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HEMODYNAMICS, ECHOCARDIOGRAPHY, AND BIOMARKERS IN DIFFERENT HEART FAILURE PHENOTYPES

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*To my parents **Hikmat** & **Sauhama** and my beloved family:*
Ghosoon, Vanessa, Leandro,** and **Valerio

"You give but little when you give of your possessions.
It is when you give of yourself that you truly give."
Kahlil Gibran, The Prophet

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1. ABSTRACT

Background
Heart failure (HF) is a major health problem affecting millions of patients worldwide and is associated with impaired quality of life and poor prognosis. Different approaches including invasive and non-invasive methods can be used to provide information about the cardiac performance in HF patients. The overall aim of this thesis was to study a number of different but complementary invasive and non-invasive methods that are currently used to assess cardiac performance in different HF phenotypes, mainly in advanced HF.

Methods and results
Study I
Twenty-three patients with advanced stable chronic HF received a single 24 h levosimendan infusion. Levosimendan had the following effects (median change ± SD): a significant increase in cardiac output (+9.8 ± 21.6%; P = 0.026) and decrease in N-terminal pro-brain natriuretic peptide (-28.1 ± 16.3%, P < 0.001), estimated total peripheral resistance (-16.9 ± 18.3%, P = 0.004), and mean arterial pressure (-5.9 ± 8.2%, P = 0.007). There was no change in estimated glomerular filtration rate (+0.89 ± 14.0%, P = 0.955). No significant associations between baseline clinical and/or hemodynamic factors and the change in CO were found.

Study II
Levels of soluble suppression of tumorigenicity 2 (sST2) were investigated in HF with reduced ejection fraction (HFrEF), preserved EF (HFpEF) and in healthy controls. Crude sST2 levels were higher in HFrEF compared to HFpEF and controls. sST2 was associated with the composite endpoint of death or HF hospitalization in HFpEF, adjusted hazard ratio (HR) per log increase in sST2 6.62, 95% confidence interval (CI) 1.04–42.28, p=0.046, and in HFrEF with death, heart transplant or left ventricular assist device (LVAD); 3.51, 95% CI 1.05–11.69, p=0.041.

Study III
In 192 patients with hemodynamic findings indicating HF, right heart catheterization waveforms were used to measure the pulsatile and steady components of the pulmonary capillary wedge pressure (PAWP) and to assess their impact on the pulmonary arterial compliance (PAC) and pulmonary vascular resistance (PVR) relationship. PAC and PVR were hyperbolically and inversely associated. In the patient cohort with higher pulsatile PAWP component, there was a significant downward and leftward shift of the PAC-PVR curve fit. The steady PAWP component did not impact significantly on the PAC-PVR relationship.

Study IV
Data from 14 LVAD patients assessed by echocardiographic ramp test was retrospectively reviewed. Adequate left ventricular (LV) unloading was defined as no more than mild mitral regurgitation, and intermittent aortic valve (AV) opening or closed AV, and reduction of LV end-diastolic diameter, and for the follow-up measurement, decreased NT-proBNP. Ramp testing resulted in final LVAD speed increase in 79% of patients and a median net change of 200 (200; 300) revolutions per minute. Speed adjustments after ramp testing resulted in improved LV unloading in additional 21% of patients who were not originally optimized. Right ventricular function did not worsen.

Conclusion
By assessing hemodynamics, echocardiography and biomarkers, it is possible to gain a better understanding of the different HF phenotypes and the underlying physiology, which may help to optimize care and introduce potential targets for therapy.

2. SAMMANFATTNING

Bakgrund
Hjärtsvikt är en vanlig sjukdom som leder till försämrad livskvalitet och sämre prognos. Olika metoder kan användas för att utvärdera hjärtfunktionen hos hjärtsviktpatienter. Syftet med avhandlingen var att studera olika invasiva och non-invasiva metoder för att kartlägga och optimera hjärtfunktionen i olika hjärtsviktsfenotyper med primärt fokus på avancerad hjärtsvikt.

Metoder och resultat
Studie I
23 patienter med avancerad, stabil hjärtsvikt fick levosimendaninfusion i 24 timmar. Blodprover och hjärtfunktion utvärderades före och efter infusionen. Levosimendan hade positiva hemodynamiska effekter efter bara en infusion men inga prediktorer för den positiva effekten kunde identiferas.

Studie II
I en retrospektiv analys av patienter med hjärtsvikt med sänkt (HFrEF) och bevarad (HFpEF) ejektionsfraktion undersöktes nivåer av Suppression of tumorigenicity (sST2) och associationer med kliniska parametrar och outcome. Patienter med HFpEF hade lägre nivåer av sST2 jämfört med HFrEF. Högre sST2 nivåer var associerade med svårare hjärtsvikt och sämre prognos i båda hjärtsviktstyperna.

Studie III
Hos 192 patienter med HFpEF och HFrEF studerades data från hjärtkaterisering för att utvärdera effekten av de olika komponenterna av wedgetryck (PAWP) på relationen mellan pulmonell arteriell compliance (PAC) och resistans (PVR). Vi bekräftade den tidigare kända påverkan av PAWP på PAC-PVR förhållandet och vi visade att den pulsatila komponenten i PAWP hade större betydelse än den statiska.

Studie IV
Ekokardiografiskt ramp-test utfördes på 14 patienter med vänsterkammarassist (LVAD) 1-3 månader postoperativt för att se om avlastning av vänsterkammare hos patienter med LVAD kan förbättras med ekokardiografisk utvärdering (ramp-test). Ramp-testet bidrog till justering av pumphastighet och optimering av hemodynamik utan försämring av högerkammarfunktionen.

Slutsats
Invasiva och icke-invasiva metoder kan användas i olika typer av hjärtsvikt för att få bättre uppfattning om patofysiologin och bättre kunskaper om prognosen och behandlingen.

3. LIST OF SCIENTIFIC PAPERS

I. Najjar, E., M. Stalhberg, C. Hage, E. Ottenblad, A. Manouras, I. Haugen Lofman and L. H. Lund (2018). "**Haemodynamic effects of levosimendan in advanced but stable chronic heart failure.**" ESC Heart Fail 5(3): 302-308.

II. Najjar, E., U. L. Faxen, C. Hage, E. Donal, J. C. Daubert, C. Linde and L. H. Lund (2019). "**ST2 in heart failure with preserved and reduced ejection fraction.**" Scand Cardiovasc J 53(1): 21-27.

III. Najjar, E., L. H. Lund, C. Hage, J. Johnson and A. Manouras. "**Left atrial pressure pulsatility attenuates pulmonary arterial compliance in heart failure.**" In Manuscript.

IV. Najjar, E., T. Thorvaldsen, M. Dalén, P. Svenarud, A. Hallberg Kristensen, M. J. Eriksson, E. Maret and L. H. Lund. "**Validation of non-invasive ramp testing for HeartMate 3.**" Submitted to ESC Heart Failure.

4. ABBREVIATIONS

ACEI	Angiotensin-converting enzyme inhibitor
ADHF	Acute decompensated heart failure
ARB	Angiotensin receptor blocker
ARNI	Angiotensin receptor neprilysin inhibitor
AV	Aortic valve
BMI	Body mass index
BSA	Body surface area
BTT	Bridge to transplantation
CI	Cardiac index
CI	Confidence interval
CO	Cardiac output
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration
CRT	Cardiac resynchronisation therapy
CV	Coefficient of variation
CVP	Central venous pressure
DT	Destination therapy
E/e’	Left ventricular transmitral early diastolic filling velocity/ left ventricular early diastolic myocardial velocity
EF	Ejection fraction
eGFR	Estimated glomerular filtration rate
eTPR	Estimated total peripheral resistance
HF	Heart failure
HFmrEF	Heart failure with mid-range ejection fraction
HFpEF	Heart failure with preserved ejection fraction
HFrEF	Heart failure with reduced ejection fraction
HR	Hazard ratio
HRAEs	Hemocompatibility-related adverse events
HTx	Heart transplantation
Innocor®	Inert gas re-breathing method
ICD	Implantable cardioverter defibrillator
IVC	Inferior vena cava
KaRen	Karolinska-Rennes Study
LA	Left atrium
LAP	Left atrial pressure
LAVi	Left atrial volume index
LV	Left ventricle

LVAD	Left ventricular assist device
LVEDD	Left ventricular end-diastolic diameter
LVMi	Left ventricular mass index
MAP	Mean arterial pressure
MR	Mitral regurgitation
MRA	Mineralocorticoid receptor antagonist
NPs	Natriuretic peptides
NT-proBNP	N-terminal pro-brain natriuretic peptide
NYHA	New York Heart Association
PAC	Pulmonary arterial compliance
PAP	Pulmonary artery pressure
PAPD	Diastolic pulmonary artery pressure
PAP_M	Mean pulmonary artery pressure
PAPS	Systolic pulmonary artery pressure
$PAWP_M$	Mean pulmonary capillary wedge pressure
PAWPp	Pulsatile PAWP
PAWPs	Steady PAWP
PBF	Pulmonary blood flow
PH	Pulmonary hypertension
PVR	Pulmonary vascular resistance
RAAS	Renin angiotensin aldosterone system
RC-time	Time-constant of the pulmonary circulation
RHC	Right heart catheterization
RPM	Revolutions per minute
RV	Right ventricle
RVEDD	Right ventricular end-diastolic diameter
SNS	Sympathetic nervous system
ST2	Suppression of tumorigenicity
SV	Stroke volume
TAPSE	Tricuspid annular plane systolic excursion
TR	Tricuspid regurgitation
Q1	Lower quartile
Q3	Upper quartile

5. INTRODUCTION

5.1 General aspects of heart failure

Definition

Heart failure (HF) is defined by the European Society of Cardiology (ESC), as "a clinical syndrome characterized by typical symptoms (e.g. breathlessness, ankle swelling and fatigue) that may be accompanied by signs (e.g. elevated jugular venous pressure, pulmonary crackles and peripheral oedema) caused by a structural and/or functional cardiac abnormality, resulting in a reduced cardiac output (CO) and/or elevated intracardiac pressures at rest or during stress." (1).

Cardiac performance is dependent on heart rate, contractility, preload, and afterload and both diastolic and systolic function must be maintained for normal cardiac function (2). However, much of HF research and therapy has focused on systolic dysfunction, partly due to the widespread use of reduced left ventricular (LV) ejection fraction (EF) for diagnosing HF in clinical practice, acceptance of EF as a predictor of prognosis in HF patients, and the use of EF for selecting patients into clinical trials of HF therapy (3). Studies in the early 2000s explored the impact of normal or mildly reduced EF in HF patients and initially diastolic and systolic HF were considered as distinct phenotypes within the HF spectrum (4, 5). The echocardiographic *Recommendations for chamber quantification* from 2005 recommended the assessment of the LV diastolic function and considered left atrial (LA) enlargement as a marker of both the severity and chronicity of diastolic dysfunction and magnitude of LA pressure (LAP) elevation (6).

Current classification

In 2016, a new classification of chronic HF was proposed by the ESC, based on EF; HF with reduced EF (HFrEF) where EF is <40%, mid-range EF (HFmrEF) with EF 40-49%, and preserved EF (HFpEF) where EF is ≥50%. The old nomenclature of systolic and diastolic HF is abandoned in the current guidelines since diastolic dysfunction can exist throughout the EF spectrum, and vice versa, since systolic function is not necessarily normal in HFpEF (1). According to the ESC guidelines and contemporary trials, the diagnosis of HFmrEF and HFpEF also requires elevated natriuretic peptides (NPs) and relevant structural or functional heart disease such as LV hypertrophy, LA enlargement, and/or diastolic dysfunction. However, the precision and reproducibility of echocardiography, the most frequently used imaging modality to assess EF in these patients, is probably not good enough to categorize HFrEF, HFpEF, and HFmrEF reliably and reproducibly (7, 8).

Advanced HF

HF is a progressive clinical syndrome and many HF patients ultimately progress to advanced HF, refractory to evidence-based therapies (9). Patients with advanced HF comprise an estimated 5 % of the overall HF population but the prevalence is increasing along with the ageing population as well as due to more efficient therapies in ischemic heart disease and HF leading to improved survival (10, 11). Prior definitions of advanced HF have changed since 2007 when the Heart Failure Association (HFA) identified advanced HF as a stage where conventional therapies were insufficient to control the patient's symptoms, and advanced treatments [heart transplantation (HTx) or mechanical circulatory support] or palliative therapies (e.g. inotropic agents, ultrafiltration or peritoneal dialysis to control volume, or

end-of-life measures) were needed (12). In the new HFA statement, the previous definition of advanced HF has been revised to acknowledge the importance of HFpEF (13). The current guidelines include the following criteria for defining advanced HF: [1] Severe and persistent symptoms of HF [advanced New York Heart Association (NYHA) class III or IV], [2] severe cardiac dysfunction defined according to the ESC criteria, to include all HF patients independent of EF, [3] pulmonary or systemic congestion episodes, episodes of low output requiring inotropes/vasoactive drugs, malignant arrhythmias causing >1 unplanned visit or hospitalization in the last year, and [4] severe impairment of exercise capacity (13).

