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DOCTORAL DISSERTATION

A critical appraisal of Neurohumoral and Hemodynamic Alterations in Heart Failure

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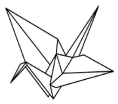

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LIST OF ABBREVIATIONS

Abbreviations:

ACE: angiotensin converting enzyme

ADHF: acute decompensated heart failure

ARB: angiotensin receptor blocker

AUC: area under the curve

AV: atrioventricular

AVP: arginine vasopressin

BSA: body surface area

BUN: blood urea nitrogen

CI: cardiac index

CI: contractility index

CRT: cardiac resynchronization therapy

CS: coronary sinus

CVP: central venous pressure

eGC: endothelial glycocalyx

eGFR: estimated glomerular filtration rate

EnNaC: endothelial sodium channel

FFR: force-frequency relationship

GAG: glycosaminoglycan

GFR: glomerular filtration rate

HA: hyaluronic acid

HF: heart failure

HFmrEF: heart failure with mid range ejection fraction

HFpEF: heart failure with preserved ejection fraction

HFrecEF: heart failure with recovered ejection fraction

HFrEF: heart failure with reduced ejection fraction

ICD: intracardiac cardioverter defibrillator

LBBB: left bundle branch block

LVEF: left ventricular ejection fraction

LVESVI: left ventricular end systolic volume index

LVOT: left ventricular outflow tract

MAP: mean arterial pressure

Na^+: sodium

NO: nitric oxide

NT-proBNP: N-terminal of the prohormone of B-type Natriuretic Peptide

NYHA: New York heart association class

PAC: pulmonary artery catheter

PAP: pulmonary arterial pressure

PAWP: pulmonary arterial wedge pressure

PRA: plasma renin activity

RAAS: renin-angiotensin-aldosterone system

RAP: right atrial pressure

RBF: renal blood flow

RCM: red cell mass

RI: resistive index

ROC: receiver-operating characteristic

RV: right ventricular

SBP: systolic blood pressure

sGAG: sulphated glycosaminoglycan

SNS: sympathetic nervous system

UA: uronic acid

VII: venous impedance index

GENERAL INTRODUCTION

Heart failure (HF) is a clinical syndrome characterized by typical symptoms that may be accompanied by signs caused by a structural and/or functional cardiac abnormality, resulting in a reduced cardiac output and/or elevated intra-cardiac pressures at rest or during stress (1). The etiologies of HF are broad and vary from a genetic condition, ischemic heart disease or other pathologies (tachycardia, pulmonary disease, etc.), over external factors (chemotherapy, ethanol, etc.) to unknown causes. The main terminology used to describe HF is historical and based on clinical signs and symptoms as well as measurement of the left ventricular ejection fraction (LVEF). Three distinct categories are defined: those with normal LVEF (considered as LVEF ≥ 50%; HF with preserved ejection fraction or HFpEF, those with reduced LVEF (<40%, HFrEF) and recently patients with an LVEF of 40-49% defined as HF with mid range ejection fraction (HFmrEF). However, LVEF is rather dynamic over time with HFpEF patients progressing to HFrEF and vice versa. The prevalence of HF is approximately 1-2% of the adult population in developed countries, rising to ≥10% among people >70 years of age (2-4). Over the last 30 years, advancement in treatments and their implementation have improved survival and reduced hospitalization rate in patients with HF. Nevertheless, the outcome of HF remains rather poor with a 12-month all-cause mortality for hospitalized and ambulatory HF patients of 17% and 7% and a 12-month hospitalization rate of 44% and 32%(5).

Currently, the most important reasons for HF-related morbidity and mortality are associated with increased cardiac filling pressures and volume overload (6). Major insights in the pathophysiology of this condition go back several decades when the importance of the neurohumoral systems – the renin-angiotensin-aldosterone system (RAAS) and sympathetic nervous system (SNS) – and the hemodynamic disturbances in the cardiovascular system were discovered (7). Unrestrained neurohumoral activation due to cardiac dysfunction is up till today considered as one of the key drivers of disease progression in HF with reduced ejection fraction (7). It has been demonstrated many years ago that the degree of increase of activity of the neurohumoral systems are directly associated with prognosis in HF patients (8). The important cardio-renal relation in HF originates from the fact that neurohumoral activation stimulates renal sodium and water retention in different nephron segments. Normally, renal sodium and water excretion parallels intake to sustain an euvolemic state. However, in patients with HF, sodium and

