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BIOMARKERS OF FIBROSIS IN HEART FAILURE AND RELATIONS TO LEFT VENTRICULAR SIZE, FUNCTION AND OUTCOME

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THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

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To my family

You got to work hard, you got to work hard

If you want anything at all

Work hard (Depeche Mode)

POPULAR SCIENCE SUMMARY OF THE THESIS

Heart failure (HF) is a syndrome characterized by fatigue, dyspnea, and swelling of the legs due to poor cardiac function. The symptoms appear partly because of compensatory effects due to the inability of the heart to pump enough blood to meet the need of the body's organs. HF can be classified into three groups according to the percentage of maximal blood volume in the left ventricle that is pumped into the aorta. This is called the ejection fraction (EF) which in a healthy person is around 55%. HF with EF ≤40% is referred to as HF with reduced EF (HFrEF) and it is the form of HF where pharmacotherapy has proven effective in improving heart function and saving lives. HF with EF between 41-49% is called HF with mildly reduced EF (HFmrEF) and HF with EF≥50% is called HF with preserved EF (HFpEF). Many heart diseases can lead to HF and the most common causes are myocardial infarction, hypertension, and valvular disease. Patients with HFrEF more often have a history of myocardial infarction compared with patients with HFpEF who more often have hypertension, diabetes, obesity, and atrial fibrillation. HFmrEF shares diseases seen both in HFrEF and HFpEF. On average, HF patients are in their mid-70s when they get the diagnosis. Quality of life is often poor with frequent hospitalizations and the 5-year survival rate after HF diagnosis is about 50%, which is worse than most forms of cancer. Heart transplantation is the ultimate option in younger patients with severe and symptomatic HF, sometimes after a period of treatment with a mechanical pump device to reduce symptoms and optimize the organs pre-transplantation.

The cardiomyocytes are the cells of the heart that are responsible for the contraction of the heart with every heartbeat, resulting in a synchronous pumping action that moves the blood through the heart and to the organs of the body. The space between the cardiomyocytes is called the interstitium and the residing cells form the so-called extracellular matrix (ECM). The ECM forms a supportive structure for the cardiomyocytes but also transduces cell-to-cell signals and force transmission. Collagen is the main supportive protein in the ECM and the fibroblast is the cell type that produces most of the collagen. During normal conditions collagen production and breakdown is stable, but in the case of a pathological stimulus, e.g., hypertension or myocardial infarction, the equilibrium is distorted. The potential excessive accumulation of collagen in the heart is called fibrosis and it can be focal, as in the scar formation after a myocardial infarction where dead cardiomyocytes are replaced by collagen, or diffusely spread in the ECM as in hypertension.

This thesis focuses on interstitial fibrosis, which is associated with various heart conditions, diabetes, hypertension, and aging alone. Fibrosis predisposes HF and is a part of the pathological remodeling process where the heart changes in shape, size, and function for the worse, but also provides compensation for loss of pump function. Myocardial fibrosis can be assessed by e.g., biopsies, magnetic resonance imaging, or through biomarkers in the blood. However, these biomarkers are not specific to cardiac fibrosis.

Patients over 60 years hospitalized for HF with impaired EF were included in the OPTIMAL study in the 1990s. We examined the participants for biomarkers of myocardial fibrosis (both at the start of the study (papers 1-III) and after 12 months (paper IV) and their associations with cardiac measurements, clinical presentation, and outcome.

In the first study, we found associations between increasing levels of the biomarkers of type I collagen synthesis (PICP) and degradation (CITP) with increasing levels of brain natriuretic peptide (BNP) which is a protein fragment released from the heart under stressful conditions, especially in HF. We also found associations between increasing PICP levels with increasing size of the left ventricle of the heart (a sign of adverse remodeling) and a sign of impaired relaxation of the heart during the filling of blood (worse diastolic function).

Atrial fibrillation (AF) is a common disturbance of the normal heart rhythm, especially in HF, which makes the heartbeat irregular and often faster than normal. In the second study, we found relations between increasing levels of both PICP and CITP to increasing size of the left atrium of the heart (a common abnormal feature seen in AF). Another finding was that increasing levels of PICP and decreasing size of the left ventricle were associated with AF.

The third study showed that higher levels of PICP and CITP at the start of the study were predictive of death from all-cause or cardiovascular disease. CITP was the best predictor, and it was also a predictor of non-cardiovascular death. We found a relationship between increasing levels of CITP and worsening functional capacity of the patients.

In the fourth and final study, we examined if changes in the biomarkers of fibrosis from the start of the study (baseline) to one year, after optimized pharmacotherapy in an outpatient HF clinic, would give additional information compared with baseline values alone. We also examined a biomarker index of collagen stability and a biomarker of type III collagen metabolism (reflecting both synthesis and degradation). The functional capacity, EF, and BNP improved after one year in the whole group. CITP was lower, and the collagen stability index was higher. The ten-year follow-up showed that the later deceased patients were older with a longer duration of HF at the study start and CITP was higher both at baseline and after a year compared with the survivors. The collagen stability index indicated lower stability after one year in the later deceased. The levels of PICP increased more after one year in the later deceased compared with the survivors. There were indications of better survival by lower levels of the one-year levels of PICP and CITP and signs of more stable collagen.

In conclusion, higher levels of biomarkers of type I collagen metabolism in HF patients with depressed EF are associated with larger cardiac size, increased BNP, atrial fibrillation, and worse outcomes. Increased pharmacotherapy improved cardiac function and functional capacity along with decreasing biomarkers of collagen metabolism. A reduction in collagen breakdown and an increase in collagen stability seem to be prognostically favorable

ABSTRACT

Background:

Heart failure (HF) is a syndrome with impaired quality of life and higher mortality than most forms of cancer. The excessive accumulation of collagen in the myocardial extracellular matrix is called myocardial fibrosis, which is a reaction to myocardial stress, myocardial injury and a part of the remodeling process that alters the shape, size, and function of the heart.

Aims:

To study (aims 1-3) myocardial fibrosis assessed by biomarkers at baseline in HF patients and their associations with 1) clinical variables, B-type natriuretic peptide (BNP), and echocardiographic findings at baseline 2) clinical variables, BNP, and echocardiographic findings in HF with or without atrial fibrillation 3) mortality outcomes 4) associations between temporal changes in biomarkers of myocardial fibrosis with clinical and echocardiographic findings and outcome.

Methods and results:

The OPTIMAL study consisted of 208 patients ≥60 years and hospitalized for HF with depressed systolic function. Patients were included from January 1996 to December 1999 and randomized to either follow-up at a nurse-led outpatient HF clinic or conventional follow-up in primary care. The main objective was to evaluate if the nurse-led outpatient HF clinic was associated with improved quality of life. The patients in this thesis are a cohort from the original study, with varying numbers of study objects due to the available numbers of echocardiographic examinations, long-term ambulatory ECG recordings, blood samples, and patients who survived the first year after inclusion.

Paper I: Echocardiography and biomarkers of type I collagen synthesis (PICP) and degradation (CITP) were available for 132 patients. Both increasing levels of PICP and CITP were independently associated with increasing levels of BNP. Increasing levels of PICP were also independently associated with increasing left ventricular size and decreasing isovolumic relaxation time.

Paper II: Long-term ambulatory ECG monitoring was available in 143 patients in addition to echocardiography, PICP, and CITP. Direct relations between levels of PICP and CITP to left atrial size were seen. Increasing levels of PICP and decreasing left ventricular end-diastolic volume were independently associated with atrial fibrillation.

Paper III: Same cohort as in paper I. PICP and CITP predicted all-cause and cardiovascular mortality after adjustment for possible confounders in two different models. CITP was also a predictor of non-cardiovascular death and directly related to NYHA functional class.

Paper IV: Sixty-eight patients from the cohort in paper I were analyzed at baseline and 12 months for PICP, CITP, matrix metalloproteinase-1 ([MMP-1], a collagenase), amino-terminal propeptide of type III collagen ([PIIINP], reflects type III collagen metabolism), and the CITP to MMP-1 ratio was calculated (an inverse index of collagen cross-linking). Pharmacotherapy was optimized over 12 months. Improved left ventricular ejection fraction (LVEF), decreased left ventricular mass index, better NYHA functional class, and lower BNP were parallel with lower levels of PICP, CITP, and CITP:MMP-1 in all patients after 12 months. Follow-up at ten years showed the later deceased (n=42) to be older and with a longer duration of HF compared with survivors. CITP at baseline and 12 months, and CITP:MMP-1 at 12 months were higher in the later deceased compared with the survivors. The temporal changes from baseline to 12 months were in favor of the survivors versus the later deceased regarding improved left ventricular dimensions and function, better NYHA functional class, lower BNP, and lower PICP.

Conclusions: Higher levels of the biomarkers of type I collagen metabolism in HF patients with depressed EF are associated with increased cardiac size, increased BNP, atrial fibrillation, and worse outcomes. Pharmacotherapy improved cardiac function and functional capacity along with decreasing biomarkers of collagen metabolism. A reduction in the biomarker of type I collagen breakdown and an increase in type I collagen cross-linking seem to be prognostically favorable.

LIST OF SCIENTIFIC PAPERS

I. Löfsjögård J, Kahan T, Díez J, López B, González A, Edner M, Henriksson P, Mejhert M, Persson H. Biomarkers of collagen type I metabolism are related to B-type natriuretic peptide, left ventricular size, and diastolic function in heart failure. *J Cardiovasc Med (Hagerstown). 2014 Jun;15(6):463-9.*

II. Löfsjögård J, Persson H, Díez J, López B, González A, Edner M, Mejhert M, Kahan T. Atrial fibrillation and biomarkers of myocardial fibrosis in heart failure. *Scand Cardiovasc J. 2014 Oct;48(5):299-303.*

III. Löfsjögård J, Kahan T, Diéz J, López B, González A, Ravassa S, Mejhert M, Edner M, Persson H. Usefulness of collagen carboxyterminal propeptide and telopeptide to predict disturbances of long-term mortality in patients ≥60 years with heart failure and reduced ejection fraction. *Am J Cardiol. 2017 Jun 15;119(12):2042-2048.*

IV. Löfsjögård J, Kahan T, Díez J, López B, González A, Ravassa S, Edner M, Persson H. Temporal changes in circulating biomarkers of myocardial fibrosis, echocardiography, and BNP during one year and the association to long-term prognosis in heart failure with an impaired ejection fraction. *Manuscript.*

CONTENTS

LIST OF ABBREVIATIONS

ACE-I	Angiotensin-converting enzyme inhibitor
ARB	Angiotensin II receptor blocker (antagonist)
AF	Atrial fibrillation
ARNI	Angiotensin receptor neprilysin inhibitor
AVPD	Atrioventricular plane displacement
BB	Beta-blocker (beta-adrenergic receptor antagonist)
BNP	Brain natriuretic peptide
CI	Confidence interval (95%)
CITP	Carboxy-terminal telopeptide of type I collagen
CITP:MMP-1	The ratio of CITP to MMP-1
CMR	Cardiovascular magnetic resonance
CO	Cardiac output
CRT	Cardiac resynchronization therapy
CV	Cardiovascular
DCM	Dilated cardiomyopathy
ECG	Electrocardiogram
eGFR	Estimated glomerular filtration rate
ESC	European Society of Cardiology
ELISA	Enzyme-linked immunosorbent assay
ECM	Extracellular matrix
HF	Heart failure
HFmrEF	Heart failure with mildly reduced ejection fraction
HFpEF	Heart failure with preserved ejection fraction
HFrEF	Heart failure with reduced ejection fraction
ICD	Implantable cardioverter defibrillator
IHD	Ischemic heart disease
IQR	Interquartile range
IVRT	Isovolumic relaxation time
LBBB	Left branch bundle block
LV	Left ventricle

LVAD	Left ventricular assist device
LVEDd	Left ventricular end-diastolic diameter
LVEF	Left ventricular ejection fraction
LVMI	Left ventricular mass index
MI	Myocardial infarction
MMP-1	Matrix metalloproteinase-1
MRA	Mineralocorticoid receptor antagonist
NT-proBNP	N-terminal pro-B-type natriuretic peptide
NYHA	New York Heart Association
OPTIMAL	The Optimising Congestive Heart Failure Outpatient Clinic Project
PIIINP	Amino-terminal propeptide of type III procollagen
PICP	Carboxy-terminal propeptide of type I procollagen
PICP:CITP	The ratio of PICP to CITP
QRS	A combination of the Q-wave, R-wave, and S-wave on ECG
RAAS	Renin-angiotensin-aldosterone system
RAS	Renin-angiotensin system
SD	Standard deviation
sGC	Soluble guanylate cyclase
SGLT-2	Sodium glucose cotransporter-2
SNS	Sympathetic nervous system
TGF-β	Transforming growth factor-beta

1 INTRODUCTION

Heart failure (HF) is commonly referred to as a clinical syndrome, meaning "a group of signs and symptoms that occur together and characterize a particular abnormality or condition" according to the Merriam-Webster dictionary. Typical signs are pulmonary rales and elevated jugular venous pressure, and symptoms of HF include shortness of breath, fatigue, and bilateral swelling of the legs. The British cardiologist Sir Thomas Lewis wrote in 1933 that "The very essence of cardiovascular practice is the early detection of heart failure". This statement is true even to this day since heart failure (HF) is a major health issue, both for patients and society, and the number of patients is expected to increase as we live longer, and medical science continues to improve.