Pathophysiology

Controversy remains as to whether HFpEF is the same disease as HFrEF albeit with preserved EF, if HFpEF is an entirely distinct entity with different pathophysiologic background, or finally if it is merely a consequence of ageing and related comorbidities (14-16). However, a prevailing view is that myocardial remodeling in HFrEF is driven by loss of myocardial function secondary to an initial injury, e.g. myocardial infarction, which triggers maladaptive neuro-hormonal activation [sympathetic nervous system (SNS), renin angiotensin aldosterone system (RAAS), and antidiuretic hormone (ADH)], causing increased load, myocardial remodeling including LV dilatation, and eccentric hypertrophy leading to manifest HF. In contrast, in HFpEF, a systemic pro-inflammatory state induced by comorbidities is believed to lead to endothelial damage and microvascular dysfunction through decreased nitric oxide and cyclic guanosine monophosphate, which ultimately results in concentric LV remodeling and reduced myocardial compliance (17), **Figure 1**. This new paradigm is mainly supported by the different patterns of structural and functional changes in HFpEF compared to HFrEF, and the disappointing results of previous trials employing neurohormonal antagonists in HFpEF (1, 18-20). Nevertheless, neurohormonal activation exists in both HF entities, but it is thought to play a less dominant role in HFpEF.

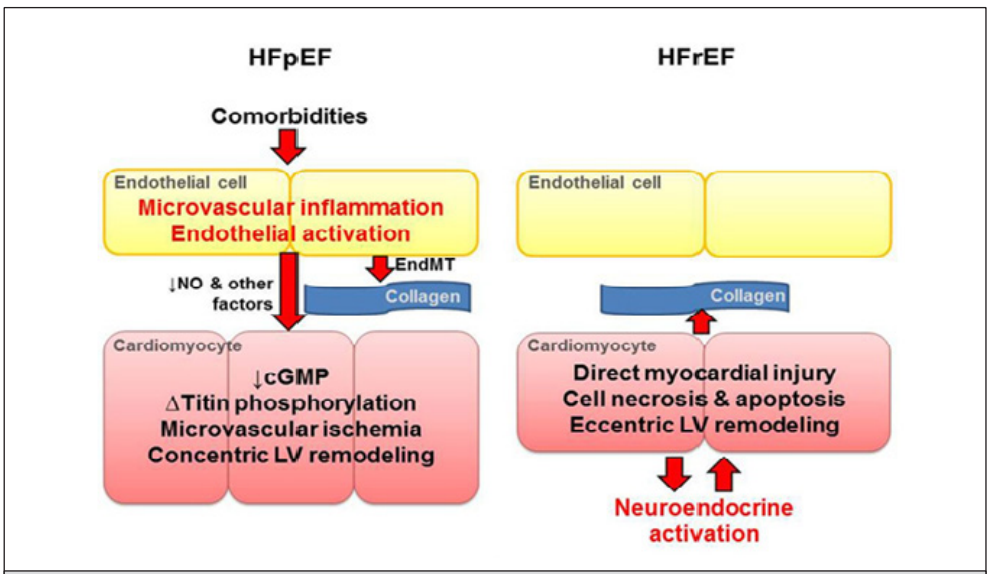

Figure 1. Overview of the pathogenesis in HFpEF and HFrEF.
Heart, Lam CSP, Lund LH, 2016, reproduced with permission from the publisher.

Epidemiology and prognosis

HF is a major and growing public health problem, affecting millions of patients worldwide with a prevalence and incidence of 3% and 0.5% respectively (21, 22). HF prevalence is rising to ≥10% among people >70 years of age (23, 24) and is estimated to increase by 25% by 2030 (25). While HFrEF incidence is declining in the last decade due to effective revascularization of patients with acute coronary syndromes (26, 27), the prevalence and incidence of HFpEF, is increasing due to improved recognition and an ageing population (28).

HF is the leading cause of hospitalization in patients >65 years (29), and is associated with severely compromised quality of life (30, 31), poor prognosis (32-34), and high socioeconomic burden (35). An analysis of the ESC Heart Failure Long-Term Registry described 1-year mortality in ambulatory HF patients in Europe, stratified by EF (HFrEF 8.8% , HFpEF 6.3%, and with HFmrEF intermediate, 7.6%) (36). Among patients hospitalized with HF, patients across the EF spectrum have a similarly 5-year mortality HFrEF (75.3%) vs. HFpEF (75.7%) vs. HFmrEF 75.7% with an increased risk for cardiovascular and HF admission (37). Repeated HF hospitalizations is a strong predictor of mortality and approximately half of patients will be dead by 1 year after 3 hospitalizations (38).

Treatment

HFrEF: Blocking the SNS and the RAAS has been the corner stone of modern HFrEF treatment since the CONSENSUS trial from 1987 which showed that the angiotensin converting enzyme inhibitor (ACEi) Enalapril vs. placebo reduced mortality by 27% in NYHA IV (39). Even the use of angiotensin receptor blockers (ARB) in patients intolerant to ACEi reduced cardiovascular mortality and morbidity (40). Similarly, the use of beta-blockers also showed a positive effect on mortality in different trials (CIBIS II, MERIT-HF, COPERNICUS) (41-43). The treatment with mineralocorticoid receptor antagonist (MRA) Spironolactone, an aldosterone antagonist blocking the RAAS, resulted in an incremental mortality reduction of 30% when added to ACEi in patients in NYHA class III-IV (44). In 2014, a new agent was introduced targeting the endogenous compensatory adaptive responses in HF, namely; the angiotensin receptor neprilysin inhibitor (ARNI) which reduces the degradation of vasoactive peptides (45) including natriuretic peptides, bradykinin and adrenomedullin. Since then a new strategy has been used to treat HFrEF based on inhibiting the maladaptive compensatory responses and enhancing the endogenous compensatory responses.

Interestingly, novel antidiabetic agents known as sodium-glucose cotransporter 2 inhibitors (SGLT2i) which act by inhibiting glucose reabsorption in proximal convoluted tubules of kidney were shown to reduce the risk for cardiovascular death and worsening HF among adults with HFrEF regardless of diabetes status (46). More recently, Vericiguat which is a novel class of drug targeting the nitric oxide (NO) pathway by stimulating the NO receptor soluble guanylate cyclase (sGC), has shown to reduce the risk of the composite endpoint of HF hospitalisation or cardiovascular death, compared to placebo in patients suffering from worsening chronic HFrEF in the VICTORIA trial (47).

Cardiac resynchronization therapy (CRT) and implantable cardioverter-defibrillators (ICDs) improve outcomes in selected patients with HFrEF in multiple clinical trials (48-51); however, the benefit of ICD in patients with CRT is less certain (51).

HFpEF: There is yet no evidence-based therapy in HFpEF and previous trials of ACEi, ARBs, beta-blockers, and MRA have all been disappointing and failed to show improved survival (1, 52-55). Recently the use of ARNI has failed to reduce the incidence of cardiovascular death or hospitalization for HF compared with valsartan in HFpEF (56). The current ESC guidelines recommend the treatment of coexisting comorbidities and measures to reduce symptoms from volume overload.

HFmrEF is increasingly recognized as phenotypically more similar to HFrEF, and post-hoc analyses from CHARM-Preserved (57), TOPCAT (58), and a beta-blocker meta-analysis (59), as well as from a sub-group analysis in PARAGON-HF (56), suggest that conventional HFrEF therapy may be effective also in patients with mildly reduced EF, patients that previously and for trial purposes were considered to have HFpEF.

Advanced HF therapy
Inotropic agents
Advanced HF therapies include durable long-term mechanical circulatory support devices (MCS) or HTx. However, in situations where the patient's clinical condition deteriorates, or end-organ function is compromised, short-term therapies like inotropic agents may be needed until MCS can be implanted or while the patient is waiting on the transplant list (13).

Intravenous inotropes (milrinone, dobutamine and dopamine) improve hemodynamics and end-organ perfusion and relieve symptoms, but due to short half-lives, they are limited to selected in-patients and may be associated with increased mortality (60-64).

Long-term or chronic treatment with inotropes for patients waiting for HTx is not routinely recommended. However, continuous or intermittent out-patient inotrope infusions may be required to prevent hospitalization and preserve perfusion and end-organ function, especially those who may not be suitable for bridging with left ventricular assist device (LVAD) (13). Yet, this practice is controversial and may also be associated with increased mortality (65-67).

Levosimendan is a relatively novel inotropic agent that was approved in Europe in the 2000s for the treatment of acute decompensated advanced HF (ADHF). It is not licensed in the USA. Its positive hemodynamic effect may last for >7 days after a 12–24 h infusion because of the pharmacologically active metabolite with a long half-life (68). Levosimendan is an intravenous inodilator agent which main effect is mediated partly by calcium sensitization, purportedly without concomitant increase in myocardial metabolic demand or intracellular calcium levels (69, 70). In early studies in ADHF, levosimendan improved hemodynamics and mortality compared to dobutamine (71) and placebo (72). In later studies, it was not superior to dobutamine (73), and improved symptoms but increased hypotension and arrhythmia compared to placebo (74).

Intermittent use of levosimendan for long-term symptomatic improvement or palliation has gained popularity in the last decade and meta-analyses of several small trials of a repeated infusion strategy have suggested a positive effect on survival and a reduction in hospitalizations (75, 76). Even the LION-HEART pilot study that randomized 69 patients with advanced HF to placebo or levosimendan over 6 hours every 2 weeks for 12 weeks showed a significantly lower NT-proBNP over time in the levosimendan group compared to the placebo group (77). However, the beneficial effects of repetitive levosimendan use in advanced HF are still uncertain and a positive survival effect needs to be demonstrated in adequately sized, prospective studies.

Mechanical circulatory support and heart transplantation
For patients with advanced HF, HTx remains the best therapeutic option with 1-year survival of almost 90 % and median survival of 12.2 years. The number of HTx procedures are nevertheless stagnant due to shortage of donors (78). Although randomized controlled trials have never been conducted for HTx, there is overwhelming consensus within the cardiology community that HTx significantly improves survival, exercise capacity, and quality of life, provided that proper selection criteria are applied (1, 13). However, HTx is still challenged by limited effectiveness and complications of immunosuppressive therapy (e.g. infections, cardiac allograft vasculopathy, antibody-mediated rejection, late graft dysfunction, and malignancy) (78).

The use of durable MCS like LVADs in advanced HF has increased during the last decade due to organ shortage and advances in bioengineering (13, 79, 80). LVADs can be used as 1) destination therapy (DT) meaning permanent therapy for patients with contraindications for HTx, 2) as a bridge to transplantation (BTT) to maintain end-organ perfusion and increase the chance of survival while waiting for a HTx, 3) as a bridge to decision (BTD) to allow time for full clinical evaluation for HTx, 4) as a bridge to candidacy (BTC) to improve end-organ function like renal function or reverse contraindications like high pulmonary vascular resistance (PVR) in order to become eligible for HTx, and 5) as bridge to recovery (BTR) as in cases of myocarditis where cardiac recovery is facilitated by LVAD unloading together with intensive neurohormonal blockade, leading to reverse remodeling (81).

LVADs improve survival, functional capacity, and quality of life in appropriately selected HF patients (82-84). However, LVADs are still limited by complications like infection, right ventricular (RV) failure, and hemocompatibility-related adverse events (HRAEs) like thrombosis, stroke, and bleeding (85-87) despite the production of more hemocompatible devices; namely, HeartMate 3 (HM3) LVAD (Abbott, Lake Bluff Illinois) (88).

The landmark trial for LVADs as destination therapy as compared to medical therapy is The Randomized Evaluation of Mechanical Assistance for the Treatment of Congestive Heart Failure (REMATCH) trial from 2001. REMATCH showed improved 1-year survival of 52% in inotrope-dependent, transplant-ineligible patients with advanced HF treated with an LVAD, but 2-year survival of 23% was not statistically different (89). Since then, the one-year survival with LVAD has improved to >80% (82) mainly due to better patient selection, improved surgical techniques, and post-operative care.

5.2 Biomarkers in HF

What we usually refer to as biomarkers are any measurement associated with a biological state, but more narrowly and more commonly, a group of biological substances (like hormones, enzymes and genes) that can be objectively measured in blood or tissue and act as indicators of normal biologic processes, pathogenic processes, or responses to a therapeutic intervention (90). The most commonly utilized biomarkers in HF belong to the natriuretic peptide (NP) family which includes a large number of peptides and peptide fragments, related to e.g. A-type NP, B-type NP (BNP), and C-type NP (91). BNP and its amino-terminal cleavage fragment (NT-proBNP) are the most clinically used biomarkers in HF and are derived from the pro-enzyme proBNP, primarily released from ventricular cardiomyocytes in response to an increase in ventricular wall tension and are valuable for the diagnosis (92,

93), prognosis (94, 95), and may potentially improve HF management when used as serial measurements guiding therapeutic interventions (96, 97). BNP has many biological functions such as vasodilation, diuresis, and natriuresis, which represent compensatory mechanisms that counteract the activation of the RAAS and SNS in HF patients (98). Nevertheless, their use is limited by the fact that the levels typically rise and fall in parallel with hemodynamic measurements such as left ventricular end-diastolic pressure, i.e. they only give us an insight into one of the different mechanisms that are involved in the development of HF namely myocyte stress/stretch (99). Furthermore, NPs discriminate well, i.e. differentiate risk, but do not calibrate well, i.e. there is no universal cut-off or criterion that can be used for specific decisions, such as who to list for heart transplantation. Plasma levels of BNP and NT-proBNP are furthermore influenced by sex, age, renal function, pulmonary disease, and obesity (100-102). Higher BNP/NT-proBNP levels are associated with severity of HF and worse prognosis in both the acute and chronic setting across all EF phenotypes (103-105), however, normal BNP is sometimes found in patients with HFpEF despite increased filling pressures, particularly those with elevated filling pressures at exercise but not at rest (106).

