, Amsterdam, The Netherlands (WJP) and RHJ Department of Veterans Affairs Medical Center, Medical University of South Carolina, Charleston SC, USA (MRZ)

Abstract

In accordance with the comorbidity-inflammation paradigm, comorbidities and especially metabolic comorbidities are presumed to drive development and severity of heart failure with preserved ejection fraction (HFpEF) through a cascade of events ranging from systemic inflammation to myocardial fibrosis. Recently, novel experimental and clinical evidence emerged, that strengthens the validity of the inflammatory/profibrotic paradigm. This evidence consists among others of:

1. Myocardial infiltration by immunocompetent cells not only because of an obesity-induced metabolic load but also because of an arterial hypertension-induced hemodynamic load. The latter is sensed by components of the extracellular matrix like basal laminin, which also interact with cardiomyocyte titin;
2. Expression in cardiomyocytes of inducible nitric oxide synthase because of circulating proinflammatory cytokines. This results in myocardial accumulation of degraded proteins because of a failing unfolded protein response;
3. Definition by machine learning algorithms of phenogroups of HFpEF patients with a distinct inflammatory/profibrotic signature;
4. Direct coupling in mediation analysis between comorbidities, inflammatory biomarkers and deranged myocardial structure/function with endothelial expression of adhesion molecules already apparent in early preclinical HFpEF (HF stage A, B).

This new evidence paves the road for future HFpEF treatments such as biologicals directed against inflammatory cytokines, stimulation of protein ubiquitylation with phosphodiesterase 1 inhibitors, correction of titin stiffness through natriuretic peptide – particulate guanylyl cyclase – PDE9 signaling and molecular/cellular regulatory mechanisms that control myocardial fibrosis.

Address for correspondence: Walter J. Paulus, M.D., Ph.D., Amsterdam Cardiovascular Sciences, Amsterdam University Medical Centers, O|2 building 10W13, De Boelelaan 1118, 1081 HV Amsterdam, The Netherlands, Tel.: 31 6 273 39 91 0, wj.paulus52@gmail.com, Michael R. Zile, M.D., Charles Ezra Daniel Professor of Medicine, Distinguished University Professor, Medical University of South Carolina and the RHJ, Department of Veterans Affairs Medical Center, Division of Cardiology, Thurmond/Gazes Building, Room 323, 30 Courtenay Drive, Charleston, SC 29425, Tel: (843) 792-6866, zilem@musc.edu.

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Introduction

Heart failure with a preserved ejection fraction (HFpEF) is a growing public health problem with substantial morbidity and mortality which currently affects 9% of people older than 60 years. This implies that more than 6 million patients suffer from HFpEF in the US plus European Union combined[1]. The prevalence of HFpEF relative to heart failure with a reduced ejection fraction (HFrEF) has risen steadily as evident from the Framingham study which observed a reversal of the HFpEF / HFrEF ratio from 41/59 in the decade 1985–1994 to 56/44 in the decade 2005–2014[2]. This evolution parallels concomitant trends in risk factors with a rise in the metabolic syndrome and a fall in obstructive coronary artery disease over the same decades. HFpEF is associated with a 5 year survival of 35% and the quality of life of HFpEF patients is as poor as in HFrEF patients.

Over the last decennium our understanding of HFpEF has evolved from mere left ventricular (LV) diastolic dysfunction to a multiorgan syndrome resulting from comorbidities like obesity, diabetes and arterial hypertension, which predispose to systemic inflammation affecting the myocardium, skeletal muscles, pulmonary vasculature and kidneys[3]. Although in distinct HFpEF subgroups, skeletal muscle fatigue and pulmonary hypertension impact clinical presentation, the vast majority of HFpEF patients suffer from invalidating dyspnea because of a brisk rise in LV filling pressures during exercise[4]. The exercise induced elevation in LV filling pressures mainly results from high diastolic LV myocardial stiffness with additional contributions in some patients of left atrial myopathy and of pericardial constriction because of epicardial fat accumulation. The present review will therefore first focus on pathophysiological mechanisms linking systemic inflammation to myocardial stiffness. It will subsequently address inflammatory/fibrotic signaling in distinct HFpEF phenotypes derived from machine-learning algorithms and finally apply concepts of inflammatory/fibrotic signaling to HFpEF therapy.

From Inflammation to LV Stiffness: Pathophysiological Mechanisms

Pathophysiological mechanisms linking systemic inflammation to myocardial stiffness are shown in Figure 1 and consist of: 1) Metabolic load induced proinflammatory signaling; 2) Hemodynamic load induced proinflammatory and fibrotic signaling; 3) Titin modifications and cardiomyocyte stiffness; 4) Myocardial collagen homeostasis; 5) Crosstalk between hemodynamic load, extracellular matrix and cardiomyocyte titin and 6) Myocardial accumulation of degraded proteins.

Metabolic Load and Proinflammatory Signaling

Over the years, studies assessing biomarkers have analyzed an ever increasing number of circulating biomarkers in patients with HFpEF and HFrEF[5–8] (initially just four[5] and recently up to 248[8]). Initial evidence showed that elevated growth differentiation factor-15 (GDF-15) was associated with the presence and severity of HFpEF[5]. This finding was soon to be followed by similar observations that confirmed not only high levels of GDF-15 but also of soluble interleukin 1 receptor-like 1 (IL1RL1), C-reactive protein (CRP) and interleukin 6 (IL6) to be associated with HFpEF (Figure 2)[6]. The BIOSTAT-CHF program measured plasma levels of 92 biomarkers, analyzed protein-protein interactions, and overrepresentation of biological processes[7]. Integrin Subunit Beta 2, which is involved in adhesion of immune cells, was most strongly associated with HFpEF (Figure 2) whereas cAMP-dependent transcription factor ATF2, which is involved in cell growth, was most strongly associated with HFrEF. The distinct nature of HFpEF and HFrEF was evident from the analysis of protein-protein interactions, which revealed 6 interactions specific for HFpEF and 8 for HFrEF. Finally, pathways relating to inflammation and extracellular matrix organization were overrepresented in HFpEF in contrast to HFrEF, in which pathways relating to cellular proliferation and metabolism were overexpressed.

In accordance to the comorbidity-inflammation paradigm, comorbidities and especially metabolic comorbidities were presumed to drive systemic inflammation in HFpEF[3,9]. This presumption was supported by a study that observed a progressive increase in plasma level of CRP with rising number of comorbidities in individual HFpEF patients (Figure 2)[10]. Among the comorbidities accounted for in this study were obesity (BMI>30 kg/m^2), diabetes mellitus, anemia, and chronic kidney disease. Consecutive steps of the comorbidity-inflammation paradigm were recently evaluated in the PROMIS-HFpEF study[8], which performed a mediation analysis based on associations of comorbidities with cardiac structure/function, of comorbidities with inflammation and of inflammation with cardiac structure/function (Figure 2). The strength of a mediation analysis is that it allows for causal inference within an observational study. Individual inflammatory biomarkers shown to mediate LV dysfunction were TNF receptor 1 (TNFR1), Interleukin1 receptor 1 (IL1R1) and GDF-15.

The importance of systemic inflammation for HFpEF is also evident from longitudinal observations that predicted HFpEF incidence in community based population samples (Figure 2). The earliest observation came from the Health ABC study, which observed an increased hazard ratio for incident HFpEF over a 9.4 year time span per doubling of baseline plasma level of tumor necrosis factor α (TNFα)[11]. In this study baseline TNFα predicted development of HFpEF ($p<0.001$) but not of HFrEF ($p=0.08$). In a similar experimental design, the CARDIA study demonstrated that high plasma levels of endothelial adhesion molecules E-Selectin and intercellular adhesion molecule 1 (ICAM-1) in early adulthood predicted that 15 to 23 years after the biomarker was assessed, patients would develop a depressed LV global longitudinal strain (GLS), a marker of incipient HFpEF-related LV dysfunction[12]. The MESA study looked at another endothelial adhesion molecule, vascular cell adhesion molecule (VCAM) and observed the highest incidence of HFpEF after 14.4 years in the highest quartiles of baseline VCAM[13]. Because of the early age at

which baseline plasma samples were obtained, both studies suggested endothelial adhesion molecules to be relevant for the diagnosis of stage A or stage B HFpEF (i.e. asymptomatic with risk factors (=stage A) or with LV remodeling (=stage B)) in contrast to biomarkers of myocardial stress (natriuretic peptides) or damage (troponin-I), which only become abnormal in stage C HFpEF[14]. A similar predictive value has also been attributed to urinary albumin excretion (UAE), a renal consequence of endothelial activation and dysfunction. In a community-based, middle-aged cohort raised UAE was shown to predict incident clinical HFpEF after 11 years of follow-up[15]. These findings were confirmed in two studies, which respectively associated urinary albumin to creatinine ratio (UACR) with incident HFpEF and an increasing trajectory of high UACR over time with unfavorable hypertrophic LV remodeling[16,17].