Descriptions of HF symptoms can be dated back to ancient China, and the Greek and Roman empires, and the foundation of today's knowledge were laid by Ibn al-Nafis who described the pulmonary system in the 13th century (1), and William Harvey who presented the complete description of the circulation of blood in the 17th century (2).

During the past century, some major achievements were made to help make us understand and even start treating HF, such as heart catheterization, ultrasound, thoracic surgery, pharmacotherapy, and heart transplantation to name a few. However, it is only about 40 years ago since the idea of HF as a syndrome emerged, and the deleterious importance of neurohormonal activation was recognized.

Since the interest in the cardiac fibroblast awakened in the 1990s, the importance of extracellular matrix production and deposition in the myocardium in various conditions has become a hot topic for the medical community. Myocardial fibrosis is seen in both cardiac and non-cardiac conditions and predisposes to the development of HF. The biochemical knowledge of fibrosis has come far, and there are ways to assess fibrosis, but this has not yet been translated into effective therapies or guidelines for the management of myocardial fibrosis.

2 LITERATURE REVIEW

2.1 HEART FAILURE DEFINITION

The 2021 European Society of Cardiology (ESC) guidelines for the diagnosis and treatment of acute and chronic heart failure state that "HF is due to a structural and/or functional abnormality of the heart that results in elevated intracardiac pressures and/or inadequate cardiac output at rest and/or during exercise" (3).

Evaluation of the left ventricular ejection fraction (LVEF) is the basis for the classification of HF type. The normal LVEF range is 54-74% in women and 52-72% in men (4). It was long believed that HF was primarily a lack of contractibility of the heart, but as diagnostic tools for assessment of cardiac function emerged, especially echocardiography, it became obvious that patients with signs and symptoms of HF could have a normal LVEF (5). However, studies of HF in the 1980s and the 1990s included patients mostly with LVEF<35-40%, which coincides with the evidence of higher mortality in patients with LVEF<40% (6).

Current guidelines propose the following classification of HF based on LVEF and the presence of signs and symptoms (3):

- LVEF ≤40%, HF with reduced ejection fraction (HFrEF)
- LVEF 41-49%, HF with mildly reduced ejection fraction (HFmrEF)
- LVEF ≥50%, HF with preserved ejection fraction (HFpEF)

In HFpEF, additional objective evidence must exist for the diagnosis: cardiac structural and/or functional abnormalities consistent with the presence of left ventricular diastolic dysfunction/raised filling pressures, including natriuretic peptides.

HFrEF accounts for most of all HF (55%), and the proportion of HFmrEF and HFpEF is similar (22% and 23%, respectively) (7). HFpEF seems to increase and may be more prevalent in the future (8).

The New York Heart Association (NYHA) functional classification is a way to classify HF patients according to their physical capacity (9). It comprises classes I-IV, with I meaning the patient has no symptoms despite evidence of impaired function of the heart. Patients in class II and III have a slight and marked limitation of physical capacity, respectively, and patients in class IV have symptoms even at rest.

2.2 EPIDEMIOLOGY AND PROGNOSIS

In 2017 the number of people with HF in the world was estimated to be 64.3 million (10), with the highest prevalence in Central Europe, the Middle East, and Africa (11). In western countries, the incidence of HF is considered to be stable or decreasing (12) and the prevalence is 1-3% (8), and is believed to increase due to factors such as aging populations, improved access to diagnostic tools, and effective treatment for risk factors of HF and HF itself. In Sweden, HF incidence has decreased (from 0.32% to 0.29%), but the prevalence has

increased (from 1.61% to 1.72%) in the last 10-15 years, age-adjusted (13). The mean age for the incident and prevalent patients is similar at 77 years, with women older than men (80 vs 74 years, respectively) (14). The incidence and prevalence increase with age as does death from HF, and the prevalence is almost 20% in the age group 80-89 years with a 5-year survival rate of 35% compared with 55% in the age-matched population. In Sweden in 2010, the 5-year survival of HF was 48% (14) which is substantially worse than the approximately 73% 5-year survival rate of all cancers combined (15). The observed survival after 5 years has improved over time from 35% in the period 1950-1969 (16), to 60% between 2010 and 2019 in high-income countries (17).

Important factors that give information on prognosis are age, gender, ejection fraction, diastolic dysfunction, increased natriuretic peptides, renal function, exercise ischemia, NYHA functional class, peak oxygen uptake or exercise capacity, quality of life, and frequent readmissions (18). Patients with HFmrEF have a better prognosis than patients with HFrEF (19). HF exacerbation is common and the most common cause of hospitalization in patients 65 years or older (20), and HF causes 1-2% of all hospitalizations in high-income countries (21).

The prognosis in patients with HFpEF has been reported to be as poor as in patients with HFrEF and has not changed significantly from 1990 to 2009 (42). However, it has also been reported that mortality is higher in patients with HFrEF compared with patients with LVEF≥40%, and mortality increases the lower EF is (6). Patients with HFmrEF and HFpEF had the same mortality as those with LVEF≥60% (6). Death from cardiovascular (CV) disease is more common than death from non-CV disease in HFrEF, and vice versa in HFpEF (42).

2.3 ETIOLOGY

A plethora of possible causes of HF exist, and many diseases of the heart may lead to HF. Etiology also varies around the world and some are usually located in specific regions, e.g. rheumatic fever in sub-Saharan and low-income countries and Chaga's disease in South America (8). Ischemic heart disease (IHD) is a major cause of HF and is accountable for 26.5-40% worldwide (8, 11). The incidence and severity of myocardial infarction (MI) decrease and a shift from more frequent post-MI HFrEF to HFpEF has been observed, where smoking cessation and access to early percutaneous coronary intervention are possible causes (22). Hypertension is believed to be accountable for etiology in 15-26.2% of HF patients in the world (8, 11). Tachycardia can also cause HF, and it is most often of supraventricular origin (primarily atrial fibrillation [AF]) (23). Valvular heart disease, diabetes mellitus, cardiomyopathies, and alcohol are some of the other possible causes of HF. Cardiomyopathies may have specific causes like hemochromatosis, cytostatic treatment, or infectious diseases but are most often idiopathic. Comorbidities are very common in HF, and 87% of the patients had three or more coexisting chronic diseases in 2014 (24). Only 35% of HF trials reported comorbidities, and the most common were hypertension, ischemic heart disease, hyperlipidemia, diabetes, atrial fibrillation, and chronic kidney disease (25). The

under-representation of comorbidities in HF trials makes it harder to implement study results in the large group of patients that suffers not only from HF. AF begets HF and vice versa, but AF is more prone to precede HF than the opposite (26). In the Swedish HF registry, the prevalence of AF in HF was 53% in HFrEF, 60% in HFmrEF, and 63% in HFpEF (7).

Patients with HFpEF are often older, female, and more often have hypertension and AF, but less frequent IHD compared with patients with HFrEF (6).

2.4 PATHOPHYSIOLOGY

During exercise, the healthy heart possesses the ability to react with a substantial increase in cardiac output (CO), which is the product of heart rate and stroke volume. The Frank-Starling mechanism explains that increased preload including rising filling pressure in the left ventricle (LV), stretches the myocardium resulting in more blood per time unit ejected into the aorta during systole. The HF patient normally has a lower CO already at rest, and during exercise, the failing heart is not able to increase CO in the same manner as in a healthy person. Ultimately, the elevated LV end-diastolic pressure (filling pressure) leads to an increase in pulmonary capillary mean pressure which enables fluid to exit from the capillaries leading to congestion in the lungs. Normal CO at rest is 4-6 l/min which can increase to 20 l/min during exercise, or even >35 l/min in the professional athlete. With maximal exertion, the arteriovenous oxygen extraction increases to provide further improvement of circulatory function.

There is an activation of the sympathetic nervous system (SNS) in HF and a release of catecholamines that stimulates the heart by increasing myocardial contractility and heart rate, and it also causes systemic vasoconstriction and increased vascular resistance which raises blood pressure and thereby ameliorates organ perfusion. This serves as a compensatory mechanism, to begin with, but in the long-run excessive catecholamine release worsens HF and its prognosis (27-29). The renin-angiotensin-aldosterone system (RAAS) also gets activated, in part by the increased sympathetic activity (an increase of renin by stimulation of beta receptors), leading to sodium and water retention, vasoconstriction, and aldosterone secretion by the action of angiotensin II on the angiotensin II type 1 receptor primarily (30).

The neurohormonal activation in HF, i.e., the activation of SNS and RAAS, leads to pathological remodeling of the heart. A consensus from the year 2000 stated that “cardiac remodeling may be defined as genome expression, molecular, cellular and interstitial changes that are manifested clinically as changes in size, shape, and function of the heart after cardiac injury” (31). The remodeling process renders LV dysfunction and may be asymptomatic in some cases, but there is a marked risk of progression to symptomatic HF in asymptomatic systolic and diastolic LV dysfunction compared with control subjects without LV dysfunction (32). Concentric LV remodeling/hypertrophy is seen in a state of chronic high-pressure overload as in hypertension and aortic stenosis and is characterized by hypertrophic cardiomyocytes and sarcomeres that are added in parallel. In contrast, in eccentric remodeling/hypertrophy, the sarcomeres are added in series in a chronic volume overload

situation like mitral regurgitation. With time progressive LV dilation occurs. The adverse remodeling in MI includes loss of cardiomyocytes and LV dilation to compensate for the loss of contractility. Eccentric LV hypertrophy is the most common type in patients with HFrEF.

Myocardial fibrosis is a term used to describe the pathological accumulation of collagen in the heart and it is an important part of the remodeling process. Following an MI, a fibrotic scar is formed to replace dead cardiomyocytes (33) and in hypertension, a diffuse accumulation of interstitial collagen takes place (34).

The pathophysiological importance of LV remodeling was initially demonstrated in rats with induced MI. The higher the degree of LV dilation and the lower LVEF was after the MI, the poorer the prognosis was, and for those rats who showed reverse remodeling (i.e., lessen the dilation and improvement of LVEF) survival increased (35, 36).

The pathophysiology of HFpEF is not yet fully understood, but increased oxidative stress, inflammation, and endothelial dysfunction are thought to be important elements in its development of worsening diastolic LV function. Initially, slower isovolumic relaxation (IVRT) progresses into severe restrictive diastolic dysfunction and stiffness which results in increasing LV filling pressures and low CO due to small LV despite normal or preserved LVEF. Moderate or severe diastolic dysfunction is strongly independently associated with poor prognosis in HFpEF patients (37). Metabolic and hemodynamic load and oxidative processes are causing inflammatory responses through microvascular dysfunction in the systemic and coronary circulation (38). Profibrotic processes occur, and these seem to be associated with titin changes and collagen turnover (39). See more under 2.7.

2.5 DIAGNOSIS

Sensitive measures of heart failure are a 12-lead electrocardiogram (ECG) and blood levels of natriuretic peptides. If the ECG is normal, the probability of HF is low (40). The blood level of a B-type natriuretic peptide (NT-proBNP or BNP) is analyzed, and a low value indicates a low probability for HF in the non-acute setting (ESC cut-off point of <125 pg/mL for NT-proBNP or <35 pg/mL for BNP) (41). NT-proBNP is an inactive fragment that is cleaved off when the active BNP is formed, and it is mostly released from the cardiac ventricles as a reaction to volume expansion or pressure overload (42). Obesity, acute renal failure, and AF are a few conditions that may affect the sensitivity of the test (43, 44). Besides testing for B-type natriuretic peptides, blood tests are performed to rule out other conditions with similar symptoms as HF, to give information on prognosis, and to give advice on appropriate treatment. Echocardiography is the key player to diagnose HF and provides information on the size, shape, and function of the heart and the possible etiology. A chest X-ray may reveal other conditions that cause shortness of breath such as pneumonia or chronic obstructive pulmonary disease, but also pulmonary congestion or enlargement of the heart which are found in HF.

If the initial tests indicate HF, then additional tests to determine the etiology should be considered.

2.6 TREATMENT

2.6.1 Pharmacotherapy

The pharmacological studies that laid the foundation for today´s evidence-based treatment of HF, were performed in patients with LVEF mainly <40%. At that time HFpEF was not fully recognized and there is still no evidence or consensus on how it should best be treated, and traditionally treatment has often been the same as in HFrEF.

HF is a progressive disease and HF patients are recognized by high mortality rates, periodic exacerbations with hospitalizations, low functional capacity, and impaired quality of life. Pharmacological treatment aims to improve these factors.

2.6.1.1 HFrEF

The basis for the treatment is derived from the pathophysiology of HF previously discussed, which means that the target is to counteract the detrimental effects of increased RAAS and SNS activation. Randomized controlled trials have proved treatment options in patients with symptomatic HFrEF (NYHA II-IV), but not for HFpEF.