The exploration of novel applications of established biomarkers and research on new reliable biomarkers that reflect different pathophysiological pathways and markers of potential therapeutic targets in HF have been expanding over the last decades (107). An example of such new biomarkers is Galectin-3, secreted by activated macrophages and closely associated with cardiac remodelling. Galectin-3 level is usually elevated in HF patients, associated with prognosis but is inferior to BNP and NT-proBNP at diagnosing HF (108, 109). Another example is Adrenomedullin (ADM), which is expressed in all body tissues and reflects the neurohormonal activation pathway. ADM has vasodilative, antiproliferating, and inotropic effects (110). ADM activation is more accurately reflected by its stable precursor fragment mid regional pro-ADM. ADM levels are elevated in HF and correlate to disease severity but has limited clinical application due to instability and short half-life in plasma (111-113). A third example is growth differentiation factor-15 (GDF-15), a marker of cell injury and inflammation (114). Elevated GDF-15 level is strongly related to NYHA class in HF and serve as a strong predictor of all-cause mortality in HFrEF and HFpEF (115, 116).

5.3 Supression of tumorigenicity 2

Suppression of tumorigenicity 2 (ST2) is a member of the interleukin (IL) 1 receptor family and has two main isoforms: a transmembrane receptor (ST2L) and a soluble receptor that can be detected in plasma (sST2) (117). It was first described in 1989 in inflammatory and autoimmune diseases (118, 119), is secreted by many cells in response to damage (120) and is part of a cardioprotective signaling system. When the ligand for ST2, Interleukin 33 (IL-33) is bound to ST2L, IL-33 exerts anti-fibrotic and anti-hypertrophic effects while the presence of sST2 inhibits these beneficial effects by neutralizing the beneficial activity of circulating IL-33 and thus leading to fibrosis (121, 122). Interestingly, sST2 is markedly induced in mechanically overloaded cardiac myocytes and reflects myocardial stress, ventricular remodelling, and fibrosis (123). Unlike NPs, it reflects not only load but also inflammatory and fibrotic pathways (124). sST2 is elevated in various cardiovascular conditions such as worsening of previous chronic HF, new onset HF, and myocardial infarction. However, its diagnostic ability for acute HF is inferior to NT-proBNP making sST2 less used as a diagnostic tool for HF (125). On the other hand, in acute HF independent of EF and in chronic HFrEF patients, sST2 levels are strongly associated with the severity of HF and are

strong and independent predictors of mortality (126-128). Interestingly, serial measurement of sST2 with higher levels at follow-up predict mortality in acute and chronic HF independent of natriuretic peptides (129-131). sST2 is less studied in HFpEF, especially in patients with chronic HFpEF (132-134).

The pathway of ST2 and its ligand IL-33 has emerged as a novel area of interest in HF and may be a therapeutic target in the prevention and treatment of cardiovascular diseases including HF (124, 135, 136).

5.4 Cardiovascular hemodynamics

5.4.1 Right heart catheterization

Right heart catheterization (RHC) is a widely used invasive procedure providing direct and accurate measurements of hemodynamics of the cardiovascular system. It was performed for the first time by Forssmann in 1929 (137). RHC enables direct and accurate assessment of the pulmonary and right heart pressures as well as an indirect yet accurate measurement of the left atrial pressure (LAP). Quantification of CO and PVR are critical components of invasive hemodynamic assessment. RHC is most commonly used in the following conditions: 1) diagnosis and differentiation of pulmonary hypertension (PH), 2) assessment of eligibility for LVAD, and heart and/or lung transplantation 3) evaluation prior to correction of cardiac shunt defects (138-140).

It is usually carried out using a 6F balloon-tipped fluid-filled Swan-Ganz catheter through the internal jugular vein. Mean right atrial pressure, pulmonary artery pressures (PAP), pulmonary capillary wedge pressure (PAWP) and RV systolic pressure are usually recorded under fluoroscopy at end-expirium during spontaneous breathing after calibration with the zero-level set at the mid-thoracic line, **Figure 2**. Thermodilution or Fick are usally used to measure CO (141-143).

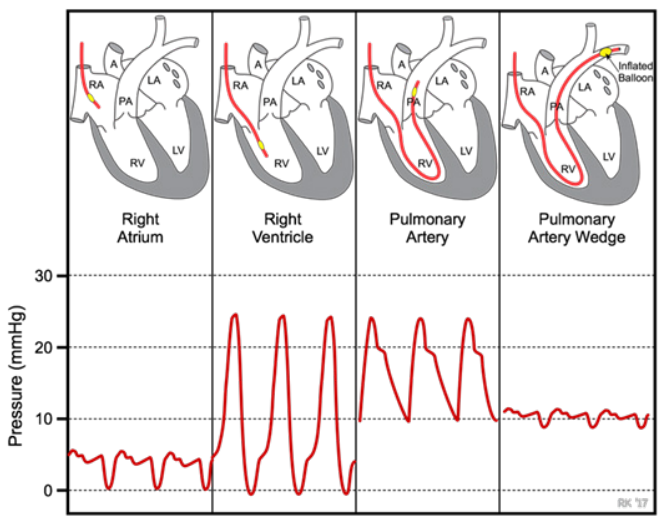

Figure 2. Illustrates RHC and normal pressure waves, https://www.cvphysiology.com, reproduced with permission from the publisher.

In general, the RHC is more time consuming and associated with a risk for complications. In the acute and advanced HF setting, trials suggest that RHC does not improve outcomes (126, 127), but it remains widely used particularly in the intensive care setting. For these reasons, non-invasive techniques, e.g. inert gas rebreathing (Innocor®, Innovision A/S, Denmark) and echocardiography, have been developed to assess CO.

5.4.2 Cardiac output

CO is the amount of blood pumped by the heart in one minute and is the product of heart rate (HR) and stroke volume (SV). The SV is the amount of blood ejected during each ventricular contraction and is dependent on preload, contractility, and afterload (144).

Various methods can be used to measure the SV and CO; some of them are invasive like angiography and cardiac catheterization (Direct Fick method, dye dilution or thermodilution) while others are non-invasive like echocardiography, cardiac magnetic resonance imaging, and inert gas rebreathing such as with the Innocor® device.

The direct Fick method is often considered the gold standard for the quantification of CO and is based upon measurements of the oxygen uptake together with calculation of the difference in oxygen saturation between arterial and venous blood. However, the clinical use of the Fick technique is less practical and the development of the thermodilution technique made this method the practical gold standard in the cardiac catheterization laboratory and intensive care settings (144).

5.4.3 Echocardiography

Assessment of the LV systolic and diastolic functions is the most clinically important task of echocardiography because of the potential association between all forms of heart disease and abnormalities of LV function, and because of the use of EF for determining indications for evidence based HF therapy. The difficulty in assessing the LV systolic function is related to the complexity of the heart, which is always in a dynamic state. Moreover, cardiac performance is continuously influenced by the prevailing hemodynamic conditions i.e. preload and afterload (145). Several echocardiographic approaches can be used to evaluate the LV systolic function. The most commonly used is the measurement of EF, which represents the proportion of blood within the LV that is ejected during each cardiac cycle. The biplane modified Simpson's rule is the recommended method for determining LV volumes and EF, taking into account the ventricular geometry by measuring LV cavity area in 2 planes perpendicular to each other (6). Other echocardiographic methods to determine the LV systolic function include determination of the SV by Doppler echocardiography, the use of tissue Doppler, the use of strain echocardiography, and the determination of the intraventricular pressure changes during early systole (dP/dt) (146-149).

Echocardiographic assessment of LV diastolic function is also an integral part of the routine evaluation of patients presenting with symptoms of dyspnea or HF and the current guidelines recommend a number of parameters to grade diastolic dysfunction and to estimate LV filling pressures (150).

5.4.4 Inert gas rebreathing method

Innocor® (Innovision A/S, Denmark) is an established, safe and non-invasive inert gas rebreathing technique used for assessing CO, pulmonary blood flow (PBF), SV, and other

parameters and is based on the Fick principle (151), **Figure 3**. The Innocor® uses a gas mixture of two inert gases in a closed rebreathing system; one completely and immediately blood soluble (nitrous oxide) and one insoluble (sulfur hexafluoride). The soluble gas dissolves in the blood of the lung capillaries and is subsequently washed out by the blood perfusing the lungs. The PBF, which in the absence of shunting equals CO, is proportional to the rate by which the soluble gas is washed out and is measured by a photoacoustic gas analyser. The insoluble gas is used to determine the lung volume needed for the calculation of the CO. This technique has been shown to correlate well with invasive measurements of CO (direct Fick, thermodilution and dye dilution), to be safe, easy to use, and comfortable for the patients in different clinical settings (152-154).

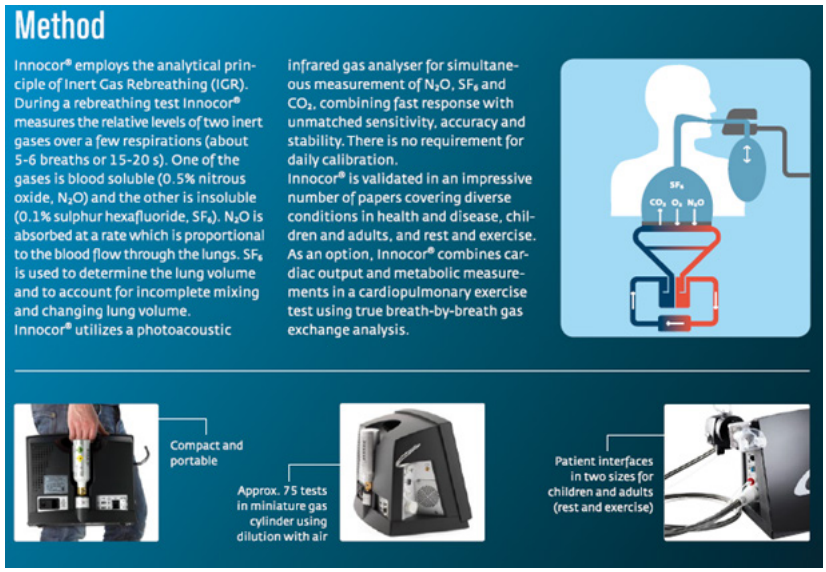

Figure 3. Measuring CO using Innocor®, http://www.innocor.dk/#Product, reproduced with permission from the publisher.

5.4.5 The cardiopulmonary unit

Increased RV afterload is the main pathophysiologic mechanism for RV failure and is mainly caused by LV systolic or diastolic dysfunction. Essentially all myocardial diseases involving the left heart and independent of EF may affect RV function like myocardial ischaemia/infarction, myocarditis/septic cardiomyopathy, takotsubo cardiomyopathy, dilated cardiomyopathy, and hypertrophic cardiomyopathy (155, 156).

The RV and the pulmonary vascular system are referred to as *the cardiopulmonary unit* where there is an interaction between the ventricular pump and its afterload (157). The RV afterload is composed of resistive and elastic components. The resistive component represents the opposition to forward flow and is usually described as the pulmonary vascular resistance (PVR) while the elastic component represents the energy required to overcome increased systolic pressure during ejection and is often evaluated using pulmonary arterial compliance (PAC) (158, 159).

In clinical practice and trials, PVR is usually used to describe the RV afterload and the potential improvement following a specific therapy. However, using only PVR to determine RV afterload is an inaccurate measure since it does not include the contributions of the elastic component (160, 161). Moreover, PAC decreases earlier in the disease process, when PVR is still normal (161).

Previous studies have shown that there is an inverse and hyperbolic relationship between PAC and PVR and advocated for a constancy of their product (RC-time) (162-165). This RC-time constancy has been previously explained by the contribution to PAC by the vessels responsible for PVR (163). In addition, increased PVR will lead to increased pressure (Ohm's law), which will lead to a decrease in PAC by a shift in position on the pressure-volume relationship of the large pulmonary artery (165). This general rule of constancy has been questioned and a number of modifying factors have been suggested to reduce RC-time like increasing age, increased pulmonary capillary wedge pressure (PAWP) and exercise (166-168).

5.4.6 Ramp test to optimize LV unloading

A key element in the postoperative management of LVAD patients is to optimize pump speed in order to achieve LV unloading, optimal hemodynamics and to reduce LVAD-related complications (169, 170). Echocardiography-guided LVAD speed optimization protocols (Ramp test) entail a gradual increase of the LVAD speed by 100-400 revolutions per minute (RPM) at 1-2 minute intervals with repeated acquisition of all echocardiographic and device parameters at each speed step in order to optimize pump speed and thus achieve adequate LV unloading (169, 171).