Hemodynamic Load, Proinflammatory and Profibrotic Signaling

In addition to metabolic load-induced activation of inflammatory signaling, the increased hemodynamic load resulting from arterial hypertension and aortic valve stenosis also activates proinflammatory and profibrotic signaling. Activation of these hemodynamic load-induced signaling cascades are evidenced by the alterations in myocardial and circulating biomarkers, including proteins, peptides and microRNAs. Within the myocardium, endothelial cells, fibroblasts, endogenous macrophages as well as cardiomyocytes are hemodynamic load sensitive and each may participate in this load-induced inflammatory and fibrosis signaling. Data from clinically relevant animal models of hemodynamic overload have shown that increased load alters the balance of histone acetylation (HDAC) induced control of microRNAs (miR) which in turn control myocardial specific proinflammatory and profibrotic signaling[18]. Murine transverse aortic constriction induced pressure-overload resulted in increased HDAC 1 and 2 with decreased miRs. MicroRNAs remain stable when circulating in plasma and can be assessed as biomarker evidence of epigenetic control of inflammatory and fibrotic signaling. Accordingly, decreased miR 1, 21, 29 and 133 have been identified in HFpEF patients. These hemodynamic load-induced changes are also believed to alter the cellular phenotype of resident cells such as fibroblasts and lead to fibroblast activation. In addition, increased hemodynamic load leads to proinflammatory and profibrotic specific cell recruitment into the myocardium such as activated macrophages and T-cells[19–23]. These cellular and molecular changes are summarized schematically in Figure 3.

Some of the mechanisms displayed in this schematic are supported by clinical and basic studies, others await further study; however, it was our intent in this review to propose a comprehensive overview that not only summarizes past evidence but also guides future investigation. Increased hemodynamic load (Figure 3 Panel B), is sensed by cardiomyocytes, fibroblasts and resident macrophages and leads to alterations in the basal lamina (also termed basement membrane) structures that in part facilitate cardiomyocyte remodeling. Basal lamina changes include changes in laminin isoform to a more compliant form, with increases in perlican, nitogen and collagen IV which may compliment structural remodeling. In addition, cardiomyocytes undergo parallel addition of sarcomeres with increased cross-sectional area; these cellular changes lead to concentric LV hypertrophy.

Increased hemodynamic load also leads to proinflammatory signaling (Panel C) with cytokines or chemokines and inflammatory cell recruitment such as macrophages and T/B cells[19–22]. In relevant animal models and in patients with pressure-overload, myocardially produced cytokines and chemokines enter the circulation. Circulating monocytes, both from the bone marrow and the spleen, migrate to myocardial endothelial cell surfaces, with attachment and extravasation into the interstitial space facilitated by vascular cell adhesion proteins and become activated macrophages. These macrophages both secrete matricellular proteins that facilitate procollagen processing and collagen fiber assembly and may further activate fibroblasts[19–22]. These changes in aggregate alter the interstitial ECM in a profibrotic manner with concomitant increase in myocardial stiffness (Panel D)[24].

Titin Modifications and Cardiomyocyte Stiffness

High LV myocardial diastolic stiffness as characteristically observed in HFpEF, results from both myocardial fibrosis and low cardiomyocyte and myocardial distensibility. These findings have been directly measured in cardiomyocytes retrieved from LV endomyocardial biopsies and LV myocardial muscle samples obtained from LV epicardial biopsies[24,25,26]. Myocardial fibrosis has been detected from the frequent presence of an elevated collagen content in the LV or septal myocardium of HFpEF patients[27,28]. The giant sarcomeric protein titin is responsible for the diastolic distensibility of cardiomyocytes and also plays a role in mechanical stress sensing. Titin spans Z disc, I band, A band and M band sarcomeric regions. Its elasticity resides in the I-band region, which consists of a series of segments (proximal IgG, N2B, PEVK, distal IgG), each of which can contribute to overall elasticity[29,30]. Deletion of the N2B segment increases titin stiffness and induces LV atrophy[31] whereas deletion of the PEVK segment also increases titin stiffness but induces LV hypertrophy[32]. Titin modulates diastolic distensibility of cardiomyocytes through transcriptional and posttranslational modifications. Differential splicing gives rise to two titin isoforms of different size and stiffness: a short, less compliant N2B isoform and a long, more compliant N2BA isoform. RNA binding motif-20 (RBM20) is a major splicing factor of titin and its inhibition upregulates expression of long compliant isoforms capable of correcting diastolic LV compliance in HFpEF mouse models[33,34]. Posttranslational modifications of titin mainly consist of phosphorylation and oxidation. Phosphorylation of the N2B segment by PKA or PKG decreases and phosphorylation of the PEVK segment increases distensibility of titin[35–37]. Finally, reactive oxygen species modulate titin elasticity through formation of disulfide bonds, which interfere with protein folding and lead to titin aggregation in the distal IgG segment[38].

All of the aforementioned mechanisms that modulate titin distensibility are involved in the high myocardual stiffness in HFpEF: 1) the N2BA/N2B titin isoform ratio is lower in HFpEF than in HFrEF[25,39]; 2) the N2B titin isoform is hypophosphorylated in clinical HFpEF[39] and in the ZSF1 or Seipin-KO HFpEF rat models titin is respectively hypophosphorylated in the N2B segment or hyperphosphorylated in the PEVK segment[40,41]; 3) oxidative changes of titin account for the ageing-induced rise in cardiomyocyte stiffness and are paralleled by a protective build-up in the sarcomeres of anti-oxidant HSP27 or αB-crystallin[42,43].

Two novel aspects of titin pathophysiology are especially relevant to HFpEF namely lack of stretch-induced titin phosporylation, which turns the left ventricle into a fixed filling pressure pump[44] and modifications of titin as a result of diabetes mellitus[45]. In response to an acute volume load, the myocardium slowly adjusts its diastolic compliance over a 15 minute period. This gradually lowers LV filling pressures to values well below the initial values after imposition of the volume load. This myocardial property has previously been labelled stress relaxation, myocardial viscosity or creep. The adjustment of diastolic LV compliance results from PKG-induced phosphorylation of titin, disappears following inhibition of NO synthase or PKG and is absent in hypertrophied myocardium[44] (Figure 4).

In HFpEF, myocardial cGMP content and PKG activity are low mainly because of microvascular inflammation and rarefaction[46,47] and the aforementioned adjustment of diastolic LV compliance following an acute volume load is therefore compromised. This results in a sustained elevation of LV filling pressures, which contributes to the poor tolerance of volume expanion in HFpEF. The same mechanism also affects exercise tolerance in HFpEF especially in the presence of chronotropic incompetence, as occurs with betablockade, when an inadequate rise in heart rate leads to LV end-diastolic volume expansion during the initial phase of exercise.

Diabetes mellitus worsens diastolic LV dysfunction in HFrEF, HFpEF and aortic stenosis (AS) through increased fibrosis in HFrEF, higher cardiomyocyte resting tension in HFpEF and both augmented fibrosis and cardiomyocyte resting tension in AS[48,49]. The mechanisms responsible for the higher cardiomyocyte resting tension have recently been unraveled and consist of hypophosphorylation of titin in the I-band N2B segment and hyperphosphorylation in the I-band PEVK segment[50]. Administration of insulin and metformin restored these posttranslational modifications of titin and a similar effect was recently also described for empagliflozin[51].

Myocardial Collagen Homeostasis and Myocardial Stiffness

Both increased metabolic and hemodynamic load lead to abnormalities in the myocardial extracellular matrix (ECM) in HFpEF patients. These changes include altered fibrillar proteins such as increased fibrillar collagen content, a shift toward less compliant collagen types (increased collagen I vs III), increased collagen cross-linking, increase in collagen fiber size, changed basal lamina structures, glycosaminoglycans and proteoglycans (often referred as ground substance). In aggregate, these ECM changes in HFpEF patients result in both myocardial fibrosis and increased myocardial stiffness[24,27]. Myocardial biopsies in clinical studies and in animal model HFpEF studies have shown that a substantial portion of HFpEF patients have an increase in collagen volume fraction by histology and/or MRI using LGE and T1 mapping studies[24,27].