Angiotensin-converting enzyme inhibitors (ACE-I) are a cornerstone in the treatment of HF. It has multiple effects such as decreasing preload and afterload, decreasing sodium and water retention, and increasing CO. ACE-I reduces mortality and improves symptoms (45, 46). Angiotensin II receptor blockers (ARB) have similar effects (47) as ACE-I in HF treatment and are often used if a patient has the common side effect of a dry cough or has experienced angioedema with ACE-I because of impaired degradation of bradykinin by ACE-I. A combined angiotensin receptor-neprilysin inhibitor (ARNI) proved to be superior to ACE-I to reduce mortality and hospitalization in HFrEF (48). Neprilysin is an enzyme that degrades vasoactive peptides like natriuretic peptides, and inhibition of its action thereby causes blood vessel dilation and reduction of extracellular fluid volume via sodium excretion (49). Soluble neprilysin predicts outcome in HFrEF (50).

Beta-blocker (BB) treatment in HF is also well documented in randomized controlled trials (51-53). By blocking beta-adrenergic receptors in the heart, the effects of an overstimulated SNS in HF are reduced. Heart rate is lowered allowing optimal ventricular filling and a more efficient contraction.

Aldosterone synthesis is increased in the final step of the RAAS and acts on the distal part of the nephron of the kidneys. Stimulation of the aldosterone receptor results in sodium reabsorption, water retention, and potassium excretion Mineralocorticoid receptor antagonists (MRAs) act by blocking receptors to aldosterone and other corticoids, and studies have shown a reduction in mortality and hospitalization (54, 55).

Empagliflozin and dapagliflozin are inhibitors of sodium-glucose cotransporter 2 (SGLT-2) and are used to treat patients with type 2 diabetes. Blood glucose levels are decreased by glucosuria through osmotic diuresis. A significant reduction in CV death and hospitalization

for HF was seen in patients with type 2 diabetes who received empagliflozin vs placebo (56). Patients with HFrEF, with or without type 2 diabetes, had a lower risk of worsening HF or CV death if treated with dapagliflozin (57). Apart from the effect on diuresis, the mechanism of action in HF is not fully understood but there is some evidence of reduced myocardial fibrosis (58), and attenuated reversed remodeling has been observed (59).

LV reverse remodeling is associated with a better prognosis and quality of life and can be obtained by all the drugs mentioned above.

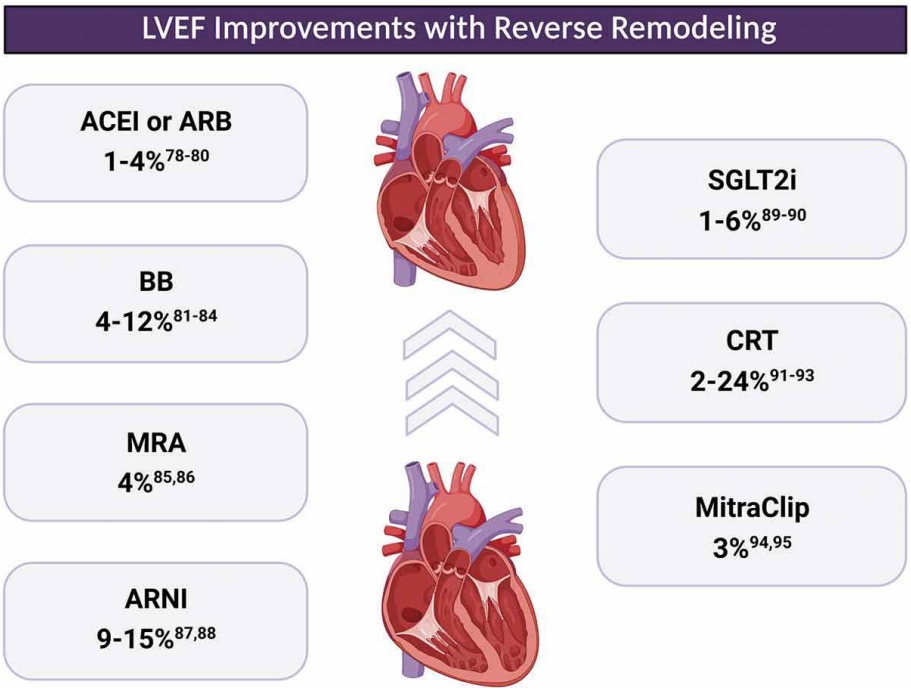

Figure 1. LVEF improvements with reverse remodeling attributable to therapies. References refer to the original article. This article was published in Structural Heart, 5, Boulet J and Mehra M R, Left Ventricular Reverse Remodeling in Heart Failure: Remission to Recovery, 466-481, Copyright Elsevier (2021).

Diuretics are used to relieve symptoms of volume overload. They reduce filling pressure and decrease symptoms of pulmonary congestion and edema, but the treatment has a negative side to it since it increases the activation of RAAS and SNS. The use of diuretics may lead to low levels of potassium, sodium, and magnesium and thereby promoting potentially lethal arrhythmias (60, 61).

In addition to the recommended pharmacotherapy, some drugs may be used in symptomatic HF. They are only meant to be added after optimized standard therapy and not on their own.

Ivabradine, a direct sinus node inhibitor, should be considered in patients in NYHA class II-IV who still have a heart rate of at least 70 beats per minute and a maximum tolerable dose of a BB. Ivabradine showed a significant reduction in the composite outcome of CV death and HF hospitalization, on top of treatment with ACE-I/ARB, BB, and MRA (62).

Vericiguat, a soluble guanylate cyclase (sGC) stimulator, may be considered since it has proved to reduce CV death and hospitalization for HF in patients with HF and EF<45% compared to placebo (63). Vericiguat does two things: It sensitizes sGC to endogenous nitric oxide and if there is no nitric oxide sGC activity can be increased. The effect is the widening of the pulmonary arteries which makes pumping blood through the lungs easier.

Digoxin is a glycoside with an inotropic effect and thereby a reduction in heart rate. To reduce the risk of hospitalization, it may be considered in patients with SR (64)

2.6.1.2 HFpEF

Treatment of patients with HFpEF is mainly addressed to reduce symptoms of HF with diuretics since ACE-I/ARNI, BB, and MRA have failed to show a reduction in mortality or morbidity. Conventional treatment of risk factors such as hypertension, coronary heart disease, and AF should be performed. The SGLT-2 inhibitor empagliflozin recently showed a reduction in the composite endpoint of CV death or hospitalization for HF in patients with HF and LVEF>40% with or without diabetes compared with placebo (65). The result was primarily achieved by a reduced risk of hospitalization for HF in the group treated with the SGLT-2 inhibitor.

2.6.2 Device treatment

The presence of a left branch bundle block (LBBB) and QRS≥150 ms on the ECG and LVEF≤35% in symptomatic patients with SR and optimized medical therapy implies a recommendation for cardiac resynchronization therapy (CRT) (3). LV diastolic filling time and LVEF (by decreased septal activity) is reduced in patients with LBBB (66). CRT has a wide range of effects, e.g., improved functional capacity, quality of life, LV diastolic and systolic function, and it induces LV reverse remodeling and a decrease in mitral regurgitation (67-71).

The risk of sudden death is increased in HFrEF, but with improved treatment of HF, it has decreased over time since the mid-1990s (72). ICD is recommended for HFrEF patients with optimized medical therapy, NYHA class II-III. and LVEF≤35% of an ischemic etiology (3). ICD both reduces the risk of sudden cardiac death and all-cause mortality (73, 74).

Implantation of a CRT alone or combined with an ICD reduces death from HF, all-cause mortality, and hospitalizations for HF to a similar degree (75).

Left ventricular assist device (LVAD) is a mechanical circulatory support device that helps the unloading of the failing LV. LVAD treatment has been shown to reverse remodeling (76). It can be used short-term in cardiogenic shock or long-term as a bridge to transplantation or to reverse some contraindications to transplantation (3). Patients with advanced HFrEF are currently enrolled in the SweVAD-study to evaluate the potential use of LVAD implantation as destination therapy in patients who have the need but are ineligible for heart

transplantation in Sweden (77). LVAD destination therapy is available in other parts of the world and previously also in Sweden.

2.7 MYOCARDIAL FIBROSIS DEFINITION

The extracellular matrix (ECM) is a three-dimensional network in which cardiomyocytes and coronary vessels are embedded. It has a structural backbone formed by the type I and III collagens, and in addition to providing structural support to the myocytes, the ECM also facilitates force transmission and transduction of signals between matrix macromolecules and cardiomyocytes, regulating the latter. Fibrosis is the term used when there is an excessive accumulation of fibrillar collagen in the ECM, and it occurs in many different organs and diseases such as liver cirrhosis, diabetic nephropathy, and interstitial lung diseases (78) as well as aging (79-81). Myocardial fibrosis is present in various cardiovascular diseases, in reaction to a variety of stimuli. Two different types of fibrosis are described: focal and diffuse interstitial fibrosis. Focal fibrosis, or replacement fibrosis, is seen after a myocardial infarction where a collagen-based scar is formed to replace dead cardiomyocytes. Diffuse interstitial fibrosis, or reactive fibrosis, is seen in most CV diseases and can also be promoted by diabetes, obesity, and aging.

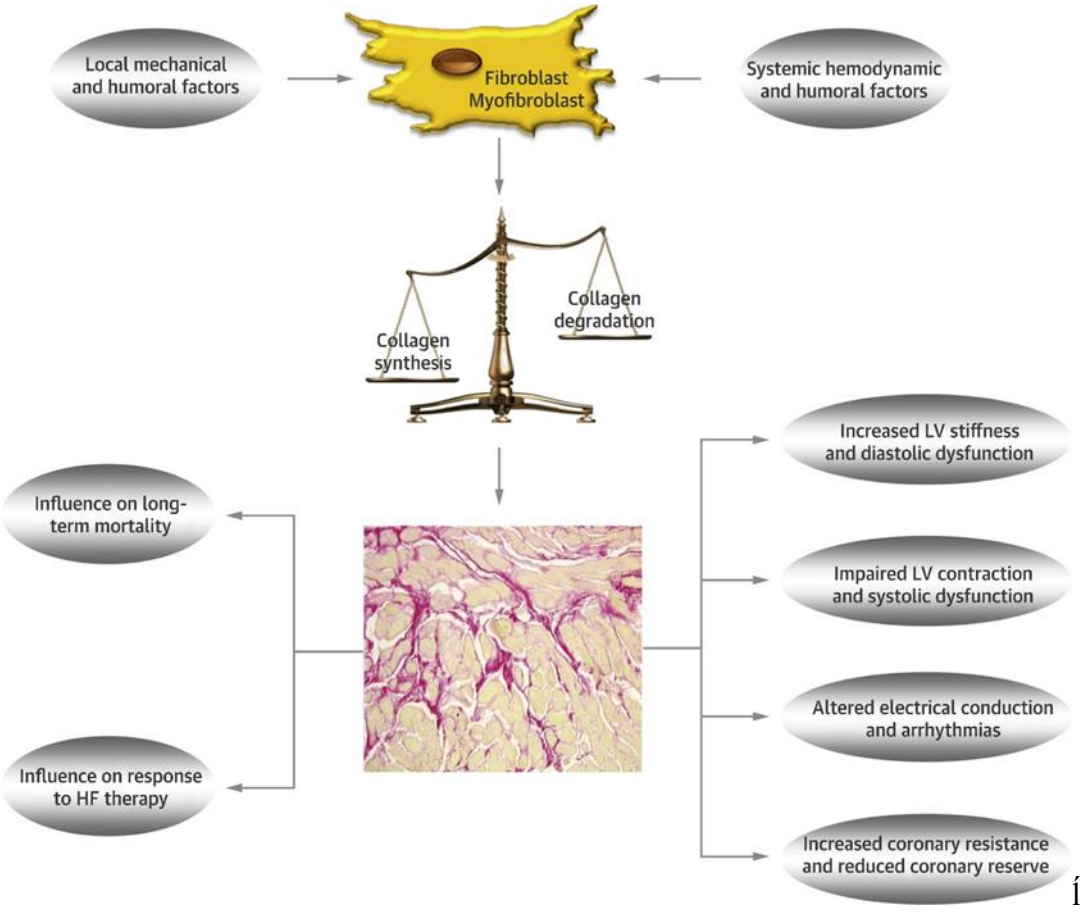

Figure 2. Pathophysiology of myocardial fibrosis. Reprinted from Journal of the American College of Cardiology, 65 /22, Lopez B, Gonzalez A, Ravassa S, Beaumont J, Moreno MU, San Jose G, et al, Circulating Biomarkers of Myocardial Fibrosis: The Need for a Reappraisal, 2449-56, Copyright (2015), with permission from Elsevier.

2.8 COMPONENTS, STRUCTURE, AND BIOMARKERS OF FIBROSIS

Cardiomyocytes are the cell type that occupies most of the volume of the human heart and accounts for about 49% and 30% of the total number of cells in the ventricles and atria, respectively (82). Cardiac fibroblasts are the main manufacturer of collagen and account for about 16% of the total number of cells in the ventricles, and 24% in the atria (82). Five types of collagens (I, III, IV, V, and VI) have been found in the heart (83, 84), and types I and III are present in the ECM. About 85% of total myocardial collagen is type I and 11% is type III (85). Type I collagen fibers are thick and stiff and type III fibers are more compliant. Cardiac fibroblasts differentiate into their activated form called myofibroblasts upon myocardial stress, by the cooperation of cytokines, growth factors (especially transforming growth factor-beta [TGF-β] and the signaling pathways), hormones, enzymes, and other agents (86). This results in an increased production of collagen and other ECM proteins which alters the normal ECM composition (i.e., matrix remodeling), and ultimately impairment of cardiac function. The myofibroblasts also secrete molecules involved in the formation of the final collagen fiber as well as its degradation (87).impairment of cardiac function. The myofibroblasts also secrete molecules involved in the formation of the final collagen fiber as well as its degradation (87).