The current echocardiography guidelines do not recommend echocardiography-guided ramp test as a standard protocol for postoperative follow-up due to lack of evidence of impact on the short- and long-term clinical outcomes (172), and there are still significant differences in assessment and management of LVADs between hospitals. On the other hand, invasive hemodynamic ramp testing by RHC appears to potentially increase the likelihood of achieving optimal hemodynamics, and reduce readmission rates (173), reduce HRAEs (174), and improve functional capacity assessed by 6-minute walk distance (175).

However, it is still a controversial issue whether to routinely use echocardiographic ramp test or invasive hemodynamic ramp test and there is an ongoing trial (The Ramp-it-Up study, Unique identifier: NCT03021239, https://www.clinicaltrials.gov) trying to answer this question by randomizing patients to speed optimization using either echocardiography or invasive hemodynamic catheterization.

6. AIMS

The overall aim of this thesis was to study a number of different but complementary invasive (**RHC**) and non-invasive methods (**sST2**, **echocardiography**, and **Innocor**®) that are currently used to assess cardiac performance in different HF phenotypes, mainly in advanced HF.

Specific aims were:

Study I
To assess whether a single infusion of levosimendan improves non-invasively measured hemodynamics in advanced, stable chronic HF, and whether the response is dependent on baseline clinical and hemodynamic factors.

Study II
To assess sST2 concentrations and associations with other biomarkers, echocardiographic measures of diastolic and systolic function, and outcomes in HFpEF and HFrEF.

Study III
To assess the differential impact of the steady and pulsatile components of the PAWP on the PAC-PVR relationship in HF patients using RHC.

Study IV
To test if echocardiographic ramp test post LVAD implantation improves LV unloading and if speed adjustment worsen RV function immediately after and 1-3 months after a ramp test as compared to before the test.

7.THESIS AT A GLANCE

Study	I	II	III	IV
Design	Cohort study	Cohort study	Cohort study	Cohort study
Data source	Patients scheduled for elective intravenous levosimendan infusion at Karolinska University Hospital	KaRen, MetAnEnd	Patients referred for diagnostic RHC at Karolinska University Hospital	Patients followed at Karolinska University Hospital after LVAD implantation
Time of data collection	2010-2015	HFpEF: 2007-2011 HFrEF: 2009-2014	2014-2018	2017-2019
Study population	Advanced stable chronic HFrEF with NYHA III-IV and EF <40%	Chronic HFpEF, HFrEF and healthy controls	Patients with hemodynamic findings indicating HF defined as elevated LAP at rest or during exercise (PAWP rest >15 mmHg, PAWP exercise ≥23 mmHg)	Patients with LVAD undergoing an echocardiographic ramp test pre discharge or as outpatient
Principal method	Innocor®	sST2	RHC	Echocardiography
Numbers included in analyses	N=23	N=193 (HFpEF: 86, HFrEF: 86, controls: 21)	N=192	N=14
Outcome or principal measurement	Change in CO after treatment with levosimendan.	HFpEF: HF hospitalisation or death from any cause HFrEF: Transplantation, LVAD or death from any cause	PAC-PVR relationship	LV unloading, RV function
Main statistical analyses	Mann–Whitney U test Wilcoxon's paired test Spearman's correlations	Cox proportional hazards models Fisher's exact test Mann-Whitney U test Kruskal-Wallis test	Mann-Whitney U test	Wilcoxon's paired test
Results/ Conclusions	In patients with advanced but stable chronic HFrEF levosimendan was associated with improved hemodynamics but no predictors of response could be identified.	In patients with HFpEF compared to HFrEF, levels of sST2 were lower but potentially more strongly associated with outcomes.	The pulsatile rather than the steady PAWP component stands for the previously documented PAWP impact on the PAC-PVR relationship in HF patients.	Echocardiography-guided ramp tests improved LV unloading with no evidence of worsening of RV function.

8. METHODS

8.1 Study population/data sources

8.1.1 Study I

Patients with advanced but not acutely decompensated HFrEF with NYHA III and IV symptoms and an LVEF of < 40% were included. They were scheduled for elective intravenous levosimendan infusion based on consensus clinical indication at the Karolinska University Hospital. Patients were either listed for or undergoing evaluation for HTx or LVAD, or in palliative care.

8.1.2 Study II

Patients with HFpEF were obtained from the Karolinska Rennes (KaRen) study, which was a prospective, bi-national, observational, multicenter study enrolling patients in France and Sweden during 2007-2011. The primary aim was to examine the role of electrical dyssynchrony in patients with HFpEF. The KaRen Biomarker Study **(Study II)** was a pre-specified sub-study including Swedish sites.

Patients were included at hospital presentation for acute decompensated HFpEF with symptoms and signs of acute HF, NT-proBNP >300 ng/L and LVEF ≥45% (by echocardiography during the first 72 h). The patients returned to the hospital in a stable condition 4–8 weeks after enrolment for a follow-up visit including blood samples, clinical assessment and echocardiography and were then followed until September 2012 whereupon patient's status was assessed by chart review, telephone contact or by the Swedish National Patient and Population Registers. The primary outcome was a composite of death from any cause or hospitalization for HF (176).

Patients with HFrEF with LVEF <40%, were recruited from referrals to Karolinska University Hospital for advanced assessment of HF between January 2009 and September 2014 (The Metabolic Anabolic Endothelial Function Heart Failure study cohort (MetAnEnd-HF)). Exclusion criteria were inability to participate or participation in a pharmacological intervention study. Blood samples were collected and clinical assessment including echocardiography was performed at enrolment and outcome data was obtained from chart review and the Swedish National Patient and Population Registers. The primary composite endpoint in this group was death from any cause, implantation of LVAD or HTx.

8.1.3 Study III

Patients undergoing diagnostic RHC at Karolinska University Hospital for unexplained dyspnea, suspected PH or for assessment prior to decision for HTx or LVAD were included and studied between February 2014 and August 2018. The study population included 192 patients with hemodynamic findings indicating HF, defined as elevated LAP at rest or during exercise (PAWPrest >15 mmHg, PAWPexercise ≥23 mmHg). Patients with normal RHC, pre-capillary PH, constrictive pericarditis, arrythmogenic RV cardiomyopathy, and post HTx were excluded. A complete echocardiographic examination and RHC were performed during the same day. All participants provided morning, fasting blood samples.

8.1.4 Study IV

We retrospectively reviewed data from 14 patients followed at the Karolinska University Hospital after LVAD (HM3) implantation between December 2017 and April 2019, who

underwent an echocardiographic ramp test when they were clinically stable. Patients were classified as clinically stable based on assessment of the treating cardiologist, had no inotropic or vasopressor therapy, and were ambulatory.

8.2 Description of study methodology

8.2.1 Study I

Aim:

To test the hypothesis that (1) levosimendan improves hemodynamics in advanced but stable, chronic, advanced HF and (2) that the response is dependent on baseline clinical and hemodynamic factors.

Methods:

All patients (n=23) received a single 24 h levosimendan infusion initially at a rate of 0.1 μg/kg/min without bolus. Prior to, and immediately after the 24 h infusion, non-invasive hemodynamic evaluation was performed using Innocor® and blood samples for analyses of creatinine and NT-proBNP were collected. Estimated total peripheral resistance (eTPR) was calculated using the formula eTPR = [mean arterial pressure (MAP) – central venous pressure]/CO × 80. The estimated glomerular filtration rate (eGFR) was determined using the chronic kidney disease epidemiology collaboration formula (CKD-EPI) (177).

8.2.2 Study II

Aim:

We evaluated sST2 concentrations, correlations with biomarkers and echocardiographic measures of diastolic and systolic function, and associations with outcomes in HFpEF and HFrEF.

Methods:

The patients [HFpEF (n=86), HFrEF (n=86) and healthy controls (n=21)] underwent echocardiography in a stable state. Fasting blood samples were collected for measurement of sST2 and NT-proBNP. sST2 was analyzed by using Presage® ST2 Assay kit that quantitatively measures sST2 by enzyme-linked immunosorbant assay. NT-proBNP was analyzed by fully automated quantitative assay using Cobas®. Concentrations of sST2 and the associations with other relevant biomarkers and echocardiographic measures of diastolic and systolic function, and outcomes were assessed in HFpEF and HFrEF.

8.2.3 Study III

Aim:

To assess the differential impact of the steady (PAWPs) and pulsatile components (PAWPp) of PAWP on the PAC-PVR relationship in patients with HF.

Methods:

In 192 patients with echocardiographic and hemodynamic findings indicating HF, RHC waveforms were used to measure the pulsatile and steady components of the PAWP. PVR was calculated as [mean pulmonary artery pressure (PAP_M) – mean pulmonary capillary wedge pressures ($PAWP_M$)]/CO and expressed as mmHg•seconds•mL^{-1}. PAC was calculated as per the equation: PAC = SV/PA_{PP} in mL•$mmHg^{-1}$; where: PA_{PP}= pulmonary arterial pulse

pressure. The raw data of PAWP and PAP waveforms was futher analysed to measure the steady and pulsatile PAWP components. All patients underwent a standard echocardiographic examination 1-hour prior to RHC and provided fasting blood samples for analyses.

8.2.4 Study IV

Aim:

To test the hypotheses that echocardiographic ramp test post HM3 LVAD implantation improves LV unloading immediately after and 1-3 months after as compared to before the test and that speed adjustments do not worsen RV function.

Methods:

Echocardiographic ramp tests were performed pre discharge or as outpatient in the early postoperative phase when patients (n=14) were clinically stable. The ramp test was carried out by gradual increase of the LVAD speed by 100-400 revolutions per minute (RPM) at 1-2-minute intervals with repeated acquisition of all echocardiographic and device parameters at each speed step in order to optimize pump speed. Optimal LVAD speed was set according to the current recommendations to adequately unload the LV while maintaining a minimal/mild mitral regurgitation (MR), and intermittent aortic valve (AV) opening or closed AV, and reduction in left ventricular end-diastolic diameter (LVEDD), and for the follow-up measurement, also decreased NT-proBNP. Worsening RV function was defined as an increase in right ventricular end-diastolic diameter (RVEDD) or increase in tricuspid regurgitation (TR) or reduction in tricuspid annular plane systolic excursion (TAPSE) or an increase in central venous pressure (CVP) (judged by inferior vena cava (IVC) size and collapsibility) and, for the follow-up assessment, an increase in diuretic dose.

8.3 Statistics

Statistical analysis was performed using SPSS version 22.0 and 23.0 (SPSS Inc., Chicago, IL, USA). Continuous variables are displayed as median and lower and upper quartile (Q1; Q3). Categorical variables are shown as numbers (n) and percentages (%). A two-sided P value of <0.05 was considered statistically significant in all studies.

8.3.1 Study I

To test statistical significance between continuous variables the Mann–Whitney U test was used. Pre and post infusion data was compared through the Wilcoxon's paired test. Association between baseline clinical and hemodynamic factors and the outcome change in CO was analysed using Spearman's correlations. The temporal change of different variables in study I, was presented as median change and standard deviation (SD).

8.3.2 Study II

Baseline data was compared using Mann-Whitney or Kruskal-Wallis test and Fisher's exact test as appropriate. Spearman's correlations were assessed between sST2, NT-proBNP, and eGFR in HFpEF and HFrEF as were echocardiographic parameters of systolic function and diastolic function/structural heart disease. The associations between sST2 and the composite outcomes in HFpEF and HFrEF respectively, were analyzed by Cox proportional hazards models and presented as hazard ratio (HR) and 95% confidence interval (CI) per log increase in sST2, crude, and adjusted for age, sex, and NYHA class.

8.3.3 Study III

To demonstrate the relationship between PAC and PVR, a non-linear curve was fitted according to the hyperbolic formula: $y = a/b + x$. Curve fits were generated using MATLAB (version 9.4, R2018a; 2018, The MathWorks, Natick, MA). Continuous variables were compared using the Mann-Whitney U test.

8.3.4 Study IV

Wilcoxon's paired test was used to compare median values of study variables before and after ramp test.

8.4 Ethical considerations

All studies complied with the Declaration of Helsinki and were approved by the regional ethical review board (178). Written informed consent was obtained from all participants in **study I-II**. Individual patient consent was not required or obtained in **study III-IV** since the studies were a retrospective analysis.

9. RESULTS

9.1 Study I

Table 1 details selected baseline characteristics of the 23 included patients. Patients had advanced HFrEF with a median (Q1; Q3) EF of 20 (15; 31) %, CO was 3.05 (2.8; 3.4) L/min, and NT-proBNP 3400 (1882; 6597) pg/ml.

Table 1. Selected baseline characteristics for patients in Study 1. Continuous variables are presented as median, lower and upper quartiles (Q1; Q3). Categorical variables as numbers (n) and percentages.

Variable	
Demographics (N=23)	
Age (years)	56 (49; 64)
Female gender (n/%)	4/17
Hemodynamics and heart failure characteristics	
Heart rate (beats/min)	69 (61; 73)
Mean blood pressure (mmHg)	79 (74; 87)
eGFR (ml/min/1.73 m^2)	62 (35; 78)
eTPR (dyn·s·cm^{-5})	1628 (1405; 2034)
BSA (m^2)	2.0 (1.9; 2.2)
NYHA-class: IIIA / IIIB / IV (n)	4/18/1
Sinus rhythm (n/%)	8 / 35
Atrial fibrillation (n/%)	15 / 65
Device therapy	
Pacemaker (n/%)	1 / 4
ICD (n/%)	6 / 21
CRT-P (n/%)	1 / 4
CRT-D (n/%)	15 / 65
Medical therapy	
B-blockers (n/%)	22 /95
ACEI/ARB (n/%)	22 /95

There was a significant increase of CO after a single levosimedan infusion from 3.05 (2.8; 3.4) L/min to 3.45 (3.1; 4.2) L/min corresponding to a median change ± standard deviation of +9.8% ± 21.6%; p=0.026 (**Figure 4**). CO increase was attributed to an increased SV from 48 (40; 53) to 52 (46; 61) mL/min, p=0.021 and heart rate was unchanged from 69 (62; 74) to 70 (61; 76) beats/min, p=0.159.