water are paradoxically retained despite an increase in intravascular volume eventually causing increased cardiac filling pressures, pulmonary congestion and peripheral edema, typical symptoms of HF (7). Both neurohumoral activation and raised cardiac filling pressures further disturb intrarenal hemodynamics and renal function, creating a vicious circle for disease progression (9). Better insights in renal sodium and water handling as well as the effect of hemodynamic alterations on renal function might identify HF patients prone for volume overload and at risk for adverse outcomes. Indeed, an intact and intricate balance between the hemodynamic and neurohumoral systems is necessary to preserve the euvolemic state regulated by the kidneys. Generally, it is assumed that edema occurs when Starling forces of the capillary membrane favor transudation into the interstitial compartment exceeding lymphatic drainage (10). However, while decompensated HF patients always present with elevated filling pressures, the occurrence of pulmonary and peripheral edema is poorly correlated with cardiac filling pressures (11, 12). Thus, the relation between intravascular volume and intra-cardiac pressure is not straightforward in many patients. Therefore, other factors, such as local interstitial and endothelial factors, might determine raised cardiac filling pressures and the occurrence of extravascular edema.

Blockers of the neurohumoral systems i.e. angiotensin-converting enzyme (ACE)-inhibitors, angiotensin receptor blockers (ARB), mineralocorticoid receptor antagonists (MRA), and beta-blockers have a class I level of evidence A recommendation in both the European and American guidelines as disease-modifying therapeutic agents in the treatment of heart failure with reduced ejection fraction (13, 14). More recently, cardiac resynchronization therapy (CRT) – an implantable cardiac device - has become an important therapeutic option too in patients with HFrEF and ventricular conduction delay. Due to the success of neurohumoral blockers and implantable devices, HF nowadays has become a treatable chronic disease. In a large part of HF patients, stabilization and often improvement of symptoms and cardiac dysfunction is possible. Moreover, in an important number of HF patients substantial or complete myocardial recovery occurs. Importantly, even in patients with complete myocardial recovery and normalization of cardiac function, neurohumoral blockers are often continued for life, while some of these patients might be taken off these drugs.

The aim of this doctoral thesis is to critically (re-)investigate the neurohumoral and hemodynamic alterations deemed responsible for typical HF symptoms and disease progression. Better understanding of the contributory role of these processes and new

insights may shed light on novel individualized diagnostic and therapeutic strategies for heart failure patients.

RESEARCH OBJECTIVES

Objective 1 | To explore the role of interstitial sodium and fluid handling in the pathophysiology, clinical presentation and prognosis of heart failure (Part I).

Objective 2 | To investigate the homeostasis of intravascular volume and to identify the effects of intravascular volume changes on central hemodynamics and renal function in Heart Failure (Part II).

Objective 3 | To study neurohumoral activation and to explore the contribution of different (medical and device-related) therapies to myocardial recovery in Heart Failure (Part III).

PART I

Interstitial Salt handling in Heart Failure

OBJECTIVE | To explore the role of interstitial sodium and fluid handling in the pathophysiology, clinical presentation and prognosis of heart failure

CHAPTER 1

The Pathophysiological Role of Interstitial Sodium in Heart Failure

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ABSTRACT

The current understanding of heart failure (HF) does not fully explain the spectrum of HF symptoms. Most HF hospitalizations are related to sodium and fluid retention resulting from neurohumoral up-regulation. Recent insights suggest that sodium is not distributed in the body solely as *free cations*, but is also *bound* to large interstitial glycosaminoglycan (GAG) networks in different tissues, which have an important regulatory function. In HF, high sodium intake and neurohumoral alterations disrupt GAG structure, leading to loss of interstitial buffer capacity and disproportionate interstitial fluid accumulation. Moreover, a diminished endothelial GAG network (the endothelial glycocalyx [eGC]) results in increased vascular resistance and disturbed endothelial nitric oxide (NO) production. New imaging modalities can help evaluate interstitial sodium and eGC integrity. Furthermore, several therapies are proven to stabilize interstitial GAG networks. Hence, a better appreciation of this new sodium "compartment" might improve current management of HF.

INTRODUCTION

Approximately 90% of heart failure (HF) hospitalizations are associated with signs and symptoms of sodium and fluid excess, which are associated with disease progression and worse prognosis (15, 16). Traditionally, the primary abnormality in HF was understood to be sodium handling, whereby water movement passively follows sodium to keep osmolality in balance. Due to neurohumoral up-regulation and increased arginine vasopressin (AVP) production, the kidneys are not capable of adjusting sodium excretion to sodium intake. The resulting imbalance leads to sodium accumulation, followed by interstitial and intravascular volume retention, and, eventually, to edema and increased cardiac filling pressures (10). However, prior to admission for acute decompensated HF (ADHF) patients display a wide spectrum of weight changes, with less than 50% gaining substantial weight (>1 kg)(11). Moreover, although a significant increase in cardiac filling pressure is consistently observed days before an ADHF admission, a broad range of plasma volumes was observed in ADHF patients (12, 17). Finally, total body sodium levels were found to be increased in observational studies of HF from more than 60 years ago (18). Interestingly, this increase was found in patients both with overt peripheral edema and without edema (19, 20). Important changes in total body sodium occur over extended periods of time, even in healthy subjects on a stable sodium diet, and are not accompanied by changes in total body water (TBW) (21, 22). Therefore, the classic idea of simultaneous sodium and fluid retention may not always hold true as an explanation for fluid overload and increased cardiac filling pressures in ADHF.