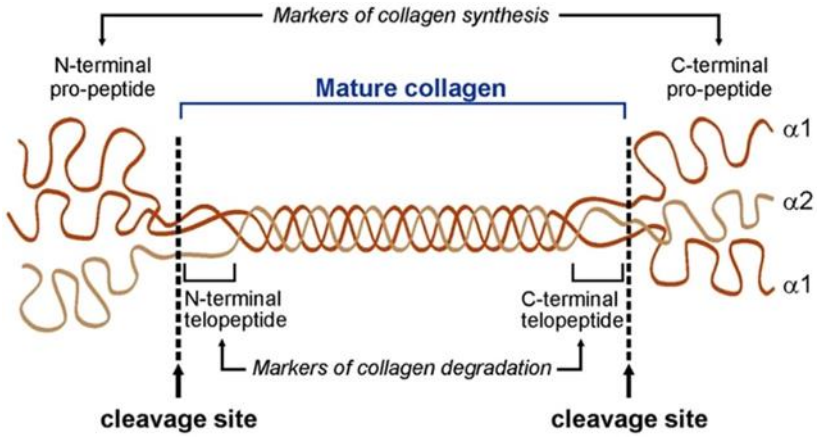

Figure 3. Structure of type I procollagen with propeptides that are cleaved off to form mature collagen. C-terminal propeptide =PICP. C-terminal telopeptide=CITP. Reprinted from J Thorac Cardiovasc Surg, 151/6, Edens RE, Gruber PJ, Fibrosis in Fontan physiology, 1527-8, Copyright (2016), with permission from Elsevier.

Fibrillar collagen is synthesized in the fibroblasts as procollagen which is a triple helical structure containing an amino-terminal and a carboxy-terminal propeptide. When procollagen is released from the fibroblast into the ECM, the propeptides are cleaved off by proteinases and the collagen triple helix can take part in the formation of the growing collagen fibril with other collagen chains. To stabilize the final collagen fiber, the fibrils can be linked to each other, and this cross-linking process makes the collagen fiber thicker and stiffer and less susceptible to degradation (88).

Blood levels of the propeptides of types I and III procollagens ([PICP], carboxy-terminal propeptide of type I procollagen, and [PIIINP], amino-terminal propeptide of type III procollagen) that are cleaved off can be measured in blood.

Collagen degradation in the heart is mainly mediated by Zn^{2+}-dependent matrix metalloproteinases (MMPs), preferentially MMP-1 that degrades collagens I and III (89), which cleaves collagen into a big and a small telopeptide. The small telopeptide is released into the bloodstream ([CITP], carboxy-terminal telopeptide of type I collagen). MMP-1 has an inhibitor called tissue inhibitor of matrix metalloproteinases (TIMPs), and the interaction between MMP-1 and TIMP-1 regulates the architecture of the ECM (90). There is a stoichiometric ratio of 1:1 between the number of type I collagen molecules produced and degraded and that of PICP and CITP released. Serum PIIINP can either be derived from newly synthesized type III collagen or the degradation of existing type III collagen fibrils. PICP and PIIINP are cleared from the blood mainly by the liver but can also be cleared via the kidneys (91, 92). CITP is cleared via glomerular filtration (93).

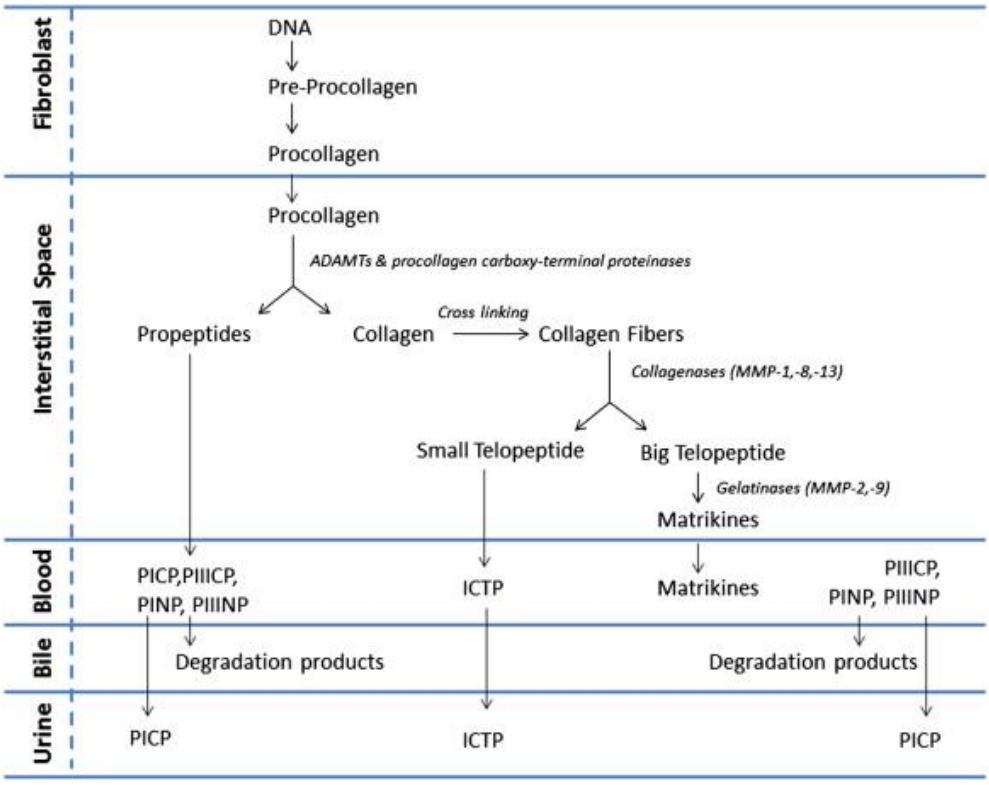

Figure 4. Synthesis and degradation of collagen I and III. ICTP=CITP. Reprinted from Clin Chim Acta, 443, Chalikias GK, Tziakas DN, Biomarkers of the extracellular matrix and of collagen fragments, 39-47, Copyright (2015), with permission from Elsevier.

Cross-linking makes the collagen fiber more resistant to degradation by MMP-1 (90), and subsequently less degradation of type I collagen takes place (less CITP formation). The CITP

to MMP-1 ratio (CITP:MMP-1) is a proposed biomarker of collagen cross-linking (94). It inversely correlates to the degree of cross-linking.

In this thesis, focus is on the biomarkers PICP, CITP, PIIINP, MMP-1, and the calculated ratios of PICP to CITP and CITP to MMP-1, which all aim to reflect collagen metabolism. Other molecules have been proposed as biomarkers of fibrosis as well, but they are related to the regulation of collagen turnover (e.g., microRNA-21 and TGF-β1) or activation of fibrosis by inflammation or myocardial stress (e.g., galectin-3 and suppression of tumorigenicity 2 [ST2]) and will not be further discussed.

2.9 ROLE OF MYOCARDIAL FIBROSIS IN CARDIOVASCULAR DISEASES

In hypertensive patients with and without HF, serum concentration of PICP correlated to myocardial content assessed by biopsies, and evidence was presented that PICP is secreted via the coronary sinus into the bloodstream (95, 96). PICP correlated with diastolic dysfunction in hypertensive patients (97), but PICP was not associated with HF in other studies (98, 99). PICP proved to be a powerful predictor of restrictive-like filling as seen in the most severe form of LV diastolic dysfunction in HFpEF (100) and HFrEF (99). Baseline PICP was analyzed in patients admitted for decompensated HF, and PICP levels showed a significant correlation to death and rehospitalization, but not to LVEF (101).

Increased serum concentrations of CITP have been observed in patients with dilated cardiomyopathy (DCM) (102, 103), suggesting that collagen degradation is increased due to the dilation of the ventricles. CITP has also proved to correlate to NYHA-class and to be an independent predictor of mortality in HF (103).

The PICP:CITP ratio has been proposed as an index of coupling between the synthesis and degradation of type I collagen (i.e., collagen turnover) (104). The increased PICP:CITP ratio correlated with increased myocardial collagen content in patients with DCM (105). In hypertensive patients with HF, PICP:CITP was significantly lower in NYHA IV compared with NYHA I-III due to increased CITP (106). The patients in NYHA IV also had significantly lower EF and LV radial function compared with the other groups, suggesting that degradation is prominent in advanced disease. It has also been reported that responders to CRT had a significantly higher PICP:CITP ratio at baseline and that CRT possessed the ability to decrease PICP (107).

Biopsies have shown increased collagen content in left atria in patients with AF (108, 109), and PICP correlated with left atrial fibrosis (108). PICP was higher in patients with persistent AF compared with those with paroxysmal AF (110). The combination of a high degree of collagen cross-linking (i.e., low CITP:MMP-1) and high PICP was independently associated with both incidence and prevalence of AF in HF of hypertensive origin (111). EF was >50% in two-thirds of patients both with or without AF at baseline, and the incident cases had significantly lower EF at baseline compared with the non-incident cases.

PIIINP is a predictor of mortality in both HFrEF and HFpEF (103, 112), and correlates to NYHA-class in HFrEF (103).

Increased collagen cross-linking rather than total collagen explained increased myocardial stiffness in hypertensive rats (113). Patients with HF of hypertensive origin and a higher degree of collagen cross-linking, both measured by biopsy and in serum (CITP:MMP-1), were more likely to be hospitalized for HF (94). Hyperglycemia in diabetes is associated with increased myocardial fibrosis and collagen cross-linking (114).

2.10 METHODS OF MEASURING FIBROSIS

The gold standard technique for the detection of diffuse myocardial fibrosis is an endomyocardial biopsy. Several samples are often required due to the uneven distribution of fibrosis in the heart, and the technique is also dependent on the operator and access to a well-trained pathologist.

Cardiovascular magnetic resonance (CMR) can detect both focal and diffuse fibrosis. In diffuse fibrosis, the fraction of extracellular volume measured by CMR correlates to fibrosis diagnosed by biopsies (115). The limitations to CMR-detected fibrosis are the inability to determine the type of collagen and hence the proportions, as well as the extent of collagen cross-linking.

The idea of detecting fibrosis via circulating biomarkers is tempting as it would be cheaper, readily available, and suitable for everyday use. A biomarker is defined as “a defined characteristic that is measured as an indicator of normal biological processes, pathogenic processes, or responses to an exposure or intervention” (116). Several molecules have been suggested as biomarkers of myocardial fibrosis, but only three have proved correlation with histologically proven myocardial fibrosis: PICP, PIIINP, and CITP:MMP-1 (94, 117). However, they are not exclusively of cardiac origin and may in fact represent total body collagen or reflect disturbances in collagen metabolism by other diseases. It is also known that HF treatment can alter collagen turnover.

2.11 CAN MYOCARDIAL FIBROSIS BE REVERSED?

The focal scar formation following myocardial infarction to replace dead cardiomyocytes is crucial to the healing process and to reverse it is not an option (118). Reversing interstitial myocardial fibrosis, however, could have potential benefits in reductions of morbidity and mortality.

RAAS is activated in HF and most CV diseases. The action of angiotensin II on the angiotensin II type 1 receptor (119) and aldosterone on the mineralocorticoid receptor (120) can promote myocardial interstitial fibrosis. Angiotensin II can also stimulate TGF-β synthesis. RAS-inhibition reduced LV interstitial fibrosis in models of pressure overload (121) and myocardial infarction (122), and improved LV size and function in parallel with increased collagen cross-linking (123). Spironolactone reduced myocardial fibrosis and

improved diastolic function in dilated cardiomyopathy (105). Recently a reduction of PICP in parallel to improvements in LV size and function was seen in patients who switched from ACE-I or ATII-receptor antagonist to ARNI (124), and previously ARNI was superior to ACE-I to reduce PIIINP(125). Torasemide is a loop diuretic that reduced interstitial fibrosis in HFrEF of hypertensive origin (126). Within the torasemide group improvement of EF, NYHA functional class, and BNP was observed as well as a reduction of cross-linking, although no certain differences were observed between torasemide and furosemide treatment (127). Empagliflozin reduced collagen types I and III synthesis as well as TGF-β1 expression in diabetic mice (58).

Lower levels of biomarkers of type I collagen syntheses were seen in responders to CRT compared with non-responders (128, 129). Levels of biomarker assessed types I and III collagens increased in CRT-responders, but not in non-responders, 6 months after implantation (129),

LVAD reverses LV remodeling and almost normalizes LV chamber stiffness but increases myocardial stiffness and types I and III collagen content as well as collagen cross-linking (130).

To summarize, blocking the RAAS is the best pharmacological option available to reduce fibrosis today. SGLT-2 inhibitors are also promising and already in use in HF patients, but they are less studied than RAAS inhibitors. Targeting fibrosis via inhibition of TGF-β has been positive in pulmonary fibrosis (131) but can also lead to LV dilation and reduced EF (132). Device treatment shows many favorable effects for HF patients, but the biomarkers of collagen metabolism studied indicate increased collagen synthesis post-implantation.