Of the changes described above in the ECM, the most important in the development of HFpEF are changes in collagen. Collagen homeostasis (Figure 5) is dependent on procollagen synthesis by myocardial fibroblasts, post-synthetic procollagen processing in the interstitium facilitated by matricellular proteins (such as SPARC, thrombospondin, periostin, ADAMs, BMP), post translational collagen cross-linking (controlled by LOX enzymes, transglutaminase, AGEs) and collagen degradation (increased by MMPs, decreased by

TIMPs). In clinically relevant animal models, transgenic overexpression, transgenic null expression and conditional constructs have demonstrated the causal relationship between these collagen homeostatic determinants and the development of fibrosis[52–57]. In each of these transgenic constructs and in clinical studies of HFpEF, a clear quantitative relationship between increased fibrillar collagen content and increased myocardial stiffness has been defined. The homeostatic regulatory proteins and peptides described above can be and have been measured in plasma/serum samples of HFpEF patients and shown to be altered[58–62]. In addition, collagen propeptides (reflective of synthesis) and telopeptides (reflective of degradations) have also been measured in HFpEF patients. The circulating levels of these biomarkers reflect the presence and severity of HFpEF, fibrosis and diastolic LV stiffness, provide prognostic information and are targets for therapy (Figure 5).

The hemodynamic load dependent collagen homeostatic mechanisms described above play a role in the regression of myocardial fibrosis that occurs when hemodynamic overload is removed. The best example of regression of myocardial fibrosis occurs when the hemodynamic overload of aortic valve stenosis is removed by aortic valve replacement (AVR)[63]. Krayenbuehl and Hess used serial LV endocardial biopsies following AVR to document significant, albeit incomplete, regression of fibrosis that occurred over a 3–5 year time period[64]. The causal mechanisms, changes in collagen homeostasis, and changes in fibroblast and macrophage dependent inflammatory and fibrotic signaling that occur after removal of hemodynamic and / or metabolic load and that lead to regression of fibrosis remain however incompletely defined.

Crosstalk between Hemodynamic Load, ECM Laminin and Cardiomyocyte Titin

Hitherto, HFpEF related changes in cardiomyocyte titin were assessed independently from alterations in ECM components such as collagen and basal lamina. Recent evidence however suggests that hemodynamic load-induced changes in ECM laminin lead to changes in titin that affect cardiomyocyte and myocardial stiffness. In in-vitro and ex-vivo studies, treatment with polylaminin, a biomimetic polymer of the adult form of laminin, resulted in an increase in the compliant titin N2BA isoform expression and a decrease in the stiff titin N2B isoform[65]. In addition, polylaminin was shown to increase fibroblast secretion of MMP-2 and 9. This suggests a change in fibroblast phenotype to a less profibrotic de-activated state. Finally, polylaminin treatment was also associated with a less proinflammatory macrophage phenotype. These observations provide initial evidence on interdependence of load, laminin and titin determining overall diastolic myocardial stiffness in HFpEF.

Myocardial Accumulation of Degraded Proteins

Up till now high diastolic LV stiffness in HFpEF was presumed to result primarily from myocardial fibrosis and modifications of titin. Recently however, Schiattarella et al.[66,67] unveiled a third mechanism responsible for high LV diastolic stiffness in HFpEF namely a deficient unfolded protein response (UPR) in cardiomyocytes. The UPR consists of three parallel enzyme systems in the endoplasmic reticulum that either correct misfolded proteins or transfer them for degradation to the ubiquitin/proteasome system or to lysosomes. Deficiency of the UPR in HFpEF is caused by systemic inflammation triggering expression of inducible nitric oxide synthase (iNOS) in cardiomyocytes (Figure 1). Induction of iNOS

leads to reduced activity of inositol-requiring enzyme1α (IRE1α) which performs the initial step in one of the three UPR enzyme systems namely splicing of the messenger RNA of X-box binding protein1 (XBP1). Lower IRE1α activity in HFpEF cardiomyocytes modifies expression of XBP1s, which impairs the UPR and leads to cytoplasmic accumulation of degraded proteins. It remains unclear if sarcomeric proteins like troponin or titin are affected but some clinical observations support their involvement. Patients with HFpEF frequently have elevated plasma level of troponin[68], which is more likely to result from cytoplasmic accumulation of destabilized troponin than from cardiomyocyte cell death because the latter was never observed in biopsy or autopsy LV specimens of HFpEF patients[25,28]. Elevated plasma levels of cleaved fragments of titin have recently also been observed and similarly to troponin related to worse HFpEF[69]. The accumulation of degraded proteins in cardiomyocytes could lead to accumulation in the myocardial interstitium as occurs in amyloidosis. In the latter condition however, the flux of proteins is reversed as interstitial accumulation results from high plasma levels of proteins such as immunoglobulin light chain, transthyretin or serum amyloid A. Apart from a deficient UPR, protein degradation by the ubiquitin/proteasome system also appears to be impaired in HFpEF myocardium. Overexpression of the ubiquitin ligase E3 WWP1 in mice, as occurs at higher age in humans, resulted in a two fold increase in myocardial collagen volume fraction and diastolic LV dysfunction[70]. Finally, protein ubiquitylation is facilitated by PKA or PKG and administration of a PDE1 inhibitor attenuated HFpEF in a mouse model of proteotoxic stress induced by mutated αB-Crystallin[71].

Inflammatory/Fibrotic Signaling in HFpEF Phenogroups

Several groups of investigators applied a machine-learning based clustering strategy to identify distinct phenogroups within HFpEF populations derived either from local registries or from large trial populations[72–76]. Table 1 summarizes their findings. Despite large scatter in number of clinical and biomarker input variables, the 5 studies shared similar findings: 1) they all identified distinct phenogroups based on presence of metabolic comorbidities such as obesity (n=4), diabetes (n=5) and renal insufficiency (n=5); 2) phenogroups with CKD (n=4), high natriuretic peptides (NP) (n=4) or prominent diastolic LV dysfunction (n=3) had the poorest outcome. Studies differed widely in the incorporation of biomarker data. All studies used natriuretic peptides as input variable but only one study also included inflammatory biomarkers as input variables[76]. After establishing the distinct phenogroups, two studies analysed the distribution of biomarkers over the different phenogroups[74,75].

When inflammatory biomarkers were used as input variable[76], three distinct phenogroups appeared: 1) a phenogroup with a paninflammatory profile; 2) a phenogroup with selective elevation of CRP and Serum Amyloid A (SAA) and 3) a phenogroup with no evidence of inflammatory biomarkers. The phenogroup with the paninflammatory profile had the worst outcome and was the only one with elevated myocardial fibrosis biomarkers. The two studies[74–75], which did not incorporate biomarkers as input variables but ‘a posteriori’ assessed their distribution in the phenogroups, confirmed these findings as they observed respectively high IL1RL1, also called solubleST2 (sST2), an inflammation/fibrosis marker[74] and high TNFα[75] in the phenogroup with the worst outcome. Finally, broad

transcriptomic profiling of endomyocardial biopsies procured from HFpEF patients revealed three subgroups, one of which featured prominent proinflammatory signaling[27].

Inflammatory/Fibrotic Signaling and HFpEF Therapy

Inflammatory/Fibrotic Biomarkers and Therapeutic Guidance

There is growing evidence that metabolic and hemodynamic load induced changes in inflammation and fibrosis that are reflected in circulating biomarkers provide significant improvement in diagnostic criteria for HFpEF, provide insights into the prognosis associated with the development of HFpEF and provide actionable targets for HFpEF therapy. Changes in natriuretic peptides (NPs), a direct reflection of hemodynamic load, have become pivotal to the accuracy of the diagnosis of HFpEF[77,78]. A circulating multibiomarker panel reflecting increased collagen synthesis and decreased degradation predicted the presence of HFpEF with an area under the curve of 0.79, performed better than any single biomarker including NT-proBNP and better than using clinical covariates alone[61]. In addition, baseline biomarkers and change from baseline levels provide prognostic value in HFpEF patients predicting both morbidity and mortality. The most studied of these is NPs[77,78]. In a similar manner, high sensitivity troponins, a biomarker reflecting load-dependent cardiomyocyte injury also predicts morbidity and mortality in HFpEF patients[68]. In two recent randomized HFpEF trials, PARAGON-HF and TOPCAT, biomarkers that reflect collagen homeostasis were altered[58,59]. These trials specifically observed: 1) increased plasma levels of aldosterone, galectin-3, and sST2; 2) increased plasma levels of biomarkers reflecting collagen synthesis/processing, such as PINP, PICP and PIIINP; 3) altered plasma levels of biomarkers that reflect collagen degradation such as lower MMPs, higher TIMPs and higher CITP. In addition, both baseline and change from baseline levels of these biomarkers provided prognostic value (Figure 6). For example, the higher the baseline value of TIMP-1, the higher the rate of HF hospitalization and CV mortality and if TIMP-1 increased during the trial, morbidity and mortality also increased. Higher TIMP-1 indeed reflects less collagen degradation therefore more collagen accumulation and increased myocardial fibrosis.