Recent evidence has demonstrated that a large part of total body sodium is bound to glycosaminoglycan (GAG) networks in the interstitium, which function as sodium buffers and play an important role in fluid homeostasis and endothelial function. This review aims to provide insight in the important physiological role of interstitial sodium bound to GAGs in preserving sodium and fluid regulation, as well as endothelial function. Better understanding of the contributory role of interstitial sodium across the spectrum of HF presentations may shed light on a novel therapeutic target that has otherwise been overlooked.

THE BODY TIGHTLY REGULATES SODIUM AND WATER BALANCE

A typical Western diet contains approximately 12 g salt (sodium chloride or NaCl) per day, equivalent to ~4.5 g or ~200 mmol sodium (Na^+), which is almost completely absorbed in the gastrointestinal system. The plasma sodium concentration and osmolality start to rise

30 to 60 min after an oral sodium load (23). Because the body tightly regulates osmolality through osmoreceptors in the hypothalamus, a rise of even a few mOsm/L in plasma osmolality results in retention of free water through stimulation of thirst and AVP release. Baroreceptors in the large (aortic arch, carotid sinus) and small vasculature (pulmonary vasculature, renal afferent arteriole) subsequently sense a rise in total body water (TBW) to modulate urinary sodium and water excretion. From the plasma, sodium is freely filtered in the renal glomerulus. As tubular sodium reabsorption exceeds 99%, only a tiny fraction is excreted in the urine. In normal circumstances, extrarenal sodium loss from skin (sweat) and from the gastrointestinal tract (feces) is negligible. Nevertheless, because relatively small changes in sodium excretion by the kidneys can lead to marked alterations in TBW (24), this tiny fraction of renal sodium excretion is highly regulated to mimic dietary intake.

SODIUM BUFFERING BY GLYCOSAMINOGLYCANS

On the basis of intracellular and extracellular sodium concentrations, approximately 65% of total body sodium is assumed to reside in the extracellular fluid (plasma fluid and interstitial fluid), while only 5% to 10% is found in the intracellular fluid (24). The remaining 25% of total body sodium is sequestered in bone as sodium apatites and is not readily exchangeable, in contrast to sodium in the extracellular and intracellular fluid compartments.

Contemporary evidence indicates that sodium cations are largely bound to negative biopolymers, called glycosaminoglycans (GAGs)(25, 26). GAGs are linear polymers of disaccharide units with variable lengths that are modified by sulfation and/or acetylation/deacetylation. Thus, all GAGs have negative charges in the form of carboxyl and sulfate groups (Figure 1)(27). Multiple GAG chains can anchor to a linear linking protein, forming a large brush-shaped *proteoglycan*, which contains numerous anionic charges. They are connected via intramolecular hydrogen bonds to form a compact macromolecule (28). The extremely polyanionic nature of these macromolecules leads to electrostatic interactions between their negatively charged surfaces and, for example, collagen fibrils, proteins, and positive electrolytes, creating a network with a high oncotic pressure. In vitro studies observed that the interaction with sodium, the most abundant cation of the extracellular compartment, is favored over other ions and proteins (29). Consequently, a large amount of sodium is bound to GAGs, creating a microenvironment of hypertonic sodium concentration (30). However, the dense network exhibits a low compliance, secondary to its strong elastic and tensile force, thereby "pressing" fluid out. Importantly,

disruption of bonds within GAGs or alterations in bound molecules will have significant structural and functional consequences for proteoglycans (31, 32).