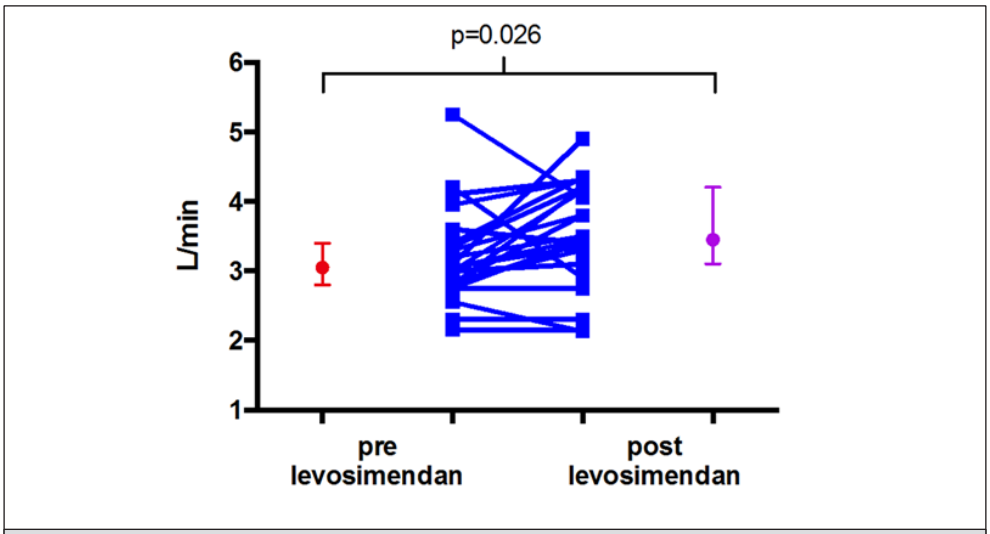

Figure 4. Individual and median (Q1; Q3) cardiac output before and after levosimendan infusion (**Study I**).

Figure 5 shows the effect of a single 24-hour levosimendan infusion on the other study variables. In summary, there was a significant decrease in NT-proBNP from 3400 (1882; 6597) to 2530 (1108; 6410) pg/ml, p<0.001, eTPR from 1628 (1405; 2034) to 1343 (1151; 1701) dyn·s·cm^{-5}, p=0.004 and MAP from 79 (74; 87) to 74 (69; 81) mmHg, p =0.007. There was no difference in eGFR; 62 (35; 78) to 61 (40; 85) ml/min/1.73 m^2, p=0.955.

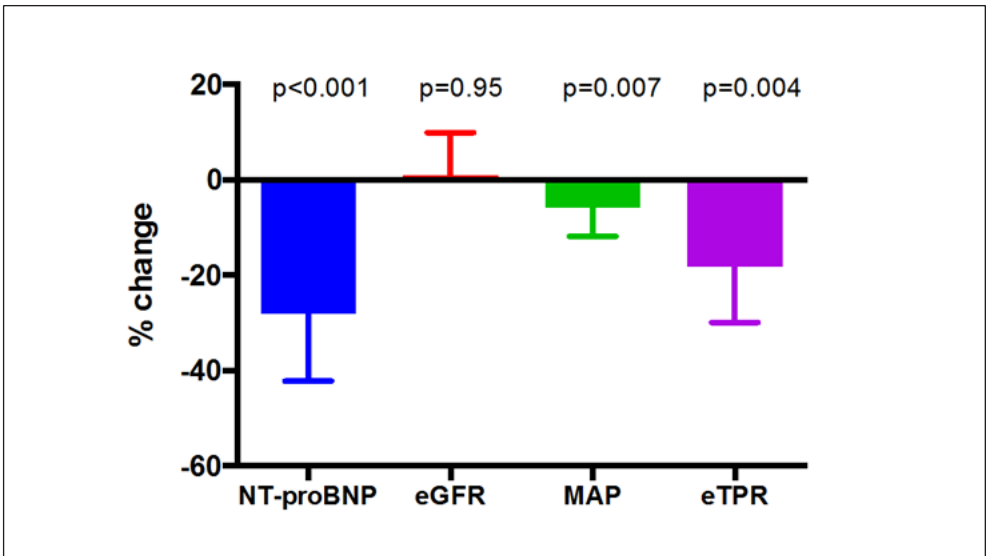

Figure 5. Effect of a single 24-hour levosimendan infusion on NT-proBNP, eGFR, MAP and eTPR (**Study I**).

Figure 6A-F shows the lack of significant correlations between baseline characteristics or hemodynamic variables and the change in CO.

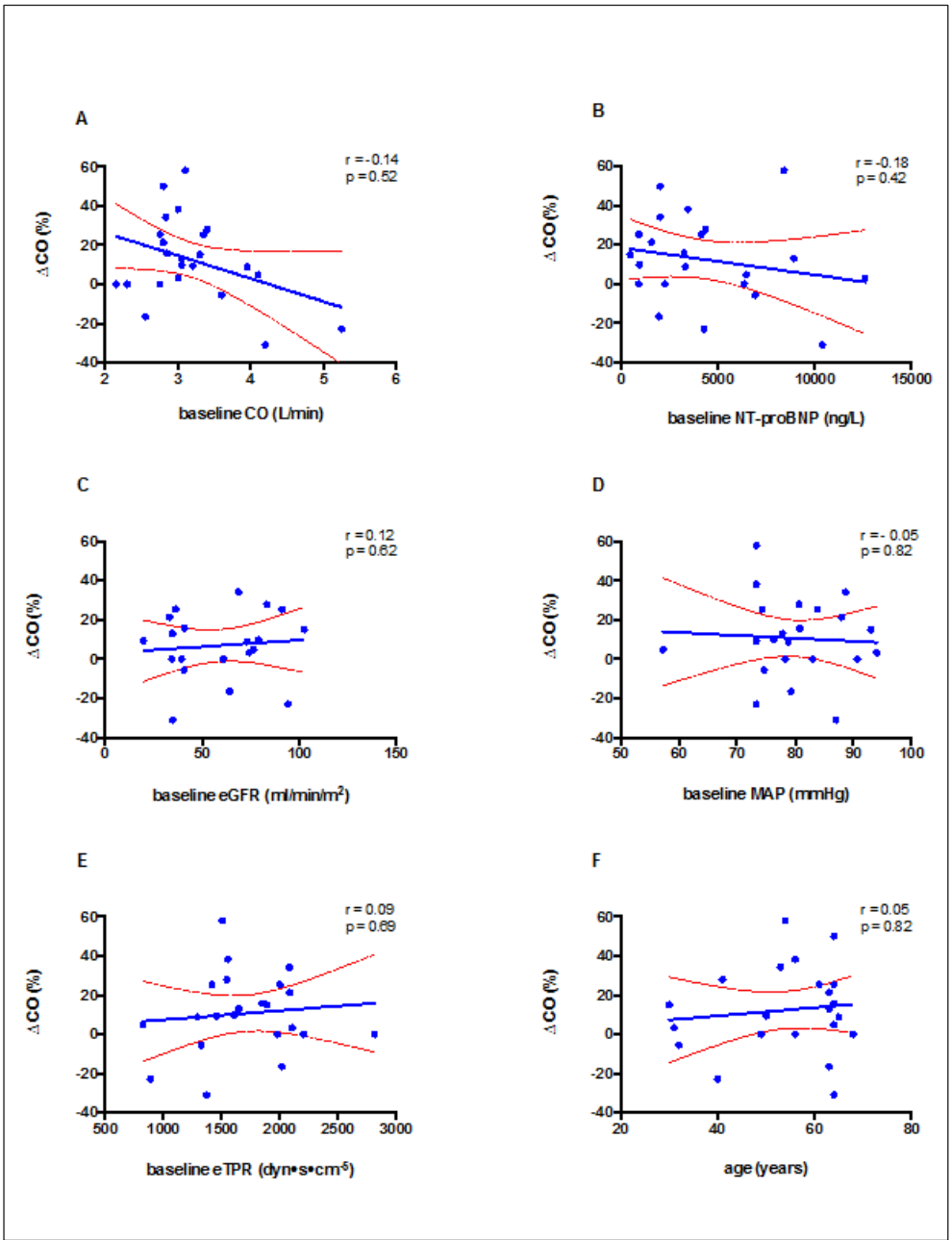

Figure 6. Absence of significant correlations between baseline cardiac output (CO) (**A**), NT-proBNP (**B**), eGFR (**C**), MAP (**D**), eTPR (**E**) and age (**F**) and change in CO in response to levosimendan (**Study I**).

9.2 Study II

Table 2 shows selected baseline characteristics of the 3 cohorts included. Patients with HFpEF were older, more often female, had lower NYHA class, higher MAP and body mass index (BMI) compared to HFrEF. Prevalence of co-morbidities such as atrial fibrillation and diabetes was similar between HFpEF and HFrEF.

In HFpEF, left atrial volume index (LAVi) was median (Q1; Q3), 43 (37; 53) mL/m^2 and LV mass index (LVMi) was 115 (95; 143) g/m^2. Corresponding data in HFrEF were; LAVi 48 (40; 73) mL/m2 and LVMi 146 (127; 184) g/m2.

Table 2. Selected baseline characteristics; median, (Q1; Q3). Categorical variables as numbers (n) and percentages (**Study II**).

Demographics	HFpEF n=86	HFrEF n=86	Control n=21	p-value			
				HFpEF: HFrEF	HFpEF: control	HFrEF: control	Overall
Age (years)	73 (67; 80)	63 (52; 68)	67 (59; 70)	< 0.001	< 0.001	0.159	< 0.001
Gender male/female	42/44 (49/51)	70/16 (81/19)	9/12 (43/57)	< 0.001	0.624	< 0.001	< 0.001
Medical history							
Atrial fibrillation/flutter	49 (57)	45 (52)		0.541			
Diabetes mellitus	28 (33)	25 (29)		0.621			
PCI	9 (11)	20 (23)		0.026			
NYHA I	19(22)	1 (1)		<0.001			
NYHA II	47(55)	4 (5)					
NYHA III	20 (23)	67 (80)					
NYHA IV	0	14 (11)					
Clinical Measurements							
BMI (kg/m^2)	29 (25;34)	27 (23;30)	25 (22;26)	0.001	<0.001	0.043	<0.001
MAP (mmHg)	100 (92;107)	82 (74;93)	94 (89;103)	<0.001	0.111	<0.001	<0.001
HR (beats/min)	70 (60;80)	70 (60;75)		0.482			
LVEF (%)	64 (58;68)	21 (15;28)		<0.001			
LVEDD (mm)	47 (43;53)	66 (61;75)		<0.001			
Treatment							
ARB/ACE-I	67 (78)	77 (90)		0.037			
Beta blocker	69 (80)	85 (99)		<0.001			
MRA	18 (21)	58 (67)		<0.001			
Loop diuretic	61 (71)	75 (87)		0.009			
Laboratory							
sST2 (µg/L)	23 (17; 31)	35 (23; 52)	25 (21; 32)	<0.001	0.145	0.029	<0.001
NT-proBNP (ng/L)	1000 (465;2335)	3290 (1405;6115)	67 (31;110)	<0.001	<0.001	<0.001	<0.001
eGFR (mL/min/1.73m^2)	68(50;81)	58 (42;73)	82 (71;91)	0.036	0.006	<0.001	<0.001

PCI; pecutaneous coronary intervention

Crude sST2 levels were higher in HFrEF compared to HFpEF and controls, median (Q1; Q3), 35 (23; 52), 23 (17; 31) and 25 (21; 32) µg/L respectively (overall p<0.001) (**Figure 7**). As depicted in **Figure 8**, levels of sST2 increased with worsening HF severity assessed as NYHA class in both LVEF categories.

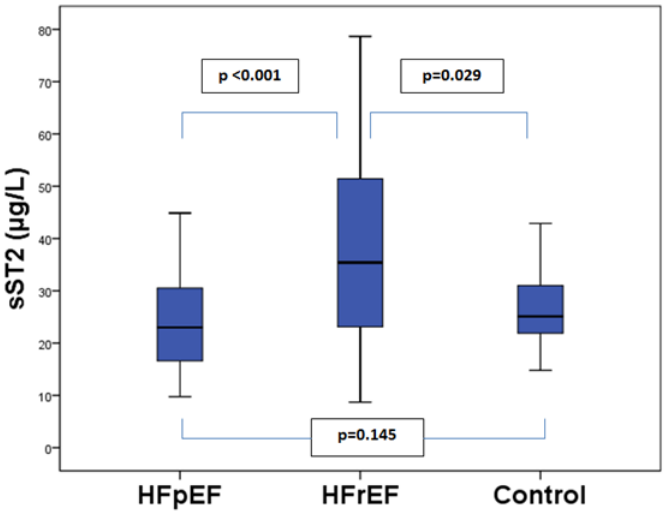

Figure 7. Crude sST2 concentrations in HFpEF, HFrEF and controls, p-value denotes comparison between groups (**Study II**).