3 RESEARCH AIMS

The overall aim was to investigate associations between primarily type I collagen metabolism, assessed by circulating biomarkers, and echocardiographic measurements of size and function, clinical findings, and outcomes in HF patients with depressed systolic function and with a mixed etiology, who were followed up at an outpatient HF clinic after hospitalization for HF.

Our main aims were:

- To explore associations between biomarkers of type I collagen metabolism and clinical findings, echocardiographic measurements of size, function, and dyssynchrony.

- To investigate associations between biomarkers of type I collagen with clinical findings, echocardiographic measurements of size, and function with or without prevalent AF.

- To examine the predictive value of biomarkers of type I collagen metabolism measured at baseline on long-term outcome.

- To evaluate the possible changes from baseline to 12 months during improved HF therapy in levels of biomarkers of type I and III collagen metabolism, and type I collagen cross-linking, and associations with echocardiographic measurements of size and function, and long-term outcome.

4 MATERIALS AND METHODS

4.1 DESIGN AND STUDY POPULATION

4.1.1 The OPTIMAL study

The Optimising Congestive Heart Failure Outpatient Clinic Project included patients with systolic HF in north-eastern Stockholm between 1996 and 1999 (133). It was a prospective randomized study that compared HF care at a nurse-led hospital outpatient clinic with conventional primary care. The primary aim was to evaluate if the nurse-led outpatient clinic improved quality of life, and secondary aims were the evaluation of cardiac function, morbidity, and mortality. The patients were recruited while hospitalized for HF, and inclusion criteria were HF with depressed EF of <45% or atrioventricular plane displacement (AVPD) <10 mm, NYHA II-IV, and age ≥60 years. Patients with valvular stenosis, MI or unstable angina pectoris in the last three months, dementia, and severe concomitant disease were excluded. After screening, 208 patients were included in the study and randomized to either follow-up at the nurse-led HF outpatient clinic or primary care, and it was noted that the excluded patients were more often older women than those who were included.

In addition to the appointments with the nurse, patients got a clinical examination and a thorough follow-up on their pharmacotherapy at 6, 12, and 18 months. They also met with the same doctor at each of these visits, but no adjustment of medication was performed. The mean follow-up was 1122 days for the whole study group.

Screening echocardiography was performed only to determine the eligibility for inclusion in the study, with the requirements described above. This was followed by a detailed baseline echocardiography examination after a few days when the patient was considered medically stabilized.

Routine blood tests were sampled at baseline and 12 months, and this study includes 132 patients who were analyzed for both PICP and CITP. No patient suffered from conditions known to affect the levels of PICP and CITP, such as chronic liver disease, clinical renal failure, or metabolic bone disease.

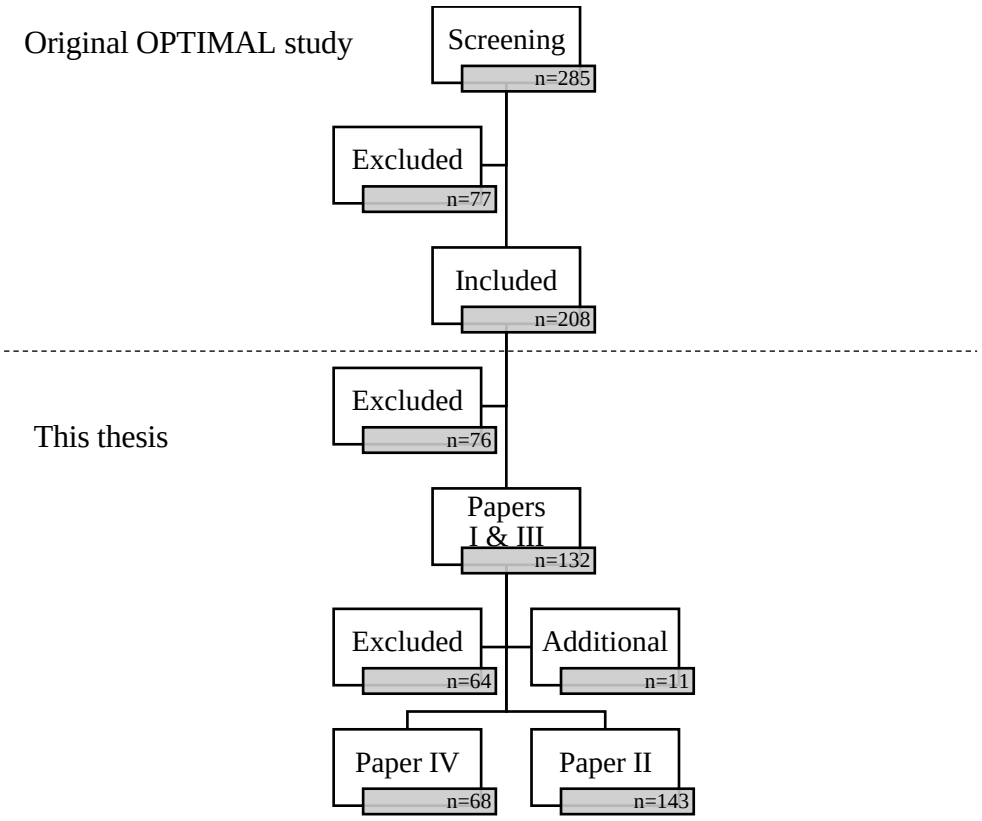

Figure 5. Flowchart of the OPTIMAL study and papers in this thesis.

4.1.2 Patients in this thesis

The combined availability of baseline blood samples analyzed for PICP and CITP, and complete echocardiographic examination yielded 132 patients for papers I and III. The mean age of the patients was 75 years and 53% were women. Registered comorbidities were hypertension (29%), ischemic heart disease (64%), diabetes mellitus (22%), and atrial fibrillation (54%).

The same cohort as in papers I and III applied to paper II, but the number of available 24-hour ambulatory ECG recordings yielded an additional 11 patients, and in total 143 patients from the original OPTIMAL study were included in study II. The additional patients were not analyzed for PICP or CITP.

Twenty-five patients from the cohort of 132 patients did not survive to make the 12-month follow-up appointment. Freezer-stored plasma and serum samples, and echocardiographic examinations, both at baseline and 12 months, were available for 68 patients of the remaining 107. These 68 patients were examined in paper IV.

Age, years	75.3 ± 7.1
Women, n (%)	57 (43)
Systolic blood pressure. mmHg	134 ± 25
Diastolic blood pressure, mmHg	80 ± 15
Hypertension, n (%)	38 (29)
Ischemic heart disease, n (%)	84 (64)
Diabetes mellitus, n (%)	29 (22)
Previously known heart failure, n (%)	71 (54)
Atrial fibrillation, n (%)	71 (54)
QRS duration, ms	117 ± 29
PICP, µg/L	73 [57-99]
CITP, µg/	7.1 [4.5-10.2]
BNP, pg/mL	210 [98-416]
Estimated glomerular filtration rate, mL/min/1.73m^2	54 ± 19
Left ventricular mass index, g/m^2	139 ± 42
Left ventricular ejection fraction, %	34 ± 11
Atrioventricular plane displacement, mm	65 ±19
Left ventricular end-diastolic diameter, mm	57 ± 10
Left ventricular end-systolic diameter, mm	47 ± 12
Left ventricular end diastolic volume, mL	115 [82-153]
Left ventricular end systolic volume, mL	77 [49-112]
Septal thickness, mm	12 ± 3
Posterior wall thickness, mm	11 ± 3
Relative wall thickness	0.40 [32-49]
E/é mean	13 ± 5
Left atrial diameter, mm	47 ± 8
Isovolumic relaxation time, ms	90 [75-110]
Transmitral E-wave deceleration time, ms	180 ± 57
Angiotensin converting enzyme inhibitor, n (%)	101 (77)
Angiotensin II receptor blocker, n (%)	6 (5)
Beta-blocker, n (%)	72 (55)
Mineralocorticoid receptor antagonist, n (%)	24 (18)
Digoxin, n (%)	71 (54)

Table 1. Baseline characteristics of the cohort of 132 patients from the original OPTIMAL study. PICP and CITP are reported with original levels from the OPTIMAL study. From paper I.

The 68 patients in paper IV were, compared with the 132 patients at baseline, less often women (35%) and previously known HF was rarer (43%). The baseline levels (original levels from OPTIMAL) of PICP and CITP were lower (69 [54-90] and 6.5 [4.1-8.9], respectively). MMP-1, CITP:MMP-1, and PIIINP were not examined before. The other variables were similar between the groups.

4.2 METHODS

4.2.1 Echocardiography

Examinations were performed with Acuson 128XP/10 (Mountain View, California, USA) at baseline and 12 months. Assessment of LV measurements and function was done according to the recommendations from the American Society of Echocardiography (134). AVPD was

calculated from apical 2- and 4-chamber projections (135). Evaluation of the diastolic function was done by a modified Mayo clinic protocol (136).

Additional measurements and calculations were done in paper I. The baseline echocardiography examinations were recorded and stored on VHS tapes, which were analyzed with offline software equipment (TomTec Imaging Systems Inc., Boulder, Colorado, USA). The mean of three beats was calculated if SR, or eight beats if AF. The LV filling pressure was estimated by the ratio of maximum early transmitral flow velocity in diastole and tissue Doppler early mitral annulus velocity (E/é), in the LV septal and lateral walls. The estimated pulmonary capillary wedge pressure (ePCWP) was calculated by the formula ePCWP=1.90 +1.24 (E/é mean) (137).

Atrioventricular dyssynchrony, i.e., the deviated timing of activation and contraction between the atria and the ventricles, was assessed by the left ventricular filling time measured from transmitral Doppler E-wave onset until the end of the transmitral A-wave in apical four-chamber view, or in case of AF until the end of E-wave. Total time and its relation to R-R interval were assessed.

Assessment of intraventricular dyssynchrony, i.e., the deviated timing of activation and contraction between different segments of the LV, was performed in three ways.

1. The activation time of the posterolateral wall was measured by M-mode in parasternal long-axis view, from the beginning of QRS to the maximum contraction of the posterolateral wall (d1). Then the time from the onset of QRS to the beginning of the E-wave by pulsed wave Doppler echocardiography at the mitral ostium was measured (d2). If d1<d2 contraction was considered delayed.
2. The time from the onset of QRS to peak systolic S-wave at basal septal and lateral segments.
3. The aortic pre-ejection time was measured from the onset of QRS to the start of outflow in the LV outflow tract by pulsed wave Dopper echocardiography.

4.2.2 Biochemical analyses

Routine blood tests were sampled at baseline and 12 months. The blood was collected 30 minutes after supine rest, put on ice, and centrifuged at +4 C before plasma and serum were frozen at -70 C until further analysis. Plasma BNP was measured by an immunoradiometric assay (Shionoria; Shionogi, Osaka, Japan) and analyzed at baseline and 12 months. Serum PICP was measured by a sandwich ELISA. Serum CITP was determined by a quantitative enzyme immunoassay (Orion Diagnostica, Espoo, Finland). PICP and CITP were analyzed at baseline.

In paper III, we added the calculated PICP to CITP ratio (an index of the balance between type I collagen synthesis and degradation) to the previous analysis.

In paper IV, we performed new analyses in 2018 on freezer-stored plasma and serum from baseline and 12 months. PICP, CITP, MMP-1 and PIIINP were analyzed. The ratio of CITP to MMP-1 was also calculated. Serum PICP was measured using the EIA MicroVue CICP (Quidel Corporation, San Diego, California, USA). Serum CITP was measured by a radioimmunoassay (Orion Diagnostica, Espoo, Finland). Total serum MMP-1 was measured by an AlphaLISA (PerkinElmer, Waltham, Massachusetts, USA). Serum PIIINP was measured by a radioimmunoassay (Orion Diagnostica, Espoo, Finland). CITP and MMP-1 levels were expressed in molarity and their ratio was calculated in each patient as previously reported (89).

4.2.3 24-hour ambulatory ECG monitoring

The results from previous recordings in the OPTIMAL study were used in paper II. Patients were considered to have AF in two ways. Firstly, all patients got a 24-hour Holter monitor recording by the time of discharge, and they were considered to have AF if AF was present at least half of the recorded time. Secondly, if AF was previously known or present on the ECG, cardiac telemetry, or echocardiography at the time of study inclusion. Paroxysmal AF was included here. Patients with recordings of <17 hours or pacemaker rhythm were excluded.

4.2.4 Outcome

Data from the Swedish Causes of Death Register was used up to December 31, 2008, for the date and cause of death in paper III and up to December 2, 2009, in paper IV for date of death when all patients had been followed for 10 years.

4.3 STATISTICS

Normally distributed variables were presented with mean values ± SD in all papers. In paper I and III the non-normally distributed variables were log-transformed (natural logarithm). In paper II and IV they were presented with median and IQR. Categorical variables were displayed with number of cases (n) and percentages. A p-value of <0.05 was considered statistically significant. When appropriate Hazard Ratios (HR) or Odds Ratio (OR) and 95% confidence intervals (CI) were calculated.