Profibrotic Signaling as Therapeutic Target

Each of the profibrotic mechanisms discussed above represents a target for development of potential effective therapies for patients with HFpEF. The results from the TOPCAT Americas data support the efficacy of mineralocorticoid receptor antagonists (Spironolactone) that reduce aldosterone induced profibrotic signaling and decrease mortality and morbidity in HFpEF patients. Particularly in HFpEF patients with diabetes, spironolactone decreased collagen propeptides (decreased synthesis), increased collagen telopeptides (increased degradation) and altered upstream signaling that controls these processes[58]. The non-steroidal mineralocorticoid receptor antagonist finerenone improved cardiovascular outcomes in diabetic kidney disease (FIDELIO-DKD[79]) and is currently also being tested in HFpEF patients (FINEARTS-HF). The results from PARAGON-HF were similar to TOPCAT and support the efficacy of sacubitril/valsartan in reducing profibrotic signaling. Sacubitril/valsartan decreased collagen propetides (decreased

synthesis), increased collagen telopeptides (increased degradation) and altered upstream signaling that controls these processes[59].

Inflammatory Signaling as Therapeutic Target

Therapies that target proinflammatory signaling have been shown to hold promise. In HFrEF, therapy with a decoy TNFα receptor (etanercept) or a TNFα antibody (infliximab) has been disappointing with lack of benefit and higher risk of HF hospitalization[80,81] probably because of loss of myocardial signaling of a key TNFα effector namely NFκB, which apart from deleterious effects also prevents mitophagy and cell death[82,83]. Prevention of cell death is relevant for the myocardium in HFrEF but not in HFpEF, which characteristically features myocardial hypertrophy[25,27]. Anti-TNFα therapy should therefore be reappraised in HFpEF. Support for a reappraisal is provided by use of anti-TNFα in chronic inflammatory joint diseases. Systemic sclerosis, systemic lupus erythematosus and rheumatoid arthritis have all been associated with an increased risk for heart failure[84]. In a Swedish registry on HF in rheumatoid arthritis, patients treated with corticosteroids had a higher incidence of non-ischemic heart failure but patients treated with biologicals showed a reverse trend namely a lower hazard ratio for non-ischemic heart failure[85]. Likewise, in rheumatoid arthritis patients with normal natriuretic peptides and LV diastolic function[86], anti-TNFα therapy lowered natriuretic peptides (p=0.10) without effect on LV ejection fraction or LV global longitudinal strain (GLS).

In contrast to anti-TNFα therapy, IL-1β blockade with anakinra has already been tested in HFpEF patients. The DHART pilot trial (Diastolic Heart Failure Anakinra Response Trial) found anakinra to increase peak VO2 at 2 weeks[87]. The subsequent DHART2 trial however reported no significant change in either peak VO2 or the VE/VCO2 slope after 12 weeks but observed a significant fall in CRP and NT-proBNP after 4 weeks[88]. The most promising result on IL-1 blockade was observed in the CANTOS trial which observed less HF hospitalization when the IL-1 antibody canakinumab succeeded to lower hsCRP below 2mg/L[89]. Although the CANTOS trial did not discriminate between HFrEF and HFpEF, many patients likely suffered from HFpEF as they were old with a high prevalence of obesity, diabetes and hypertension. In line with IL-1 blockade, IL-6 blockade (e.g. with the monoclonal antibody tocilizumab) also deserves to be addressed in HFpEF. Apart from being an intermediate step between IL1 and hepatic CRP production, IL-6 activates the epithelial sodium channel and impairs natriuresis through reabsorption of sodium in the distal renal tubule. This leads to volume expansion, renal impairment and diuretic resistance[90]. Of relevance to use of IL-6 antagonism in HFpEF are the restored cardiomyocyte titin phosphorylation in experimental myocarditis[91], the reduced myocardial fibrosis in rat pressure overload hypertrophy[92] and the regression of LV hypertrophy in rheumatoid arthritis patients[93].

The SATELLITE trial currently investigates efficacy of an orally available myeloperoxidase inhibitor (AZD4831) in HFpEF. Myeloperoxidase is abundantly present in neutrophile granulocytes and its release in the extracellular space can be triggered by presence of urate crystals. HFpEF patients frequently suffer of hyperuricemia, which can lead to deposition of urate crystals in the vasculature. The AMETHYST trial therefore investigates in HFpEF

the use of Verinurad, a novel uric acid transporter 1 inhibitor. Both trials target the coronary microvasculature, which is known to be inflamed in HFpEF as evident from endothelial expression of adhesion molecules[47] and to suffer from a blunted hyperemic response as convincingly demonstrated in the PROMIS-HFpEF study[94].

Two landmark studies demonstrated SGLT2 inhibitors to favorably modify need for HF hospitalization in type 2 diabetes and a composite endpoint of cardiovascular death and worsening HF[95,96]. Their use in HFpEF patients is currently being assessed in the EMPEROR-PRESERVED[97] and DELIVER trials. Two retrospective analyses not formally identifying HFpEF patients and only distinguishing HFrEF from nonHFrEF patients suggested canagliflozin and dapagliflozin to improve respectively new HF events and HF hospitalizations in HFpEF[98,99]. Two recent trials using sotagliflozin (SOLOIST-WHF, SCORED) identified HFpEF patients at baseline and observed a reduction of HF hospitalizations in the HFpEF subgroups[100,101]. Multiple reasons have been proposed for the beneficial effects of SGLT2 inhibitors in HF ranging from diuresis induced LV pre- and afterload reduction[102], decreased intracellular sodium[103], improved phosphorylation of titin[104] and reduction of LV mass[105,106]. Lately, anti-inflammatory effects were also reported. In a co-culture experiment of human coronary endothelial microvascular cells and adult rat cardiomyocytes, addition of TNFα impaired cardiomyocyte shortening and relaxation kinetics, both of which were restored by subsequent addition of empagliflozin[107]. In this experimental set-up, the effect of empagliflozin related to alleviation of TNFα-induced oxidative stress which resulted in restored NO signaling from endothelial cells to cardiomyocytes. An anti-inflammatory effect of canagliflozin was also observed in lipopolysaccharide-stimulated human coronary artery endothelial cells and involved reduced upregulation of the glycolytic enzyme hexokinase[108]. Finally, in the DAPA-LVH trial, dapagliflozin reduced not only LV mass but also hsCRP[106].

Statins have anti-inflammatory properties and lower CRP by 15 to 30%. These effects are largely independent of their cholesterol lowering action. In HFrEF, large outcome trials failed to observe a beneficial effect of statins on HF outcomes. In contrast, in HFpEF a series of small phase II studies or registry analyses observed positive outcomes[109–111]. Of specific interest is the reduced incidence of atrial fibrillation in HFpEF patients treated with statins[112]. Myocardial involvement in the positive outcome of statins was evident from endomyocardial biopsies, which revealed statin-treated HFpEF patients to have higher myocardial PKG activity, less cardiomyocyte hypertrophy and lower cardiomyocyte resting tension[9].

Recognizing that myocardial cGMP levels are reduced in HFpEF, drugs targeting mechanisms that increase cGMP have been evaluated. These include agents that act through NO-soluble guanylyl cyclase (sGC)-phosphodiesterase-5 (PDE5) signaling and agents that act through natriuretic peptides (NP)-particulate guanylyl cyclase (pGC)-PDE9 signaling. Trials trying to manipulate cGMP through delivery of NO (INDIE-HFpEF[113], NEAT-HFpEF[114]), stimulation of sGC (SOCRATES-Preserved[115], VITALITY-HFpEF[116], CAPACITY-HFpEF[117]) or inhibition of PDE5 (RELAX[118]) showed no benefit both overall or in specific subgroups. In contrast, sacubitril, which augments cGMP through NP signaling reached the primary outcome in women and in patients with LVEF<57%[119].