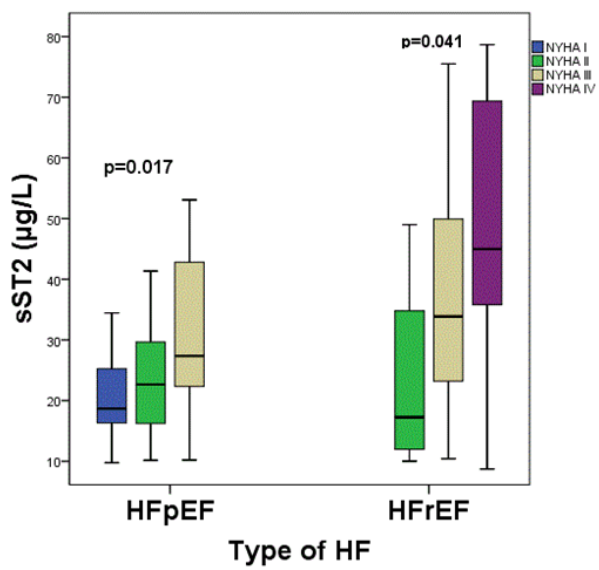

Figure 8. sST2 concentrations by NYHA class in HFpEF and HFrEF, p-values for comparison between NYHA class in HFpEF (I vs. III) and HFrEF (II vs. IV) (**Study II**).

Table 3 shows Spearman's correlations between sST2 and demographic/echocardiographic data in HFpEF, HFrEF, and controls.

Table 3. Correlations between sST2 and variables in HFpEF, HFrEF and controls (**Study II**).

Variable	sST2					
	HFpEF n=86		HFrEF n=86		Controls n=21	
	r	p-value	r	p-value	r	p-value
NT-proBNP (ng/L)	0.392	<0.001	0.466	<0.001	-0.020	0.931
Age (years)	0.116	0.295	0.043	0.698	0.215	0.350
NYHA class	0.307	0.005	0.270	0.014		
eGFR (mL/min/1.73m2)	-0.073	0.513	-0.224	0.044	-0.141	0.542
MAP (mmHg)	0.047	0.668	-0.015	0.901	-0.350	0.120
BMI (kg/m2)	0.116	0.301	-0.035	0.755	-0.115	0.501
E/e' (average)	0.148	0.248	-0.096	0.523		
LAVi (mL/m²)	0.276	0.019	0.247	0.224		
LVMi (mL/m²)	-0.005	0.795	0.622	0.018		
LVEF (%)	-0.057	0.634	0.005	0.962		

Association between sST2 and outcomes in HFpEF and HFrEF

Median (Q1; Q3) follow-up time was 522 (232; 1089) days in HFpEF and 204 (55; 421) days in HFrEF. In the HFpEF group, 11 patients (13%) died and the composite outcome of all-cause death or HF hospitalization occurred in 36 patients (42%). In the HFrEF group, 28 patients (33%) died and the composite outcome of death from any cause, implantation of LVAD or HTx occurred in 56 patients (65%).

In both HFpEF and HFrEF, sST2 was significantly associated with the composite outcome in crude analyses (HR per log increase 10.04 [95% CI 1.89-53.44], p=0.007) and (HR 3.28 [95% CI 1.06-10.16], p=0.039) respectively (**Figure 9a and b**). The association persisted after adjustment for age, sex, and NYHA class.

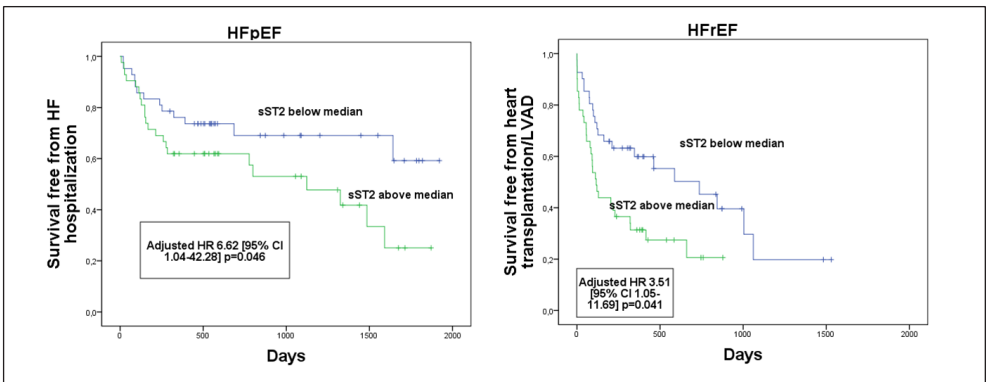

Figure 9. Kaplan-Meyer estimates of survival free from HF hospitalization in HFpEF (a) and survival free from HTx or LVAD in HFrEF (b) comparing sST2 above and below median in HFpEF and HFrEF respectively. Association with the composite endpoint in HFpEF and HFrEF analyzed with Cox Regression, HR per log increase in sST2, 95% confidence interval, and p-value depicted in each graph (**Study II**).

9.3 Study III

Selected baseline characteristics of the 192 patients are presented in **Table 4**. Median (Q1; Q3) age was 64 (52; 74) years, 81 (42%) were women, 80 (42%) patients were classified as HFpEF, and the majority being significantly symptomatic (75% in NYHA III).

Table 4. Selected baseline clinical characteristics; median, (Q1; Q3). Categorical variables as numbers (n) and percentages (%) (**Study III**).

Demographics (n=192)	
Age (years)	64 (52;74)
Gender male/female (n/%)	111/81 (58/42)
Medical history	
Ischemic heart disease (n/%)	50 (26)
Diabetes mellitus (n/%)	39 (20)
Hypertension (n/%)	109 (57)
Atrial fibrillation/flutter (n/%)	104 (54)
NYHA I	7 (3.5)
NYHA II	32 (17)
NYHA III	144 (75)
NYHA IV	9 (4.5)
Diagnosis	
HFpEF	80 (42)
Ischemic cardiomyopathy	33 (17.2)
Dilated cardiomyopathy	51 (26.6)
Hypertrophic cardiomyopathy	5 (2.6)
Restrictive cardiomyopathy	16 (8.3)
Clinical measurements	
BMI (kg/m^2)	27 (23;30)
SBP (mmHg)	110 (95;134)
DBP (mmHg)	63 (57;72)
HR (beats/min)	69 (59;79)
Treatment	
ARB/ACE-I	149 (78)
Beta blocker	163 (85)
MRA	116 (60)
CRT-P/CRT-D	34 (18)
Laboratory	
NT-proBNP (ng/L)	2120 (868;3580)
eGFR ($mL/min/1.73m^2$)	58 (41;79)

Table 5 shows selected baseline echocardiographic and hemodynamic data. Median (Q1; Q3) EF was 46 (25; 60) %, cardiac index (CI) 2.2 (1.8; 2.6) L/min/m², PVR 0.15 (0.10; 0.22) mmHg•seconds•mL^{-1}, and PAC 2.4 (1.7; 3.5) mL•mmHg^{-1}.

Table 5. Selected baseline echocardiographic data and hemodynamics; median (Q1; Q3) (**Study III**).

Echocardiographic measurements	
LVEF (%)	46(25;60)
LVEDD (mm)	53(45;65)
LVMi (g/m²)	108 (80;150)
LAVi (mL/m²)	53 (40;67)
E'(cm/sec)	7 (5.5;8.5)
E/A	1.7 (1.3;2.2)
E/e' (average)	13 (10;18)
RA area (cm²)	22 (17;27)
RVSP (mmHg)	45 (37;58)
TAPSE (mm)	15 (11;19)
Hemodynamic measurements	
CI (L/min/m²)	2.2 (1.8;2.6)
PVR (mmHg•seconds/mL)	0.15 (0.096;0.216)
PAC (mL/mmHg)	2.4 (1.7;3.5)
PAWP-mean (mmHg)	18 (14;24)
PAWP-A (mmHg)	19 (15;25)
PAWP-V (mmHg)	24 (17;32)
PAP-mean (mmHg)	30 (22;37)
PAP-systolic (mmHg)	44 (35;57)
PAP-diastolic (mmHg)	18 (13;24)
RAP-mean (mmHg)	8.5 (5;14)

E, transmitral early diastolic filling velocity; A, transmitral late diastolic filling velocity; E', average value of lateral and septal early diastolic myocardial velocity; E/A, ratio between the early transmitral diastolic filling velocity to late diastolic filling velocity; RA , right atrium; RVSP, right ventricular systolic pressure; RAP, right atrial pressure.

Impact of the $PAWP_M$ and the V-wave amplitude on the PAC-PVR relationship

In accordance with previous findings, there was a hyperbolic and inverse relationship between PAC and PVR (Curve fit: y=0.587/0.073 + x, R^2=0.56); (**Figure 10**).

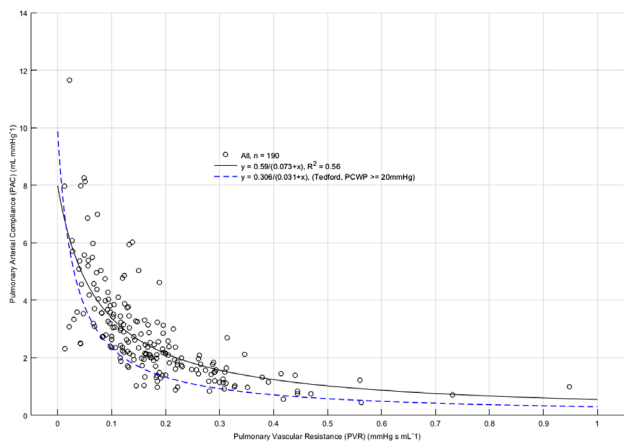

Figure 10. PAC-PVR relationship in the study cohort compared to best curve fit given by Tedford and colleagues (**Study III**).

As illustrated in **Figure 11**, elevated $PAWP_M$ (>18 mmHg) yielded a shift of the hyperbolic curve fit downward and to the left, such that PAC was lower for similar PVR values in patients with higher $PAWP_M$. Similarly, patients with peak systolic LAP above the median value for the whole group (V-wave > 24 mmHg) demonstrated a curve fit shifted downward and to the left as compared to the corresponding group with lower peak V-waves (**Figure 11**).

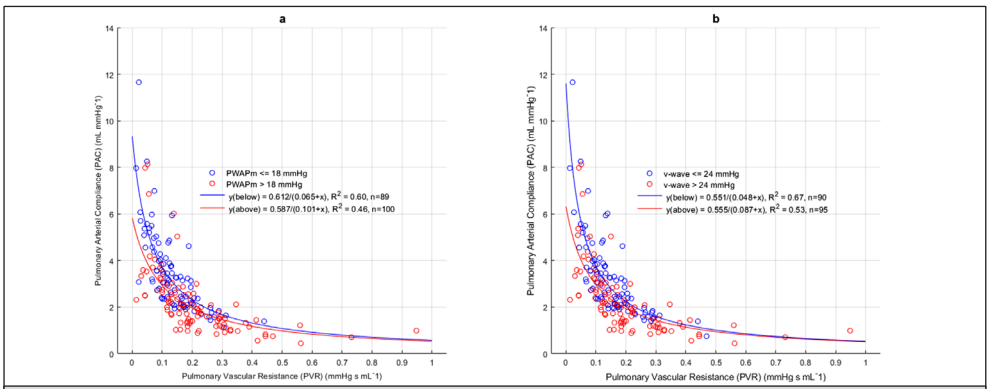

Figure 11. Effects of elevated $PAWP_M$ and V-wave on PAC-PVR relationship (**Study III**).

Impact of LAP pulsatility on the PAC-PVR relationship

As depicted in **figure 12a**, the group of patients with higher levels of steady PAWP (> 16.7 mmHg) demonstrated a slight shift of the PAC-PVR fit curve downward and to the left (y=0.671/0.119 + x, R^2=0.41) compared to those with lower steady PAWP values (y=0.570/0.059 + x, R^2=0.61). However, the RC-time did not differ significantly between the two groups (0.35 (0.23; 0.48) vs. 0.38 (0.31; 0.47)) p= 0.078 (**Table 6**).

As illustrated in **figure 12b**, the shift of the curve fit for the PAC-PVR association for the patient cohort with higher pulsatile PAWP component (> 3.5 mmHg) was more pronounced (y=0.584/0.102 + x, R^2=0.53) as compared to the corresponding group with lower levels of systolic pulsatility (y=0.683/0.078 + x, R^2=0.54). Additionally, the subgroup with higher pulsatile PAWP component displayed a significantly shorter RC-time [0.31 (0.23; 0.40) vs. 0.44 (0.34; 0.54)] p<0.001 (**Table 6**).