4.3.1 Paper I

Patients were divided into two groups twice. Firstly, below or above median PICP, and secondly, below or above median CITP. The statistical analyses were performed first for PICP and then for CITP. The Chi^2-test and Student's t-test were used for comparison of independent variables between the groups, as appropriate. Univariate correlations were examined between the biomarkers of myocardial fibrosis and the studied variables. Univariate regression analyses were performed by Pearson's correlation coefficient in the between-groups analyses. Two methods of multivariate regression analysis were used to demonstrate relations between the biomarkers and variables: cluster analysis and Cox regression. Adjustments were made for the variables with $p \leq 0.10$ in the univariate analysis (QRS-duration, BNP, LVEDd, AVPD, APE-/R-R, LVFT/R-R, IVRT, and RWT) and age.

The SIMCA-P+ software (Umetrics AB, Umeå, Sweden) was used for cluster analysis, and the STATISTICA 12 (StatSoft, Tulsa, Oklahoma, USA) was used for all the other analyses.

4.3.2 Paper II

Two groups were created based on co-existing atrial fibrillation or not. Comparisons of groups were done by the Chi^2-test and Student's t-test, and Pearson's correlation coefficient was the univariate regression test used for evaluation of correlations. Non-normally distributed log-transformed variables included BNP, C-reactive protein, and isovolumic relaxation time (IVRT). A logistic regression analysis was performed and included variables with $p<0.20$ in the group comparison (age, hypertension, coronary artery disease, diabetes mellitus, BNP, PICP, LVMI, LV end-diastolic volume, and ePCWP).

The JMP v 10 (SAS Institute Inc., Cary, North Carolina, USA) was used.

4.3.3 Paper III

The Student's t-test was used for comparison of continuous and independent variables between groups. The Chi^2-test was used for the same purpose for the categorical variables. Kaplan-Meier survival curves were plotted, and log-rank tests were performed for CV, non-CV, and all-cause mortality, to compare survival between groups. Multivariate Cox regression analysis with adjustment to MAGGIC (the Meta-Analysis Global Group in Chronic Heart Failure) and our own model parameters was used for the evaluation of the outcome. Univariate receiver-operating characteristic (ROC) curves were created to assess the ability of PICP and CITP to detect mortality (CV, non-CV, and all-cause).

The STATISTICA 12 (StatSoft, Tulsa, Oklahoma, USA) was used.

4.3.4 Paper IV

Group comparisons were performed by Student's t-test (for independent continuous and normally distributed variables), Mann-Whitney U-test (for independent continuous and non-normally distributed variables), and the Chi^2-test (for independent categorical variables). Paired tests were used for group comparisons from baseline to 12 months: Paired t-test (for dependent continuous and normally distributed variables), Wilcoxon signed-rank test (for dependent and non-normally distributed variables), and McNemar's test (for dependent categorical variables). Correlations were studied by Spearman's rank-order correlation coefficient. Survival curves for all-cause mortality were created, and log-rank tests were analyzed for comparisons of survival between groups. Uni- and multivariate Cox proportional hazard regression analyses for all-cause mortality were examined.

The STATISTICA 14 (StatSoft, Inc., Tulsa, Oklahoma, USA) was used.

4.4 ETHICAL CONSIDERATION

The OPTIMAL study on which this thesis is based was approved by the ethics committee in Stockholm and complied with the declaration of Helsinki. It was an open and randomized

controlled trial and informed consent was obtained for all patients. In 2017 our application was granted by the local ethics committee to analyze freezer-stored blood samples from baseline and 12 months.

5 RESULTS

5.1 PAPER I

5.1.1 Patient characteristics divided by median PICP

BNP ($p<0.001$) and CITP ($p=0.002$) were higher in the patients above or equal to median compared with patients below median PICP. They also had larger left ventricular end-diastolic diameter ([LVEDd], $p=0.015$), lower AVPD ($p=0.001$), and shorter IVRT ($p=0.021$).

The PICP:CITP ratio in the patients above or equal to median indicated collagen synthesis was predominant over degradation and significantly higher than in the group with PICP below median (13.1 [8.9-18.1] vs 8.8 [5.8-16.3], $p=0.006$).

Previously known HF was more common in the group with PICP above the median ($p=0.054$).

5.1.2 Patient characteristics divided by median CITP

QRS duration ($p=0.046$), BNP ($p<0,001$), and septal E/e' ($p=0.043$) were increased in patients with above or equal to median CITP compared with patients below the median.

There was no difference in previously known HF between groups.

5.1.3 Independent associations

Our main finding was that multivariate analysis (cluster analysis) confirmed independent associations between PICP and IVRT, BNP, LVEDd, and relative wall thickness (RWT). Traditional multivariable analyses also demonstrated positive independent associations to BNP ($r=0.24$, $p=0.018$) and LVEDd ($r=0.22$, $p=0.027$), and a negative association to IVRT ($r=-0.31$, $p=0.002$).

BNP was the only variable that was independently associated with CITP in the cluster analysis (VIP>1) and traditional multivariable analysis ($r=0.23$, $p<0.001$)

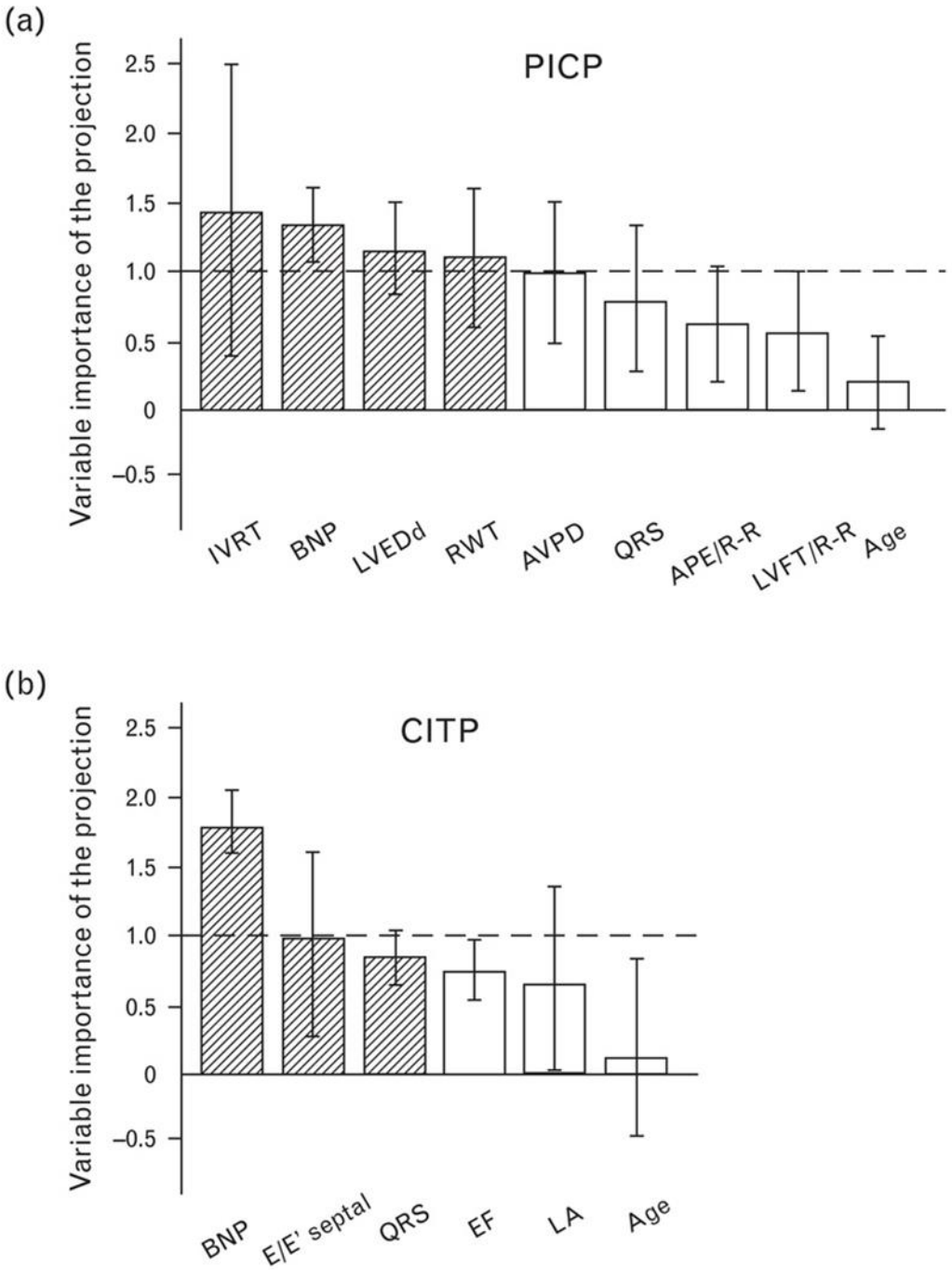

Figure 6. Cluster analysis for PICP (a) and CITP (b). Variable importance of the projection [VIP] >1 is considered significant (hashed bars). From paper I.

5.2 PAPER II

5.2.1 Patient characteristics divided by atrial fibrillation or sinus rhythm, and correlations

Co-existing AF or not was the criteria for group division. The AF group had smaller LV end-diastolic volume ($p<0.001$), greater EF ($p<0.001$) and lower LVMI ($p=0.004$) compared with the group with SR. Also, E/e' mean and estimated pulmonary capillary wedge pressure

(ePCWP) were lower (both p=0.026), and the use of warfarin and digoxin was more common (both p<0.001). PICP and CITP correlated with left atrial diameter (r=0.22, p=0.013 and r=0.26, p=0,003, respectively), and CITP also correlated with ePCWP (r=0,19, p=0.044) and C-reactive protein (r=0.29, p=0.003).

5.2.2 Independent associations

Our main finding was that the logistic regression analyses for both ways (see section 4.2.3) to define prevalent AF showed that LV end-diastolic volume and PICP were independently associated with AF, with a higher risk of AF with lower LV end-diastolic volume and higher PICP.

In the first model (Chi^2=34.1, R^2=0.18, p<0.001) LV end-diastolic volume (odds ratio for 1 mL change 0.98, 95% confidence interval 0.96;0.99, p<0.001) and PICP (OR per 1 µg/L change 1.01, CI 1.00-1.02, p=0.012) were independently associated with AF.

In the second model (Chi^2=43.5, R^2=0.24, p<0.001) LV end-diastolic volume (OR for 1 mL change 0.98, CI 0.97-0.99, p<0.001) and PICP (OR per 1 µg/L change 1.01, CI 1.00-1.02, p=0.049), were independently associated with AF.

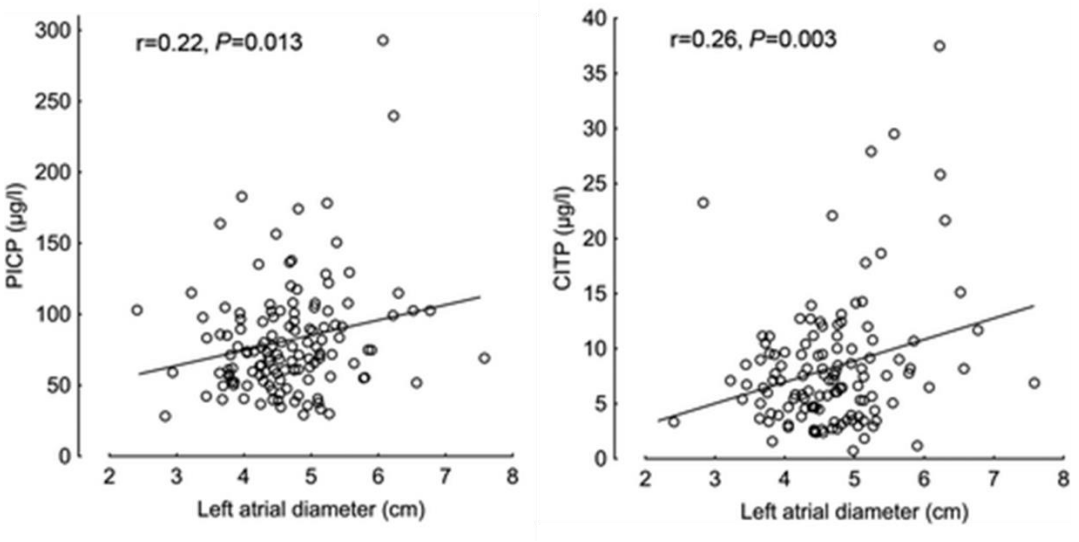

Figure 7. Correlations between left atrial size and PICP (left) and CITP (right). From paper II.

5.3 PAPER III

5.3.1 Patient characteristics divided by alive or later deceased

The deceased patients were at baseline older (p=<0.001), and more often had previously known HF (p=0.03) compared with the patients who were still alive. The QRS duration was also longer (p=0.008). Echocardiography showed that the LV septum was thicker (p=0.02), and LVMI was higher (p=0.02) in the later deceased. Blood samples in the later deceased were significantly higher for CITP (p<0.001), and lower for the PICP to CITP (PICP:CITP) ratio (p=0.006), and hemoglobin (p=0.007). Renal function was worse in the later deceased

patients, with higher creatine (p=0.003) and lower estimated glomerular filtration rate ([eGFR], p=0.001), compared with the alive. PICP (p=0.10) and the left atrial diameter (p=0.05) tended to be higher and larger, respectively, in the later deceased.