This disparity could result from compartimentalization in cardiomyocytes of both signaling routes and from counterregulatory mechanisms present in the NO-sGC-PDE5 route and absent in the NP-pGC-PDE9 route (Figure 7). Compartimentalization and counterregulatory mechanisms could explain why manipulation of the NO-sGC-PDE5 route was less successful to raise plasma or urinary cGMP in contrast to use of the NP-pGC-PDE9 route. In the INDIE-HFpEF trial, trough level of plasma cGMP was comparable in the inorganic nitrate and placebo groups and in SOCRATES-Preserved no trend in plasma cGMP was observed. In the RELAX trial a paired comparison of plasma cGMP in control and sildenafil groups showed higher plasma cGMP in the sildenafil group, which however failed to reach statistical significance (p=0.11). In contrast, in the PARAGON trial the sacubitril group had a significant increase over a 1 year period in urinary cGMP/creatinine ratio[120] and in an ovine model of heart failure PDE9 inhibition significantly raised plasma cGMP/NP ratio[121]. The counterregulatory feedback in the NO-sGC-PDE5 signaling route consists among other mechanisms, of a cGMP-binding allosteric regulatory region in PDE5 capable of raising PDE5 activity at higher cytosolic cGMP[122]. A similar site is absent on PDE9[123] and use of the NP-pGC-PDE9 route can therefore provide a sustained elevation of cGMP. Using biosensors, the elevation of cGMP was recently visualized in cardiomyocytes following NP dependent activation of pGC[124]. Elevated cGMP was specifically localized around the sarcoplasmic reticulum, myofilaments and outer mitochondrial membrane in contrast to stimulation of the NO-sGC-PDE5 signaling route, which elevates cGMP around the Z-disc. Especially the myofilamentary localization is of interest as it could relate to phosphorylation of the N2Bus segment of titin, which is known to occur following NP administration and to improve cardiomyocyte distensibility[125]. Furthermore, in an epidemiological study of a population free of cardiovascular disease or heart failure, plasma cGMP closely tracked NP signaling[126]. Finally, it is fair to conclude that the use of cGMP enhancing drugs in HFpEF has so far been disappointing. The outcome of studies using PDE9 inhibitors in HFpEF patients and sacubitril in hospitalized HFpEF patients (PARAGLIDE-HF) are therefore eagerly awaited to evaluate the importance of cGMP signaling for the inflammatory/profibrotic paradigm.

Conclusions and Future Directions

Over the past 10 years, novel experimental and clinical evidence emerged, that supports the inflammatory paradigm for HFpEF. This evidence consists of: 1) Hemodynamic overload-induced myocardial infiltration with immunocompetent cells triggered by components of the extracellular matrix like basal laminin; 2) Crosstalk between components of the extracellular matrix and cardiomyocyte titin resulting in altered titin isoform splicing; 3) Myocardial accumulation of degraded proteins because of failing UPR or ubiquitin/proteasome system; 4) Definition by machine learning algorithms of phenogroups of HFpEF patients with a distinct inflammatory/fibrotic signature; 5) Direct coupling in mediation analysis between comorbidities, inflammatory biomarkers and myocardial structure/function; 6) Endothelial expression of adhesion molecules in early HFpEF (stage A or B). In future clinical studies, the existing knowledge on the inflammatory/profibrotic paradigm in HFpEF needs to integrate new evidence from preclinical studies on metabolic substrate utilization, lipotoxicity and proteotoxicity[127]. Some specific topics which could be valuable subjects

of future research efforts are listed in Table 2. Finally, insights gained from current evidence and future research efforts will pave the road for novel treatments of HFpEF such as antagonizing proinflammatory cytokines, promoting protein ubiquitylation with PDE1 inhibitors or correcting titin hypophosphorylation through stimulation of NP-pGC-PDE9 signaling.

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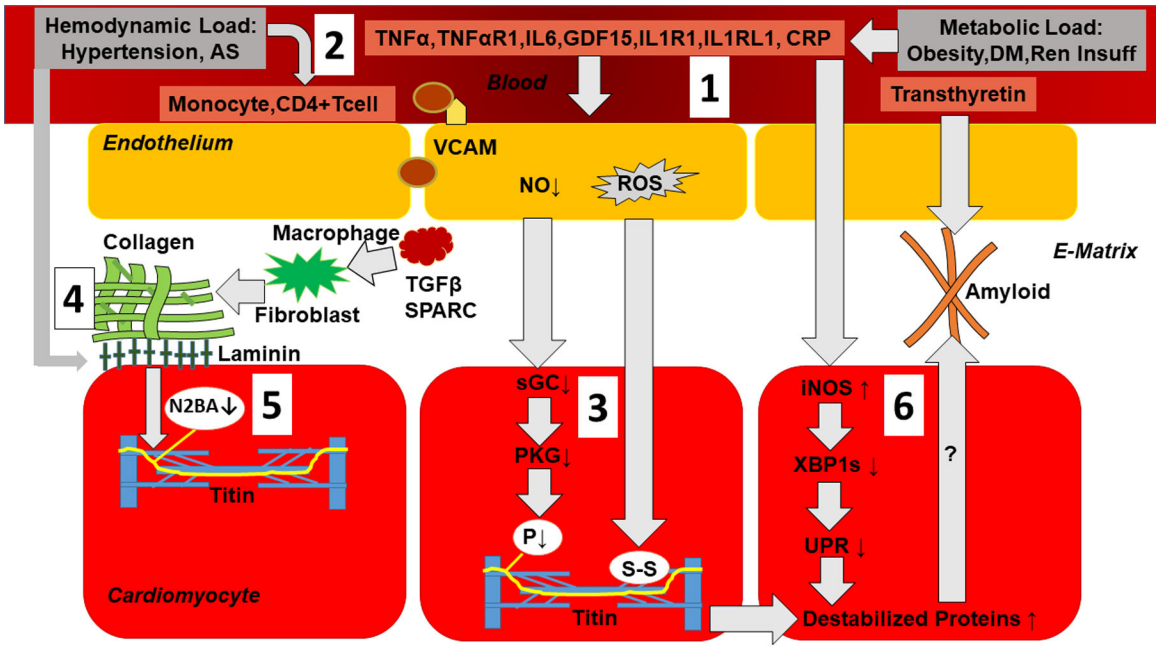

Figure 1. Pathophysiological mechanisms linking systemic inflammation to myocardial stiffness.

1) Metabolic load induces proinflammatory signaling: Metabolic load related to obesity, diabetes mellitus (DM) and renal insufficiency (Ren Insuff) triggers systemic inflammation evident from raised plasma levels of TNFα (Tumor Necrosis Factor α), TNFαR1 (Tumor Necrosis Factor α Receptor 1), IL6 (Interleukin 6), GDF15 (Growth Differentiation Factor 15), IL1R1 (Interleukin 1 Receptor 1), IL1RL1 (Interleukin 1 Receptor Like 1) and CRP (C-Reactive Protein). Systemic inflammation triggers endothelial expression of adhesion molecules (VCAM: Vascular Cell Adhesion Molecule), which attracts monocytes, lowers endothelial production of nitric oxide (NO) and raises endothelial production of Reactive Oxygen Species (ROS); **2)** Hemodynamic load as occurs in arterial hypertension and aortic stenosis induces proinflammatory and fibrotic signaling evident from myocardial infiltration of monocytes and CD4+ T cells; **3)** Low NO reduces activity of soluble Guanylyl Cyclase (sGC) and Protein Kinase G (PKG). This leads to hypophosphorylation of titin (P↓). ROS cause formation of disulfide bonds within titin. Both these titin modifications raise cardiomyocyte stiffness; **4)** Myocardial collagen homeostasis: Infiltrating monocytes become macrophages with production of Transforming Growth Factor β (TGFβ) and secreted protein acidic and rich in cysteine (SPARC) which stimulate collagen production by fibroblasts; **5)** Crosstalk between hemodynamic load, extracellular matrix basal laminin and cardiomyocyte titin results in changed titin isoform expression with less N2BA isoform (N2BA↓); **6)** Myocardial accumulation of degraded proteins: Expression in cardiomyocytes of inducible Nitric Oxide Synthase (iNOS) lowers inositol-requiring enzyme1α (IRE1α), spliced X-box binding protein1 (XBP1s) and the Unfolded Protein Response (UPR). The latter leads to build-up of destabilized proteins which could potentially also accumulate in the extracellular matrix as occurs in transthyretin amyloidosis. (Illustration credit: Ben Smith)