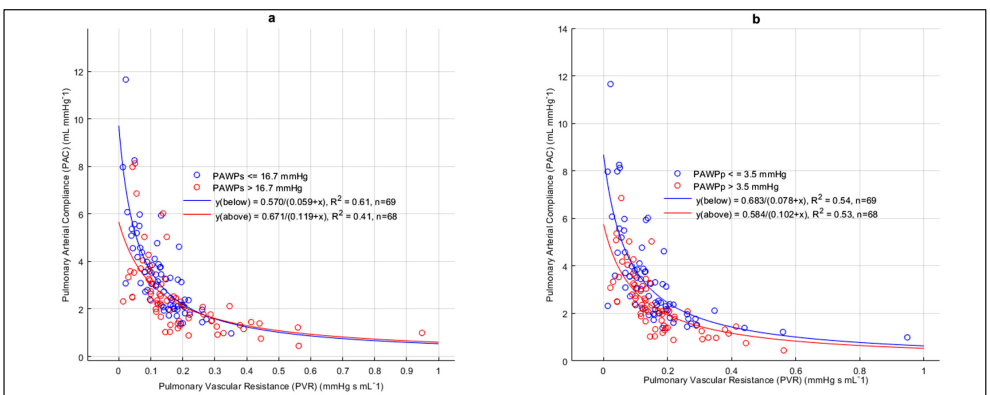

Figure 12 a-b. Effects of elevated PAWP steady and pulsatile components on PAC-PVR relationship (**Study III**).

We proceeded by dichotomizing the study cohort as based upon the ratio of the steady and pulsatile PAWP components. As provided in the **Table 6**, there was no significant difference in the $PAWP_M$ between the 2 groups. However, the RC time was significantly lower in the subgroup with elevated ratio, which in turn was driven by a significant rise in pulmonary pulse pressure (PP) (p=0.006).

Table 6. Selected baseline characteristics stratified according to pulsatile and steady PAWP pressures below and above median. Presented as median, (Q1; Q3), (**Study III**).

	Low steady PAWP n = 69	High steady PAWP n = 68	p-value	Low pulsatile PAWP n = 69	High pulsatile PAWP n = 68	p-value	Low PAWPp/ PAWPs ratio	High PAWPp/ PAWPs ratio	p-value
CI (L/min/m²)	2.35 (1.87; 2.65)	2.71 (1.73; 2.59)	0.198	2.20 (1.79; 2,47)	2.32 (1.88; 2.66)	0.140	2.15 (1.64; 2.45)	2.37 (1.94; 2.70)	0.010
PAWP-mean (mmHg)	14 (12;16)	24 (19; 27)	<0.001	15 (12; 18)	21 (17; 26)	<0.001	17 (14; 23)	18 (15; 25)	0.342
PVR (mmHg•seconds/ mL)	0.13 (0.08; 0.18)	0.15 (0.09; 0.24)	0.067	0.14 (0.08; 0.19)	0.13 (0.09; 0.19)	0.585	0.16 (0.10; 0.21)	0.13 (0.09; 0.18)	0.206
PAC (mL/mmHg)	3.1 (2.1; 4.3)	2.2 (1.4; 3.2)	<0.001	3.1 (2.1; 4.0)	2.2 (1.5; 3.2)	0.001	2.5 (1,9; 3.8)	2.5 (1.8; 3.5)	0.355
PAP-mean (mmHg)	23 (19; 26)	34 (30; 42)	<0.001	24 (19; 31)	32 (26; 38)	<0.001	26 (22; 38)	30 (23; 36)	0.490
PA_{PP} (mmHg)	22 (17; 28)	27 (21; 37)	0.003	21 (17; 27)	27 (22; 40)	<0.001	22 (18; 28)	26 (21; 38)	0.006
RC time (seconds)	0.38 (0.31; 0.47)	0.35 (0.23; 0.48)	0.078	0.44 (0.34; 0.54)	0.31 (0.23; 0.40)	<0.001	0.44 (0.34; 0.56)	0.31 (0.24; 0.39)	<0.001

9.4 Study IV

Baseline characteristics at implantation

The majority of patients included in this study were men (93%), median (Q1; Q3) age was 49 (41; 59) years. Median LVEF was 16 (14; 20) %, LVEDD 67 (61; 77) mm, and TAPSE was 13 (13; 20) mm. Dilated cardiomyopathy was the main underlying cause of HF and accounted for 57 % of all causes and 64 % had Interagency Registry for Mechanically Assisted Circulatory Support (INTERMACS) III. The main indication for LVAD was bridge to decision (BTD) (50 %).

Pre-RAMP characteristics

Intraoperatively, the patients had a low median LVAD speed of 4800 (4500; 4850) RPM, which was gradually increased in the post-operative period during the time preceding the ramp test to 4950 (4875; 5225) RPM as depicted in **table 7**. Median pre-RAMP LVEDD was 58 (55; 70) mm, RVEDD 42 (40; 48) mm, and TAPSE 8 (6.5; 9.5) mm. Median time from implantation to ramp test was 27 (16; 56) days. Adequate LV unloading was present in only 6 (43%) patients out of 14 patients at pre-RAMP when applying all the 3 criteria for LV unloading (**Table 8**).

LV unloading and RV function during ramp testing

Maximum speed limit was not achieved during ramp testing; however, the achieved median (Q1; Q3) upper LVAD speed (Ramp-High) was 5550 (5375; 6025) RPM, which was significantly higher than pre-RAMP LVAD speed (p<0.001). Increases in LVAD speed were associated with a significant reduction of LVEDD to 53 (46; 63) mm (p<0.001) in 13 (93%) patients. **Table 8** demonstrates the impact of acute changes in LVAD speed on LV unloading where 10 (71%) patients had adequate LV unloading at Ramp-High when applying all the 3 criteria for LV unloading. Out of 14 patients, 1 patient had no reduction of LVEDD, 1 patient had moderate MR, 1 patient which had the AV constantly open and in 1 patient the AV was not visualized.

Ramp testing resulted in direct LVAD speed increase in 13 (93%) patients with a median speed change of 100 (100; 200) RPM. At Ramp-High, RV function did not worsen significantly; however, seven (50%) patients had increased TR severity but only 1 out of 7 increased to severe TR. **Tables 7-8** and **figure 15** illustrate the acute impact of RPM changes on LV unloading and RV function.

Table 7. Characteristics before, during ramp testing, and at follow-up (final LVAD speed); median, (Q1; Q3) (**Study IV**).

	Pre-RAMP	Ramp-High	Follow-up (final speed)	p-value	
				Ramp-High vs. Pre-RAMP	Final Speed vs. Pre-RAMP
Pump speed (RPM)	4950 (4875;5225)	5550 (5375;6025)	5200 (5000;5425)	<0.001	0.001
LVEDD (mm)	58 (55;70)	53 (46;63)	57 (49;62)	<0.001	0.125
RVEDD (mm)	42 (40;48)	46 (42;50)	46 (40;47)	0.391	0.625
TAPSE (mm)	8 (6.5;9.5)	8 (6.5;10.5)	8 (7.5;10)	0.425	0.200
CVP (mmHg)	5 (4;10)	5 (5;11)	5 (5;10)	0.437	1.000
Furosemide (mg)	120 (80;260)		80 (20;220)		0.072
eGFR (mL/min/1.73m^2)	45 (31;71)		52 (32;66)		0.855
NTproBNP (ng/L)	2320 (1845;3593)		1310 (812;2653)		0.002

Table 8. LV unloading at different time points; pre-RAMP, ramp-High, and final follow-up LVAD speed; n (%) (**Study IV**).

LVAD speed	n	Reduced LVEDD	≤ mild MR	Closed or intermittently opened AV	All three criteria	Reduced NT-proBNP	All four criteria
Pre-RAMP vs. pre-implant	14	13 (93%)	13 (93%)	8 (57%)	6 (43%)	11 (79%)	5 (36%)
Ramp-High vs. pre-RAMP	14	13 (93%)	13 (93%)	12 (86%)	10 (71%)		
Final vs. pre-RAMP	13	9 (69%)	13 (100%)	7 (54%)	6 (46%)	12 (86%)	4 (31%)

LV unloading and RV function at final LVAD speed (Speed at follow-up)
Median time from ramp test to follow-up echocardiography was 55 (47; 102) days. The median LVAD speed at the time of follow-up echocardiography was 5200 (5000; 5425) RPM, which was significantly higher than pre-RAMP LVAD speed (p=0.001) but lower than goal RPM which was 5375 (5100; 5700) RPM. One patient was transplanted before doing follow-up echocardiographic examination and this patient was excluded from echocardiographic analysis at final LVAD speed. Ramp testing resulted in final follow-up LVAD speed increase in 11 (79%) patients and a median net change at the time of follow-up echocardiography of 200 (200; 300) RPM. **Figure 13** shows the median change of different study variables at final follow-up LVAD speed compared to pre-RAMP pump speed.

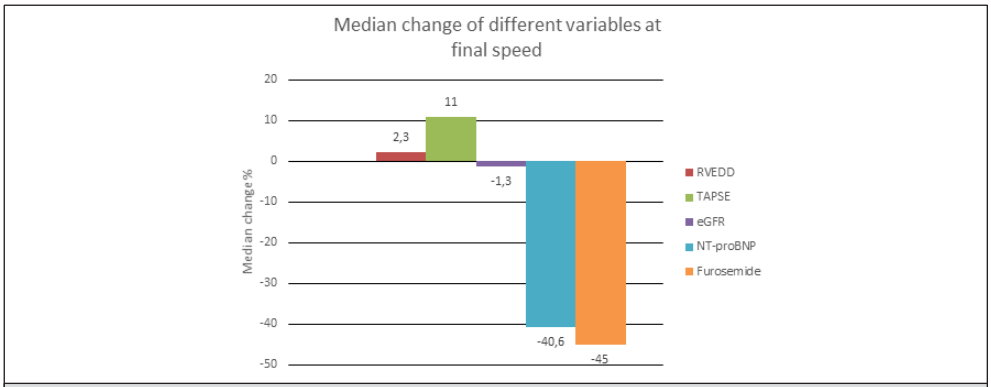

Figure 13. Median change of RVEDD, TAPSE, eGFR, NTproBNP, and Furosemide dosage at final follow-up LVAD speed compared to pre-RAMP pump speed (**Study IV**).

Figure 14 illustrates a reduction of LVEDD to 57 (49; 62) mm in association with increased LVAD speed but the reduction was not significant (p=0.125) compared to pre-RAMP, despite a significantly higher final follow-up LVAD speed.

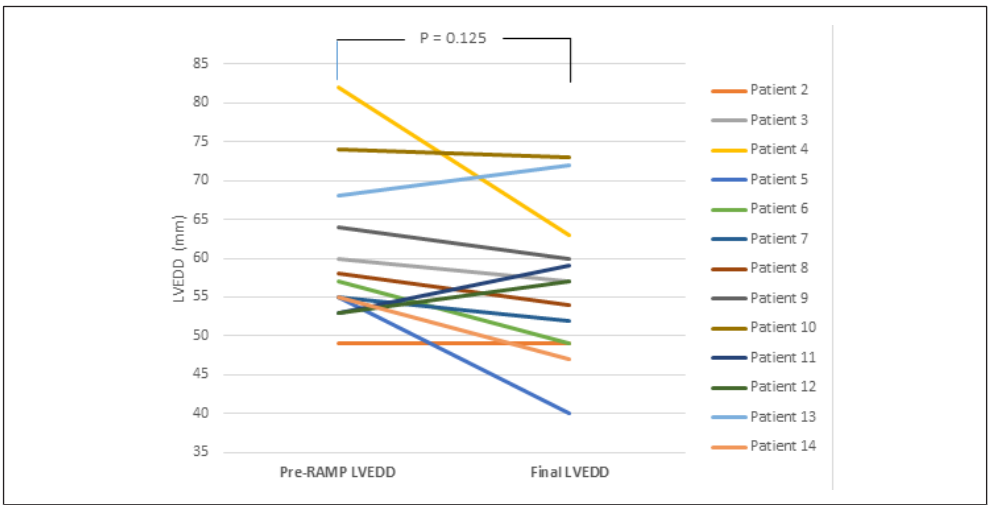

Figure 14. Individual LVEDD at pre-RAMP and final follow-up LVAD speed (**Study IV**).

Adequate LV unloading was achieved in 6 (46%) patients when applying all the 3 echocardiographic criteria for LV unloading at the time of follow-up, and in 4 (31%) patients when all 4 criteria (including NT-proBNP) were applied (all of them had increased RPM 100-300 at final speed compared to pre-RAMP (**Table 8**)).

At the time of follow-up echocardiography, RV function had not worsened significantly and only one (8%) patient had increased TR severity (**Table 7** and **figure 15**). Additionally, there was a non-significant reduction in daily Furosemide dose from 120 (80; 260) mg to 80 (20; 220) mg (p=0.070).