5.3.2 Outcome

The mean survival time was 5.5 years during follow-up, which was 9 to 13 years. Survival curves and log-rank tests between groups showed that the highest quartiles of PICP and CITP had the worse outcome for all-cause (p=0.014 and p<0.001, respectively) and CV mortality (p=0.012 and p<0.001, respectively). In the case of non-CV mortality, the highest quartile of CITP (p=0.002) and the lowest quartile of PICP:CITP (p=0.026) predicted mortality.

According to a report on how to interpret the calculated area under the curve (AUC) in ROC analyses (138), CITP proved to be an excellent predictor of non-CV mortality (AUC 0.81, p<0.001) and an acceptable predictor of CV and all-cause mortality (AUC 0.72, p=0.001 and AUC 0.75, p<0.001, respectively).

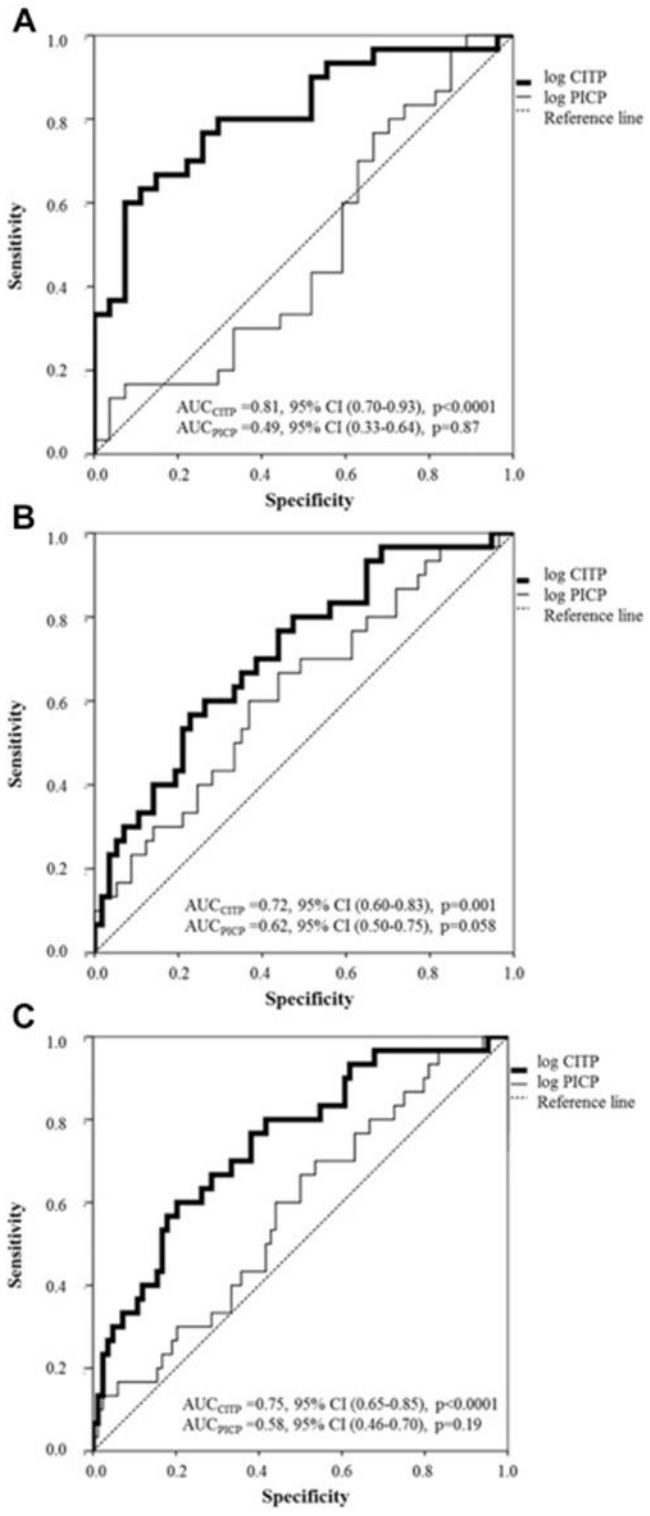

Figure 8. ROC-curves for PICP and CITP versus non-CV mortality (A), CV mortality (B), and all-cause mortality (C). From paper III.

Our main finding was that PICP and CITP were independent predictors of all-cause and CV mortality by multivariate regression analyses, in which two established sets of variables associated with HF outcome (5 variables for OPTIMAL and 12 for MAGGIC) with possible confounding effects were included. CITP was also an independent predictor of non-CV mortality.

CITP and PICP:CITP were also independently related to NYHA functional class.

5.4 PAPER IV

For the sake of simplicity, the comparisons listed in the following paragraphs are meant to be read as compared with the second group mentioned in each paragraph. The first time a comparison is mentioned in each paragraph states the way the groups are compared, if not stated otherwise.

5.4.1 Patient characteristics at baseline and 12 months in all patients

	Baseline	**12 months**	**p**
Diastolic blood pressure, mm Hg	82 ± 15	77 ± 10	0.020
Heart rate, bpm	86 ± 18	75 ± 12	<0.001
Proportion of target dose RAS-inhibitor, %	29 [13-88]	50 [29-100]	<0.001
NYHA class			<0.001
I, n (%)	0 (0)	23 (34)	
II, n (%)	49 (72)	32 (47)	
III, n (%)	18 (27)	12 (18)	
IV, n (%)	1 (1)	1 (1)	
PICP, µg/L	62 [54-86]	68 [56-86]	0.104
CITP, µg/L	6.9 [5.1-11.5]	5.0 [3.6-6.6]	<0.001
MMP-1, µg/L	7.6 [4.2-12.1]	7.1 [3.6-10.6]	0.253
CITP:MMP-1	3.5 [1.9-7.9]	2.9 [1.6-5.0]	0.002
PIIINP, µg/L	3.6 [2.7-5.1]	4.0 [2.9-5.1]	0.656
BNP, pg/mL	191 [99-302]	118 [48-290]	0.009
Hemoglobin, g/L	134 ± 13	131 ± 14	0.049
LVEF, %	32 ± 9	37 ± 11	0.001
AVPD, mm	66 ± 19	89 ± 27	<0.001
LVESd, mm	48 ± 12	43 ± 13	<0.001
LVPWd, mm	11 ± 2	10 ± 2	0.009
LVMI, g/m^2	140 ± 34	127 ± 37	0.001

Table 2. Biomarkers of myocardial fibrosis and variables with significant changes from baseline to 12 months. From paper IV.

RAS-inhibitor target dose increased, and NYHA functional class improved from baseline to 12 months. Further, systolic function improved with lower left ventricular end-systolic diameter (LVESd), higher LVEF and AVPD. The LV posterior wall thickness decreased (as did LVMI). CITP and CITP:MMP-1 decreased, as well as BNP.

5.4.2 Patient characteristics divided by alive or later deceased 10 years after inclusion

All patients were followed up until the date of death, or 10 years if they were alive. The median survival time was 8.4 [4.1-10.0]years. The later deceased (n=42) were older (p=0.012) and more often had previously known heart failure (p=0.013), and QRS duration

on ECG was longer according to their status at baseline (p=0.012) compared with the patients who were still alive (n=26). There were no differences in NYHA functional class, pharmacotherapy, or echocardiographic measures including LVEF at baseline. CITP was higher in the later deceased, both at baseline and 12 months (p=0.037 and p=0.004, respectively). CITP:MMP-1 at 12 months was also higher in the later deceased compared with the alive (p=0.041). Furthermore, hemoglobin (p=0.001) and renal function (creatinine p=0.004 and eGFR p=0.011) were lower in the later deceased patients.

5.4.3 Temporal changes between patients alive or later deceased 10 years after inclusion

	Alive (n=26)	Later deceased (n=42)	p
NYHA	-1 [-1-0]	0 [-1-0]	0.028
PICP, µg/L	0.2 [-9.1-6.3]	8.7 [-8.0-23.6]	0.044
CITP, µg/L	-1.9 [-3.9-(-0.7)]	-1.9 [-4.4-(-0.5)]	0.984
MMP-1, pg/mL	-1.0 [-5.9-1.0]	0.1 [-2.9-3.2]	0.129
CITP:MMP-1	-0.6 [-3.0-0.2]	-0.9 [-4.0-0.9]	0.840
PIIINP, µg/L	-0.1 [-0.7-1.1]	0.2 [-1.0-1.6]	0.955
BNP, pg/mL	-96 [-177-(-28)]	0 [-112-140]	0.005
LVEF, %	8 (21)	0 (13)	0.014
AVPD, mm	2.9 [1.6-6.2]	0.6 [-0.1-2.9]	<0.001
LVEDd, mm	-3.2 ± 5.9	0.5 ± 6.2	0.028
LVESd, mm	-8.2 ± 8.5	-1.8 ± 7.3	0.004
LVESV, mL	-10 ± 36	9 ± 30	0.027
LVESVI, mL/m2	-6 ± 21	4 ± 15	0.030
IVRT, ms	14.0 ± 35.4	11.0 ± 48.5	0.029
Left atrial diameter, mm	-3.6 ± 5.0	0.4 ± 5.4	0.004

Table 3. Biomarkers of myocardial fibrosis and variables with significant temporal changes between the alive and the later deceased 10-years after inclusion. From paper IV.

The patients who were still alive improved their systolic LV function, both LVEF and AVPD, more than the later deceased, from baseline to 12 months. The LV dimensions also improved in the alive with a reduction in LVEDd and LVESd. IVRT was numerically lower in the alive at baseline, but not statistically different compared with the later deceased, and the temporal change was greater in the alive. PICP was the only marker of fibrosis that showed a temporal change, and it increased more in the later deceased. Signs of better improvement of HF were seen in the alive with lower BNP and better NYHA functional class.

5.4.4 Correlations with temporal changes of markers of fibrosis

An increase in PICP correlated with an increase in BNP (r=0.40, p>0.001) and a decrease in AVPD (r=-0.32, p=0.01), and if CITP increased there was a deterioration in NYHA functional class (r=0.25, p=0.045). A change in CITP:MMP-1 correlated positively with a

change in left atrial diameter (r=0.26, p=0.04). LVMI, LVESd, and BNP increased greatest in patients with the greatest increase in PIIINP (r=0.38, p=0.004 and r=0.29, p=0.03 and r=0.25, p=0.04, respectively).

5.4.5 Outcome (all-cause mortality)

Univariate, but not multivariate, analyses indicated that higher baseline CITP was associated with all-cause mortality (HR for 1 µg/L change 1.05, CI 1.01-1.09, p=0.013). A shorter lifespan was also proposed by higher 12-month levels of PICP (HR for 1 µg/L change 1.01, CI 1.00-1.01, p=0.022), CITP (HR for 1 µg/L change 1.22, CI 1.10-1.35, p<0.001), and CITP:MMP-1 (HR for 1 µg/L change 1.06, CI 1.00-1.12, p=0.045).

6 DISCUSSION

6.1 BIOMARKERS OF MYOCARDIAL FIBROSIS AND ASSOCIATIONS WITH LV SIZE AND FUNCTION

6.1.1 At baseline

We showed that PICP was independently associated with LVEDd and IVRT, but other studies have failed to show this (98, 101). However, our results suggest that with increasing LV size, there is an increase in type I collagen synthesis and increased myocardial fibrosis. Myocardial fibrosis is associated with diastolic dysfunction (97), and a shorter IVRT indicates a more severe form of diastolic dysfunction. Although IVRT was within the normal range in our study, it was shorter in the group with PICP above median. There was also a weak and direct correlation between PICP and left atrial diameter, and left atrial enlargement is associated with LV diastolic dysfunction (139). Measuring volume is a more accurate estimation of left atrial size, but a diameter more than 47 mm indicated left atrial enlargement (140).

We showed no association of CITP with echocardiographic variables of the LV which is in line with other studies of primarily ischemic HF and DCM (98, 141). CITP was higher in the group above or equal to median PICP compared with the group below median, to restore collagen homeostasis, but the PICP to CITP ratio indicated type I collagen synthesis to be predominant over degradation. Thus, since there was an increase in collagen accumulation CITP was not associated with LV size or function.

In conclusion, increased type I collagen accumulation is associated with increased LV dilation and diastolic dysfunction, indicating progressive LV remodeling.

6.1.2 Changes from baseline to 12 months

Improved indices of LV function and size in all patients were noticed and expected after the optimizing period at the nurse-led outpatient HF clinic. These findings were in parallel with a lower degree of biomarker assessed type I collagen degradation and increased type I collagen cross-linking, compared with baseline. I.e., signs of reverse LV remodeling were in parallel with decreased type I collagen degradation, indicating of a more stable type I collagen scaffold in the ECM.

LV dilation and depressed LVEF were associated with decreased collagen cross-linking, and after the administration of an ACE-I, the reduction of collagen cross-linking was stopped and LV size and function improved (123). This is most likely seen in our study too.

There were also signs of reverse LV remodeling in the changes from baseline to 12 months in the alive, paralleled with lower type I collagen synthesis, but not in the later deceased.