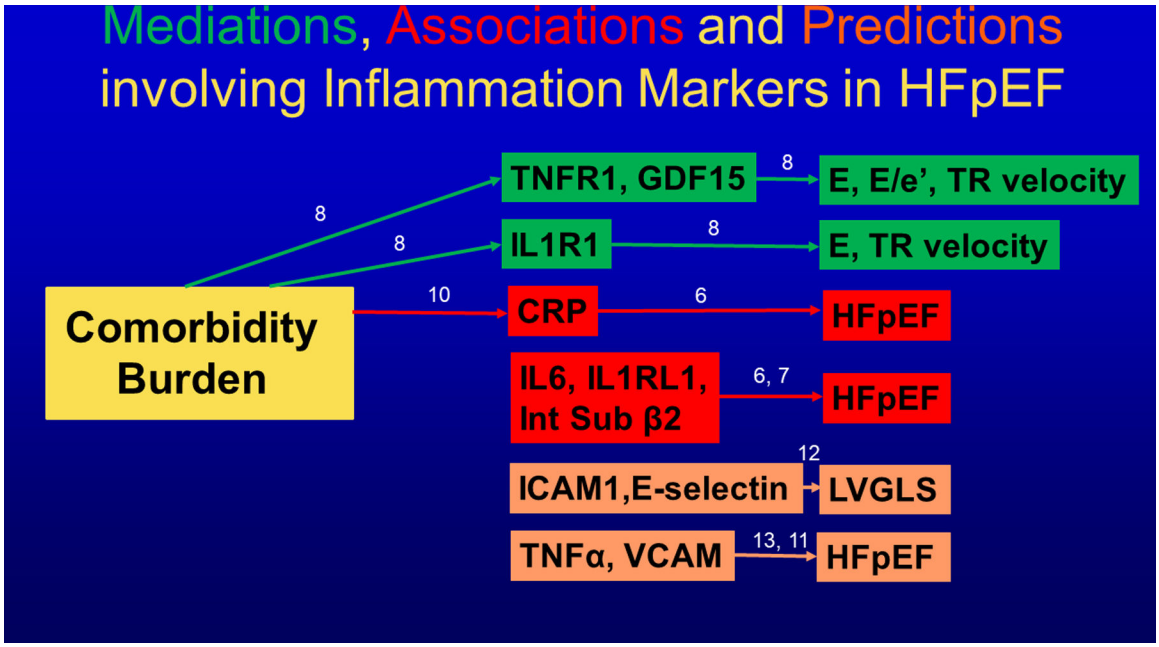

Figure 2. Mediations, associations and predictions involving inflammation markers in HFpEF.

Mediations (Green) between comorbidity burden, inflammatory biomarkers and echocardiographic cardiac function (TNFR1: Tumor Necrosis Factor Receptor 1; GDF15: Growth Differentiation Factor 15; IL1R1: Interleukin 1 Receptor 1; E: Early diastolic mitral flow velocity; E/e': ratio of Early diastolic mitral flow velocity over Early diastolic long axis lengthening velocity; TR: Tricuspid Regurgitation velocity). Associations (Red) between inflammatory biomarkers and presence of HFpEF (CRP: C-Reactive Protein; IL6: Interleukin 6; IL1RL1: Interleukin 1 Receptor Like 1; Int Sub β 2: Integrin Subunit Beta 2). Biomarkers predicting (Orange) myocardial function or HFpEF (ICAM1: Intercellular Adhesion Molecule 1; TNFα: Tumor Necrosis Factor α; VCAM: Vascular Cell Adhesion Molecule; LVGLS: Left Ventricular Global Longitudinal Strain). Numbers indicate corresponding reference.

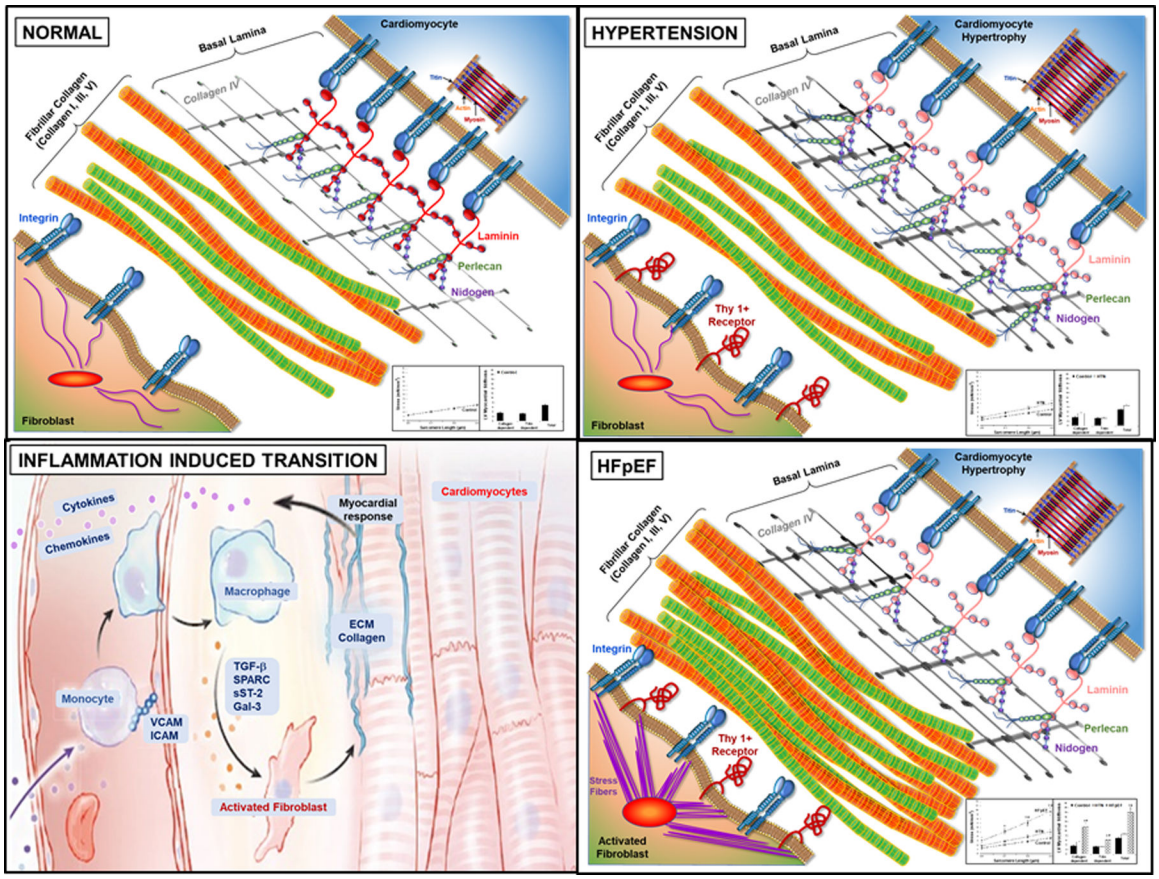

Figure 3. Hemodynamic load-induced myocardial inflammatory/fibrotic signaling.

Sequential changes in myocardial cardiomyocytes, fibroblasts, fibrillar collagen, and basal lamina structures result from the imposition of increased hemodynamic load that lead to the development of HFpEF. (Illustration credit: Ben Smith).

Compared to Panel A, showing normal myocardium, Panel B depicts changes that result from increased hemodynamic load, such as that which occurs in systemic arterial hypertension. The change in load is sensed by cardiomyocytes, fibroblasts and resident macrophages and leads to alterations in the basal lamina structures. Basal lamina changes include changes in laminin isoform to a more compliant form, with increases in perlican, nitogen and collagen IV which may compliment and compensate for these changes in laminin. Cardiomyocytes undergo parallel addition of sarcomeres and increased cross-sectional area; these cellular changes lead to concentric LV hypertrophy. However, these changes in myocardial structure does not result in increased myocardial diastolic stiffness (inset). Panel C depicts the inflammation induced transition from hypertensive heart disease to HFpEF. This transition is led by proinflammatory signaling of increased cytokines and chemokines causing cell recruitment to the myocardium of macrophages and T/B cells. These cytokines and chemokines are secreted by the myocardium and enter the circulation. Circulating monocytes, both from the bone marrow and the spleen, migrate to myocardial endothelial cell surfaces, with attachment and extravasation into the interstitial space facilitated by vascular cell adhesion proteins and become activated macrophages. These macrophages both secrete matricellular proteins that facilitate procollagen processing and

collagen fiber assembly and may further lead to fibroblasts activation. Panel D shows the results of this transition in HFpEF and the aggregate profibrotic changes in interstitial ECM and their resultant increase in myocardial diastolic stiffness (inset).