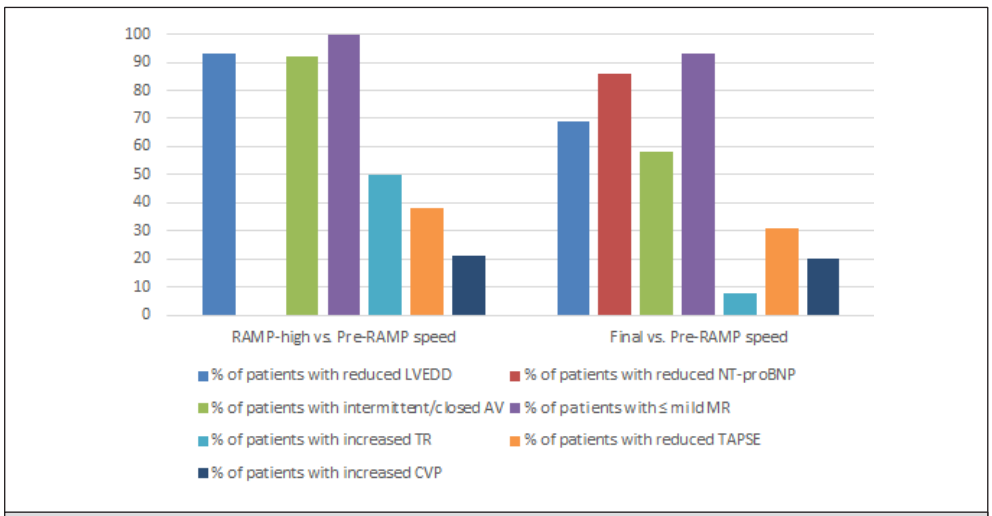

Figure 15. LV unloading and RV function at final follow-up LVAD speed vs. pre-RAMP pump speed, and at ramp-High vs. pre-RAMP pump speed (**Study IV**).

10. DISCUSSION

10.1 Major findings

Multiple different invasive and non-invasive methodologies were useful in multiple different HF settings, to assess prognosis and optimize care.The principal findings of the four studies in this thesis are:

Study I: Levosimendan improved Innocor®-assessed CO and reduced NT-proBNP, MAP, and eTPR after a single infusion in advanced stable chronic HF, but no predictors of the levosimendan effect on CO that could be used for patient selection were identified.

Study II: In patients with HFpEF, compared to HFrEF, crude levels of sST2 were lower but potentially more strongly associated with outcomes.

Study III: In HF patients, the PAC was reduced in a steeper manner for the same PVR value in patients with higher PAWP compared to patients with less pronounced PAWP elevations. We also demonstrated that the pulsatile rather than the steady PAWP component had a stronger impact on the PVR-PAC relationship in patients with HF.

Study IV: LVAD speed adjustments after echocardiographic ramp testing resulted in optimized final follow-up LVAD speed, manifested as improved LVAD unloading achieved in an additional 21% of patients who were not originally optimized. This speed optimization did not worsen RV function.

10.2 The use of levosimendan in heart failure

Levosimendan has an established role as short-term therapy in ADHF patients with low CO and evidence of end-organ dysfunction. It is the most frequently used inotropic agent in cardiology and internal medicine in Sweden (68) despite conflicting evidence on survival benefits (71-74). Furthermore, there has been an increasing interest in the last decade to use levosimendan repetitively or intermittently to provide periods of hemodynamic relief in patients with advanced HF waiting for LVAD/HTx or as a palliative care pathway. The use of levosimendan in repeated or intermittent cycles seems to have clinical benefits including reduction in NT-proBNP, and trends toward reduction in HF readmissions and probably HF-related mortality, but evidence is not uniform and there is lack of selection criteria to predict benefits from repeated or intermittent levosimendan therapy (77, 179, 180).

In **study I,** we studied the effects of a single infusion of levosimendan in advanced but stable chronic HF. Our findings of distinct improvements in hemodynamics and NT-proBNP in stable HF are novel but consistent with previous studies in ADHF, and suggest that the benefits in advanced but stable chronic HF may be mediated similarly to that in ADHF. However, we could not identify any clinical or hemodynamic predictors of levosimendan response, and thus the selection of patients for this expesnive and potentially harmful intervention remains difficult.

10.3 The role of ST2 in heart failure

Presently there is no evidence-based therapy for HFpEF and there is an increasing interest in identifying biomarkers reflecting distinct and novel pathophysiological pathways (181), both

as a diagnostic tool and as markers for targets for future therapy. As mentioned earlier, sST2 is one of the most promising novel biomarkers in HF and has a diagnostic capability and a prognostic potential independent or additive to other biomarkers in acute and chronic HF (125, 182-188). sST2 measurement is now recommended by American College of Cardiology and American Heart Association for additive risk stratification in ADHF and chronic HF (189). Furthermore, there is mounting evidence suggesting that serial sST2 measurements might have significant prognostic implications (129, 130).

Unlike NPs, sST2 is not confounded by age, renal function or BMI (190-193); however, it is inferior to NT-proBNP for diagnosis of acute HF (125, 194). Interestingly, the currently approved partition value of 35 µg/L to predict morbidity and mortality, is mainly based on studies of HFrEF or studies in which HFrEF patients were dominant (185, 195, 196). Hence, studies in HFpEF are warranted.

In **study II,** our cohort demonstrated lower sST2 levels in patients with HFpEF (23 µg/L) than in other studies including patients with HFpEF, where the median values were 25-30 µg/L (197, 198). In HFpEF patients, we found an association between sST2 levels and LAVi, which is an important prognostic predictor in HF, independent of LVEF. In both HFpEF and HFrEF sST2 was significantly associated with the composite outcome.

Study II adds to the existing evidence of sST2 as an independent prognostic marker not only in HFrEF but also in HFpEF and suggests a lower sST2 cut-off than 35 µg/L in HFpEF.

10.4 The impact of PAWP on PAC-PVR relationship

The pulmonary circulation is a high flow/low-pressure system and the RV is vulnerable to increases in afterload. Increased RV afterload will contribute to RV dysfunction, which is associated with poor prognosis (157). The concept of RV afterload is frequently used in routine clinical practice, but is somewhat simplified by measuring only PA pressure and PVR rather than combining PVR and PAC which represent RV resistive and elastic components respectively (158, 159). Considering RV afterload in terms of PAC and PVR accounts for the pulsatility imposed by the cardiac cycle.

Study III, confirmed the finding of previous studies that there is an inverse and hyperbolic relationship between PAC and PVR (162-164, 166, 199-201) and that higher PAWP shifted the curve fit downward and leftward in HF patients. In particular, we explored the effects of elevated static and pulsatile LAP components inherent to the cardiac events and showed that the pulsatile pressure component of the LAP and not the steady component had a significant impact on the PAC-PVR relationship. It appears that the mechanism by which the phasic oscillations of LAP affect the elastic properties of the pulmonary vascular tree might be ascribed to the resultant wave reflection as well as the subsequent distortion of the pulmonary pressure waveform. These findings introduce a new way of considering the hemodynamic consequences of elevated LAP in HF patients and may aid in the development of new treatments targeting both the steady and pulsatile components of PAWP.

10.5 The usefulness of echocardiographic ramp test to optimize LVAD speed

Improved postoperative management and optimal LVAD speed are key elements to reduce adverse events, optimize hemodynamics and achieve adequate LV unloading. The main advantage of using echocardiographic ramp test over the hemodynamic approach is that it is easy, non-invasive and available in many clinics.

Study IV confirmed the positive impact of echocardiographic ramp test on LVAD speed optimization and LV unloading without worsening of RV function both during ramp testing and at the time of follow-up with echocardiography. Another important finding was that clinical stability did not necessarily implicate optimal LVAD speed and LV unloading.

RV failure is a frequent and feared complication following LVAD implantation and is a major cause of postoperative morbidity and mortality (202). There is always a concern when optimizing LVAD unloading that the increase of LVAD speed will lead to increased venous return to the RV and a leftward septal shift resulting in unfavourable RV geometry. However, in **Study IV**, there were no signs of deteriorating RV function after LVAD speed optimization. This can partially be explained by decreased RV afterload secondary to increased LVAD speed (203, 204). Notably, it has been shown that higher LVAD speeds, beyond 5600 RPM in HM3, may affect the RV negatively as evidenced by increased RV preload and volumes and less favourable RV geometry using three-dimensional echocardiography during ramp testing (205). This may explain our findings of absent worsening RV function, since the achieved final speeds were lower than 5600 RPM. In conclusion, our findings advocate the use of echocardiographic ramp test to optimize LV unloading post LVAD implantation.

10.6 Limitations

10.6.1 Study I

The study was limited by the small sample size which did not allow meaningful assessment of outcomes such as quality of life or reaching transplantation. Another limitation is the lack of control group with placebo infusion and the findings may be due a placebo effect. However, in prior randomized trials, placebo has not affected CO or stroke volume (206, 207). We used PBF as a surrogate for CO, which due to shunts may entail limitations for cross-sectional between-patient comparisons but is reliable for within-patient, changes over time, which was the purpose in our study.

10.6.2 Study II

A major limitation was the small sample size with, in particular, few patients in the control group. Additionally, the three cohorts were recruited in different settings and there was no specific echocardiography protocol in the HFrEF patients. Moreover, the outcome definitions in HFrEF and HFpEF differed. Hospitalisations were not included in HFrEF due to a too high event rate, while implantation of LVAD or HTx were considered as deterioration and included in the composite endpoint for HFrEF. Including LVAD and HTx as part of a composite endpoint is standard practice in HFrEF. The alternative, to censor patient at LVAD and HTx would underestimate risk in HFrEF, since censoring precludes subsequent competing events.

Furthermore, despite multivariable adjustment, it is difficult to compare HFpEF and HFrEF considering the different demographic and comorbidity profiles. The higher hazard ratio in HFpEF than in HFrEF is suggestive of a potentially stronger prognostic role of sST2, but by no means conclusive. Finally, it remains unclear whether the association between sST2 concentrations and the prognosis of patients is HF specific, since sST2 concentrations may reflect other pathologic processes, such as pulmonary disease.

10.6.3 Study III

The study was limited by the single center nature of the study, and that RHCs were performed by different cardiologists and results must be regarded as hypothesis generating. However, the analysis of the data was performed by a single experienced invasive cardiologist whereby a standardized interpretation of hemodynamic data was performed. There was no control group of healthy individuals. Furthermore, we used the PP method to calculate PAC, is an indirect estimate which has been reported to overestimate PAC (163, 208, 209).There are alternative approaches to calculate PAC but they are impractical.

Moreover, despite the overall constant RC-time, there remains significant scatter around the curve fits which may be due to measurement error or potential unknown determinants.

10.6.4 Study IV

This study was limited by a small sample size, and despite being a single center study, the ramp tests were performed by different cardiologists. This does however; reflect real life clinical practice and the same criteria for ramp testing were applied in all patients. The echocardiographic examinations and evaluations were not blinded but we tried to reduce bias by having one single cardiologist interpreting all the examinations. The upper speed limit was not reached during ramp testing for different reasons; however, there was a significant increase of LVAD speed during ramp testing. Moreover, the duration of LVAD support before ramp test and the time to follow-up echocardiographic examinations varied which may have caused a time bias. Finally, no powerful clinical outcomes like readmission rates, functional capacity, and HRAEs were considered.

10.7 Future perspectives

HF remains a chronic, incurable, and generally irreversible syndrome despite the advances achieved in diagnosis, prognostication and treatment. Novel approaches are required to refine our diagnostic skills and improve our therapies. Considering the complexity of HF pathophysiology and care, increased awareness and better collaboration and shared care between the primary care physician and the cardiologist are needed to improve outcomes. In **Studies I-IV**, patients with HF were referred from local HF hospital or primary care units to an advanced HF center, where further evaluation and therapies were planned and implemented such as RHC, echocardiography, LVAD, and levosimendan.

In HFrEF, the main challenges are the implementation and optimal utilization of existing evidence-guided therapies (1, 210). The non-invasive ramp test may be useful to optimize LV unloading and improve LVAD therapy in patients with advanced HF. Repetitive use of levosimendan may be acceptable as a palliative measure for patients with advanced and no other treatment options.

Regarding HFpEF, challenges remain both concerning diagnosis and therapy. The use of NPs to diagnose HFpEF with and without atrial fibrillation is already established (211) and has been added to the required criteria to diagnose HFpEF in the ESC guidelines (1). However, there is an increasing interest to use novel biomarkers that reflect other distinct pathophysiological pathways. sST2 and other biomarkers may be useful to diagnose, and risk stratify as well as serve as potential therapy targets and possibly individualize therapy (212, 213).

RV failure is common in both HFpEF and HFrEF. Describing RV afterload in terms of PVR is an oversimplification of the pulmonary circulation. Beyond PVR, the measurement of PAC gives a more accurate assessment of RV afterload which may be useful for diagnosis, treatment monitoring and is a potential treatment target.

11. CONCLUSIONS

By assessing hemodynamics, echocardiography and biomarkers, it may be possible to gain a better understanding of the different HF phenotypes and the underlying physiology, which may introduce potential targets for future therapy. This current analysis of invasive and non-invasive methods used to evaluate the cardiac performance has improved our understanding of these methods and added knowledge to the already existing evidence.

Study I: Levosimendan was associated with improved hemodynamics in patients with advanced stable chronic HF. However, no predictor of the hemodynamic response was identified.

Study II: In patients with HFpEF compared to HFrEF, crude levels of sST2 were lower but potentially more strongly associated with outcomes.

Study III: The pulsatile rather than the steady PAWP component stands for the previously documented PAWP impact on the PAC-PVR relationship in HF patients.

Study IV: Echocardiographic ramp test allowed LVAD speed adjustment and optimization, and improved LV unloading with no evidence of worsening of RV function.

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