There were weak positive correlations between changes in PIIINP and LVMI, and between CITP:MMP-1 and left atrial diameter. This suggests that increased type III collagen

metabolism is a part of increased cardiac mass, and that decreased type I collagen cross-linking is accompanied by increased left atrial size.

Temporal changes of biomarkers of ECM collagens have not been examined as extensively as baseline levels. In HF with dilated LV and depressed EF and mainly of non-ischemic origin, the greatest decrease in CITP over 12 months was associated with the greatest improvement of LV size and function after patients received a BB in addition to RAS-inhibitor (156). PICP remained unchanged in the same study. Changes in collagen biomarkers is mostly investigated in acute MI (142, 143).

In conclusion, optimized pharmacotherapy during 12 months showed evidence of reverse LV remodeling and a simultaneous decrease in type I collagen degradation most likely due to increased type I collagen cross-linking rendering a more stable ECM scaffold to support the cardiomyocytes.

6.1.3 Dyssynchrony

No associations between markers of collagen metabolism and indices of mechanical dyssynchrony assessed by echocardiography were noticed. To the best of our knowledge, this has not been addressed before or after our study. The mean QRS duration was 117 ms in our patients indicating a probable low degree of interventricular dyssynchrony.

A review concluded that PICP is the best biomarker of the ECM to predict CRT response (145), and a value below the proposed cutoff of PICP increased the likelihood of CRT-response by 7.8 times and correlated with reverse remodeling (128). However, focal scarring and not interstitial fibrosis assessed by CMR predicted reverse remodeling after CRT (146). The levels of biomarkers of type I and III collagens increase after CRT implantation, as mentioned in section 2.11. Evidence of mechanical dyssynchrony has not been able to predict response to CRT (147).

In conclusion, myocardial fibrosis assessed by circulating levels of biomarkers of type I collagen, is not associated with indices of mechanical dyssynchrony which in turn are not associated with response to CRT. I.e., myocardial fibrosis is not related to mechanical LV dyssycnhrony.

6.2 BIOMARKERS OF MYOCARDIAL FIBROSIS AND ASSOCIATIONS WITH CLINICAL FINDINGS AND BNP

6.2.1 At baseline

The patients above median PICP expressed a more severe form of HF of longer duration associated with higher BNP compared with the patients below median PICP. and NYHA functional class was borderline higher.

Similarly, patients above median CITP expressed signs of worse HF with higher BNP, and worse NYHA functional class compared with patients below median CITP.

CITP, and the PICP to CITP ratio, also correlated with NYHA functional class in our study, and patients with symptoms of HF at one- and two-years post MI had higher CITP compared with patients without symptoms (155).

BNP in the acute setting was independently associated with PICP and CITP. BNP is released upon increased myocardial wall tension, both in pressure and volume overload conditions (144, 145), and is associated with both diastolic and systolic LV dysfunction (146). This means BNP can be elevated both in conditions when collagen synthesis is predominant as well as in circumstances when collagen degradation is increased. BNP has proved to attenuate types I and III collagen synthesis and other factors involved in myocardial fibrosis (147, 148). Hence, the patients in our study with HF with depressed LVEF with a mixed etiology expressed both pressure and volume overload conditions. Our results support previous findings (98). BNP is associated with NYHA functional class (149).

In conclusion, increasing type I collagen synthesis and degradation are both associated with increasing BNP in the acute setting, indicating a more severe form of symptomatic HF with increased collagen metabolism. Increasing type I collagen degradation also correlated with NYHA functional classification.

6.2.2 Changes from baseline to 12 months

At the 12-month follow-up of all patients, a significant increase in RAS-inhibitor dose was achieved and signs of clinical improvement (lower NYHA functional classification and BNP). These findings were in parallel with a lower degree of biomarker assessed type I collagen degradation and increased type I collagen cross-linking, compared with baseline.

The changes in clinical variables from baseline to 12 months, showed improvements in NYHA functional class and BNP in the alive, but not in the later deceased. PICP did not increase as much in the alive as in the later deceased, but other than this no other changes in collagen biomarkers were seen.

There were weak correlations between changes in PICP and BNP, and CITP and NYHA. We could not find any similar study that reported on this.

In conclusion, along with previously mentioned signs of reverse remodeling following optimization of pharmacotherapy during 12 months, decreasing type I collagen degradation and increasing type I collagen cross-linking were in parallel with clinical improvement. The correlations between changes in PICP and BNP, and CITP and NYHA were consistent with baseline results.

6.3 BIOMARKERS OF MYOCARDIAL FIBROSIS AND ASSOCIATIONS WITH ATRIAL FIBRILLATION

Although no significant differences in PICP were seen between the AF and SR groups, a weak and direct correlation existed between PICP and left atrial diameter in all patients. This and an independent relation of PICP with AF was confirmed in a previous study of patients

with AF (110). Increased type I collagen (and type III) has been histologically verified in the left atria of patients with AF compared with SR (108). In the dog model of HF-induced AF, histology of the atria showed myocyte loss, hypertrophy, and atrial interstitial fibrosis, with the latter interfering with atrial electrical conduction and creating a substrate for AF (150). Left atrial enlargement is a common feature in AF and it is the strongest predictor of new-onset AF (151). AF can cause left atrial enlargement (152) and vice versa (153) and can be caused by conditions with pressure or volume overload.

CITP showed a weak correlation with left atrial size in the present study, which together with an association with different types of AF was observed in another study (154).

A previous paper from the OPTIMAL study showed that cardiopulmonary exercise capacity in HF with depressed LVEF was similar between groups with AF or SR, even though LV volumes were smaller and LVEF higher in the AF group compared with the SR group (155). It was proposed that AF in HF is not a result of deteriorating systolic LV function but that patients with AF and concomitant HF are of a different phenotype.

Baseline PIIINP and CITP were predictors of incident AF in a long-term follow-up of individuals without CV disease, but not in patients with established CV disease (156).

PICP and CITP are likely of both ventricular and atrial origin, and the association of PICP in HF complicated with AF in the present study could mean that patients with HF and coexisting AF have a more pronounced fibrosis. It could be argued that in our study HF begets AF, with disrupted collagen metabolism in a set of patients with both pressure and volume overload conditions to cause HF. Left atrial size was increased and did not differ between the AF and SR groups, and increased LA size can be seen both in AF and due to diastolic dysfunction in HF. The same factors that induce increased collagen synthesis in the LV apply in the atria (e.g., TGF-β, aldosterone, and angiotensin II). Increased left atrial size could be a sign of a more severe form of myocardial fibrosis.

In conclusion, increasing type I collagen metabolism (as assessed by PICP and CITP) correlates with increasing left atrial size, which can be the result of several causes as discussed, and can promote AF. We cannot determine if there is a more severe form of fibrosis in the atria in the patients with AF.

6.4 BIOMARKERS OF MYOCARDIAL FIBROSIS AND ASSOCIATIONS WITH OUTCOME

6.4.1 At baseline

Baseline PICP and CITP were both independently associated with CV and all-cause mortality, and CITP also for non-CV mortality. Similar findings have been noticed in both HF with depressed and preserved EF (101, 103, 112, 157) and CITP also predicted mortality after MI (158, 159).

PICP:CITP is thought to be an index of the degree of collagen synthesis over degradation, and the later deceased exhibited a more degrative state than the alive with higher CITP and hence lower PICP:CITP, but that was not associated with LV dimensions or function. However clinically, baseline NYHA functional class was worse in the later deceased than in the alive.

In conclusion, increasing type I collagen metabolism (assessed by PICP and CITP) is associated with mortality. A degrative state of the ECM with reduction in the collagen scaffold, reflected by increasing type I collagen degradation, is the best predictor of mortality.

6.4.2 Changes from baseline to 12 months

Univariate, but not multivariate, analyses of 12-month levels of PICP and CITP predicted mortality and borderline baseline and 12-month levels of CITP:MMP-1. Thus, we failed to prove independent association of PICP and 12 months in addition to our previous result on baseline levels. This was at least in part due to lack of power secondary to few patients.

In conclusion, after optimization of pharmacotherapy with increased dosages of RAS-inhibitors, the type I collagen became more stable, indicating worse prognosis in patients with a lower degree of collagen cross-linking, both at baseline an at 12 months. Higher levels of type I collagen synthesis and degradation are likely predictors of mortality also at 12 months.

6.5 CONCLUDING REMARKS AND LIMITATIONS

Our studies have showed that PICP, CITP, and CITP:MMP-1 may be considered biomarkers of myocardial fibrosis in the absence of other conditions known to alter collagen metabolism. Most studies examining these biomarkers look at HF patients with a single etiology, but our patients have a mixed etiology and therefore represent the typical patients in an HF clinic. The biomarkers of type I collagen metabolism are associated with LV size and function, clinical findings, AF, and outcome. They are also altered after optimization of pharmacotherapy in parallel with objective and subjective improvements in HF.

Our studies have a small sample size, especially in study IV, and we may not have enough power to detect an effect of the temporal changes in the biomarkers of fibrosis on outcome. Thus, our studies are mainly of hypothesis-generating character. Also, we did not have a group of controls in our study, which makes it hard to extend our results beyond the participants in this study. However, our results are similar with previous research on patients with HF of mostly a single etiology and depressed EF.

In addition to being a small study, other factors limit our results The OPTIMAL study took place in the mid-1990s and at that point in time HFrEF was considered if LVEF was ≤45% and HFpEF if LVEF was higher than that. Today, HF classification includes HFrEF, HFmrEF, and HFpEF. Pharmacotherapy has also changed with guidelines recommendations on “old” and new drugs to be added to the toolbox, but undertreatment is not uncommon even today. The patients in OPTIMAL received optimized pharmacotherapy so they cannot be

considered undertreated, not by earlier standards at least. Echocardiography (hardware and software) has improved significantly with time, and recommendations on measurements have changed in some areas (e.g., atrial volume is preferred over diameter and AVPD is not frequently used).

7 CONCLUSIONS

- Increasing levels of PICP are associated with increasing LV size, and signs of worse diastolic function. Increasing levels of PICP and CITP are related to increasing left atrial size.

- Increasing levels of PICP and CITP are associated with increasing levels of BNP, and increasing levels of CITP correlate with worse NYHA functional class. BNP and NYHA are associated with each other, and mainly increased type I collagen degradation provides information on clinical HF severity.

- PICP and CITP, i.e., type I collagen metabolism does not relate to indices of mechanical dyssynchrony. Evidence of mechanical dyssynchrony is not associated with CRT response. However, lower levels of PICP have been associated with CRT response.

- Increasing levels of PICP and decreasing LVEDd are associated with HF and co-existing AF.

- Baseline PICP and CITP are independent predictors of CV- and all-cause mortality, with CITP suggested to be more important. Increased type I collagen synthesis, and especially type I collagen degradation, at baseline are associated with a worse prognosis.

- Optimization of HF pharmacotherapy during 12 months reduces CITP and CITP:MMP-1 in parallel to improvement in clinical findings (BNP and NYHA functional class,) and signs of reverse LV remodeling (LV size and function).

- In univariate analyses, higher 12-month levels of PICP and CITP are associated with a worse prognosis, and lower levels of CITP:MMP-1 at baseline and 12 months were associated with a better prognosis.

- Increasing levels of PIIINP correlated with increasing levels of BNP, larger LVESd, and greater LVMI. There were no associations of PIIINP with outcome.

8 POINTS OF PERSPECTIVE

We conclude that PICP, CITP, and CITP:MMP-1 may be considered biomarkers of myocardial fibrosis (in the absence of other conditions known to alter collagen metabolism) as discussed earlier, but they are not quite ready to be used in a clinical setting. However, biomarkers of collagen are already in use for monitoring the effect of treatment in other diseases such as osteoporosis and the potential side effect of methotrexate induced hepatic fibrosis in patients with rheumatoid arthritis. The same could apply to future pharmacotherapy aimed at reducing myocardial interstitial fibrosis. There should be "just enough" collagen in the ECM to prevent potentially harmful effects.

Many studies of myocardial fibrosis in HF are quite heterogenous in the selection of LVEF, and sometimes the different types of HF are used interchangeably. It became popular to study biomarkers of myocardial fibrosis in a wide variety of cardiac conditions some 15-20 years ago. Not all studies state exclusion of patients with conditions known to alter the markers of collagen metabolism, making it even harder to evaluate the results from the studies.

It could be useful to perform a case-control study including all types of HF patients with appropriate treatment according to existing guidelines and examine collagen markers of fibrosis in relation to the dimensions and function of the heart, and outcomes. The increased collagen accumulation in myocardial fibrosis is the result of many factors such as cytokines, growth factors, and hormones etc. A multi-panel test may possibly give information on the present status of fibrotic activity.

Today, some studies have proposed cut-off values for the markers studied, but these values can differ significantly from one study to another. Cut-off values for prognostication should be calculated, both for specific etiologies of HF and in a mixed etiology. The temporal changes of collagen biomarkers should also be addressed better with serial measurements over time since fibrosis is a dynamic process and today only a handful of studies exist on this subject.

If a biomarker of fibrosis could indicate a higher risk of future need for more advanced treatment, this could prove helpful, especially in the younger patients who could be candidates for heart transplantation.

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