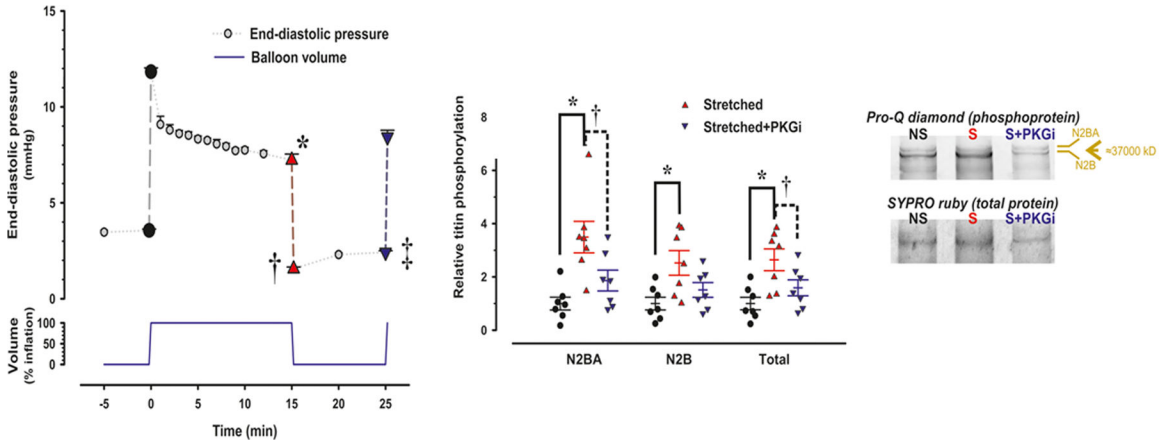

Figure 4. Stretch-induced titin phosporylation.

In a Langendorff preparation an acute volume load causes a brisk rise in LV end-diastolic pressure (LVEDP), which gradually falls over a 15 minute period. Subsequent removal of the volume load causes LVEDP to drop below baseline without full recovery over the next 10 minutes period (Left hand panel; *,†,‡: $p<0.001$). In stretched muscle strips, titin phosphorylation (total and isoform specific) was signficantly higher than in non-stretched muscle strips (Middle panel) as also evident from Pro-Q diamond staining (Right hand panel). Stretch induced effects disappeared following inhibition of PKG (PKGi) and were absent in hypertrophied myocardium.

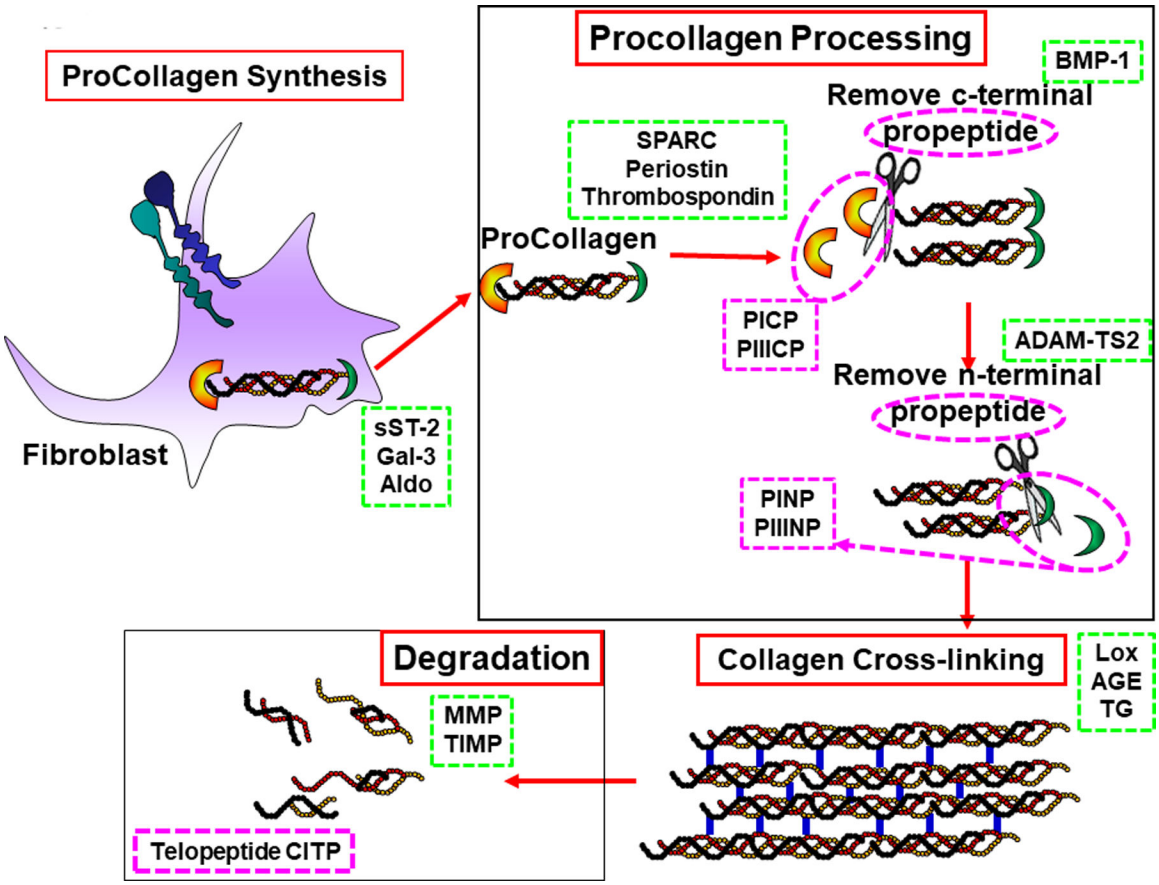

Figure 5. Molecular and cellular processes that control collagen homeostasis.

Panel A: Myocardial fibroblasts synthesize and secrete procollagen into the extracellular matrix (ECM) stimulated by sST-2, galectin-3 (gal-3), aldosterone (Aldo) and other proinflammatory and profibrotic factors (green dashed box). Panel B: Procollagen is processed in the ECM chaperoned by matricellular proteins (such as SPARC, periostin and thrombospondin) through sequential steps that remove c-terminal by bone morphometric peptide 1 (BMP-1) and n-terminal propeptides by ADAM-TS2. Plasma/serum concentrations of the resulting propeptides (PICP, PIIICP, PINP,PIIINP) reflect collagen synthesis rate. Panel C: Collagen cross-linking finalizes processing into mature insoluble collagen under the influence of lysyl oxidase (LOX), advanced glycation end products (AGE) and transglutaminase (TG). Panel D: Collagen degradation by matrix metalloproteinases (MMPs) into telopeptides (CITP) occurs under regulation of the endogenous tissue inhibitors of MMPs (TIMPs).

Each of the biomarkers within the green and purple dashed boxes can be measured in the circulation. (Illustration credit: Ben Smith).

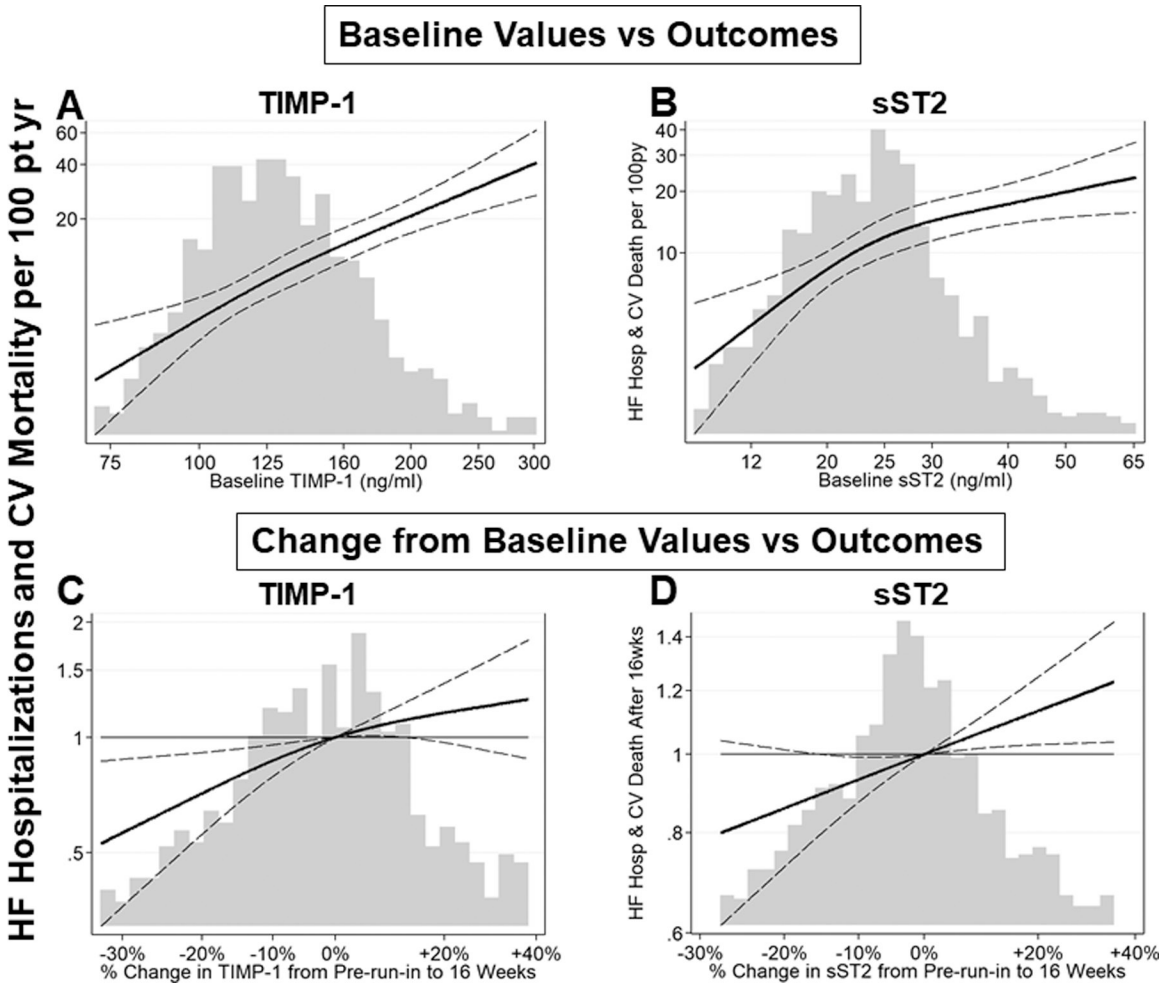

Figure 6. Prognostic value of biomarker data from PARAGON-HF Study[59].

Biomarkers that reflect mechanisms that increase procollagen synthesis, such as soluble ST2, are increased in HFpEF patients; biomarkers that reflect mechanisms that decrease collagen degradation, such as increased tissue inhibitor of matrix metalloproteinase-1 (TIMP-1) are increased in HFpEF patients. Both baseline and change from baseline levels of these profibrotic biomarkers provide prognostic value. Continuous relationships of TIMP-1 and sST2 baseline values and 16-week change from baseline values with incidence of subsequent heart failure (HF) hospitalization and cardiovascular (CV) death are plotted.

(A, B) The x-axis and histogram represent plasma biomarker level at baseline. The solid line represents estimated incidence rate of the primary endpoint, total HF hospitalizations and CV death. The dashed lines represent 95% confidence intervals for the estimated incidence rate. Incidence rate is displayed on the primary (left-sided) y-axis.

(C, D) The x-axis and histogram represent change in biomarker level between pre-run-in baseline visit and the week 16 visit. The solid line represents estimated incidence rate of the primary endpoint, total HF hospitalizations and CV death, that occurred after 16 weeks, relative to patients with no change in biomarker level, adjusted for log-transformed baseline value. The dashed lines represent 95% confidence intervals for the estimated incidence rate. Incidence rate ratio is displayed on the primary (left-sided) y-axis.

The higher the baseline value of TIMP-1 and sST2, the higher the rate of HF hospitalization and CV mortality. Over 16 weeks follow-up if TIMP-1 or sST2 decreased, the primary endpoints decreased.

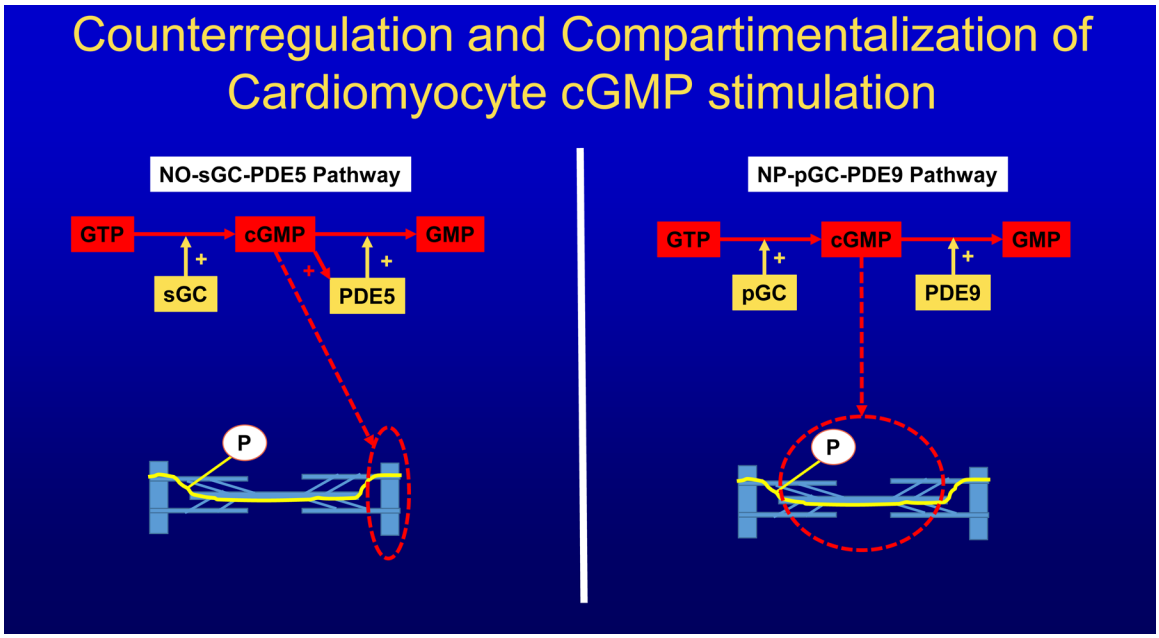

Figure 7. Compartimentalization and counterregulation of cardiomyocyte cGMP stimulation

Cyclic guanosine monophosphate (cGMP) produced by soluble guanylyl cyclase (sGC) from guanosine triphosphate (GTP) is mainly localized in sarcomeres around Z discs and preferably degraded to guanosine monophosphate (GMP) by phosphodiesterase 5 (PDE5). PDE5 activity rises when cGMP is elevated, thus providing a counterregulatory feedback. cGMP produced by particulate guanylyl cyclase (pGC) is mainly localized around myofilamentary proteins like titin and preferably degraded by PDE9. PDE9 lacks a cGMP sensitive regulatory site and is therefore not subject to counterregulation by cGMP.

Table 1:

HFpEF phenogroups derived from machine-learning clustering strategies.

	Shah SJ[72]	**Segar MW**[73] **TOPCAT**	**Hedman AK**[74] **Karolinska-Rennes**	**Cohen JB**[75] **TOPCAT**	**Sabbah M**[76] **RELAX,NEAT,INDIE**
Clin,ECG,Echo,Lab, Biomarkers	66 1 (NP)	60 1 (NP)	43 1 (NP)	60? 1 (NP)	14 13 (inflammation)
Phenogroup 1	Young Risk Low	DM,CKD,NP↑↑ E/e'↑, Risk High	DM,CKD,CAD NP↑, E/e'↑, Risk IM	COPD, Risk Low, HFpEF?	Male, NP↑↑, LAVI↑,Fibrosis Infl↑,Risk High
Phenogroup 2	Obese, DM, NP↑, Risk IM	Risk IM	COPD, NP↑↑ Risk High	AF,NP↑,E/e'↑ Risk IM	Old, Risk Low
Phenogroup 3	CKD, NP↑↑, E/e'↑, Risk High	CAD, Stroke, Risk IM	Obese, Risk Low	Obese, DM, CKD, Risk High	Female, Obese, CRP↑, Risk IM
Phenogroup 4			Obese, NP↑, Risk IM		
Phenogroup 5			Female, NP↑, Risk IM		
Phenogroup 6			Female, AF, NP↑, E/e'↑, Risk IM		

Clin, ECG, Echo, Lab: Number of clinical, electrocardiographic and laboratory characteristics used as input variables; Biomarkers: Number of biomarkers used as input variables; NP: Natriuretic Peptides; DM: Diabetes Mellitus; CKD: Chronic Kidney Disease; E/e': ratio of early diastolic mitral flow velocity over early diastolic long axis lengthening velocity; CAD: Coronary Artery Disease; Risk IM: Intermediate Risk; COPD: Chronic Obstructive Pulmonary Disease; LAVI: Left Atrial Volume Index; Infl: Inflammatory biomarkers; CRP: C-Reactive Protein; AF: Atrial Fibrillation

Table 2:

Potential Topics for Future Research Efforts on Inflammatory/Fibrotic Mechanisms in HFpEF.

1	Inflammatory signaling in obese, mostly female HFpEF patients with isolated CRP elevation
2	Involvement of myofilamentary proteins especially titin in nitrosative stress induced proteotoxicity
3	Gender related protective mechanisms against nitrosative stress induced proteotoxicity
4	Proinflammatory effects of microvascular endothelial upregulation of glycolytic enzymes
5	Blunted microvascular upregulation of adhesion molecules in the presence of elevated plasma high density lipoproteins
6	Linkage between overload induced basal lamina deformation and secretion of proinflammatory cytokines in cardiomyocytes
7	Molecular/cellular regulatory mechanisms that control myocardial fibrosis