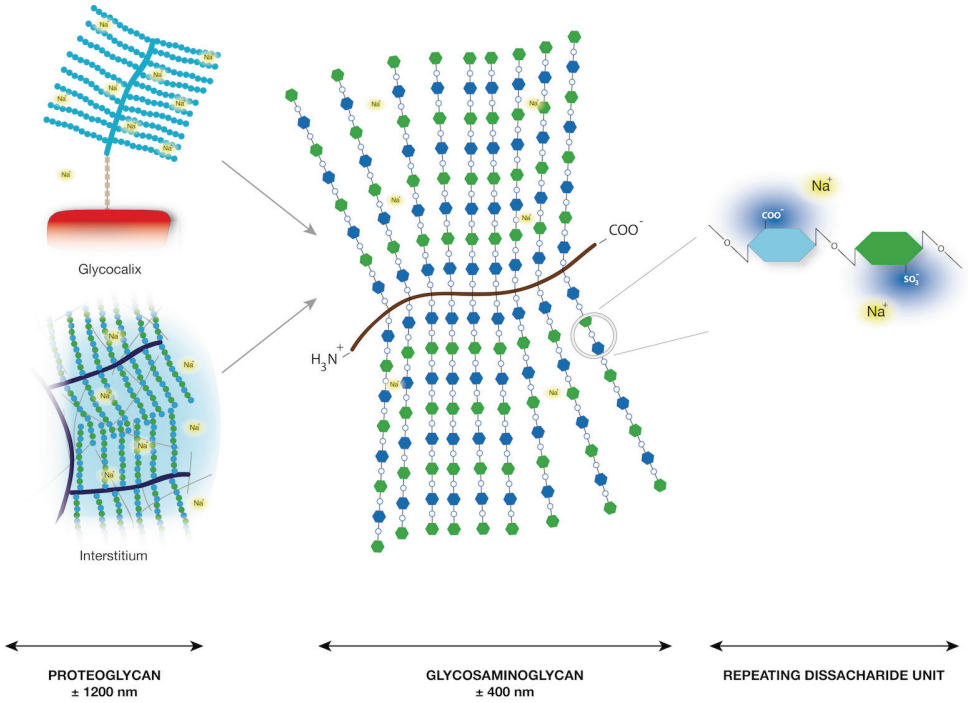

Figure 1. Proteoglycan and glycosaminoglycan. *Proteoglycans are the major structural components of the interstitium of different tissues and the first endothelial layer (the endothelial glycocalyx (eGC)). Proteoglycans consist of multiple glycosaminoglycans (GAG) attached to a linking protein. GAGs are linear polymers of disaccharide units that are modified by sulfation and/or (de)acetylation and have fixed negative charges in the form of carboxyl and sulfate groups The polyanionic nature of the GAG network leads to electrostatic interactions with different molecules, particularly sodium cations.*

INTERSTITIAL SODIUM

Sodium accumulates dynamically in interstitial GAG networks

The interstitium connects and supports tissues while serving as a transport medium for nutrients, waste products, and signaling molecules. GAGs are the main constituents of the interstitium of various tissues (25,26). Together with collagen/elastin fibers, they comprise the *solid* phase and determine the structure and compliance of the interstitium (33, 34). Since 1 GAG macromolecule can bind a large quantity of sodium cations, the interstitium

can accumulate *or buffer* a high amount of sodium (Figure 2, A)(35). Data from long-term balance studies in humans confirm that considerable amounts of sodium accumulate in the interstitium, particularly in skin and muscle tissue, *without compensatory water retention* or changes in plasma sodium concentration (22, 36, 37). Kopp et al. recently quantified sodium concentrations in skin and muscle on the basis of ^{23}Na-magnetic resonance spectroscopy (^{23}Na-MRI). Their data suggest that, in contrast to a very stable plasma sodium concentration, the tissue sodium content in humans is highly variable, and these variations are not accompanied by changes in tissue fluid content (26, 38). As a consequence, in both normal circumstances and compensated HF states, interstitial GAG networks "smooth" fluctuations in plasma sodium concentrations, and therefore conceal sodium ions for pituitary osmoreceptors, preventing AVP release and water retention. Moreover, because these secluded sodium cations do not reach the renal nephron, they also escape renal regulatory function and are more difficult to remove from the body.

In vitro studies have also shown that the interstitial GAG network can adapt to short periods of higher salt intake, as a high concentration of sodium cations changes the sulfation pattern and increases GAG charge density (28, 39). High sodium concentrations also promote gene expression of GAG polymerization enzymes, which further increases GAG content, thus activating a positive feedback pathway to expand sodium storage capacity (Figure 2, B) (21). In the reverse situation of salt scarcity, GAG polymerization and sulfation are reduced, and a subsequent reduction in matrix is associated with gradual mobilization of sodium from tissue reservoirs (40).

However, if an excessively high sodium concentration in the GAG network is prolonged, the conformation of the macromolecules will eventually be altered, leading to a dysfunctional GAG network with loss of interstitial network integrity and buffering capacity (Figure 2, C). The loss of interstitial buffering capacity may be especially important in salt-sensitive hypertension, as well as in neurohumoral activation in HF (41, 42).

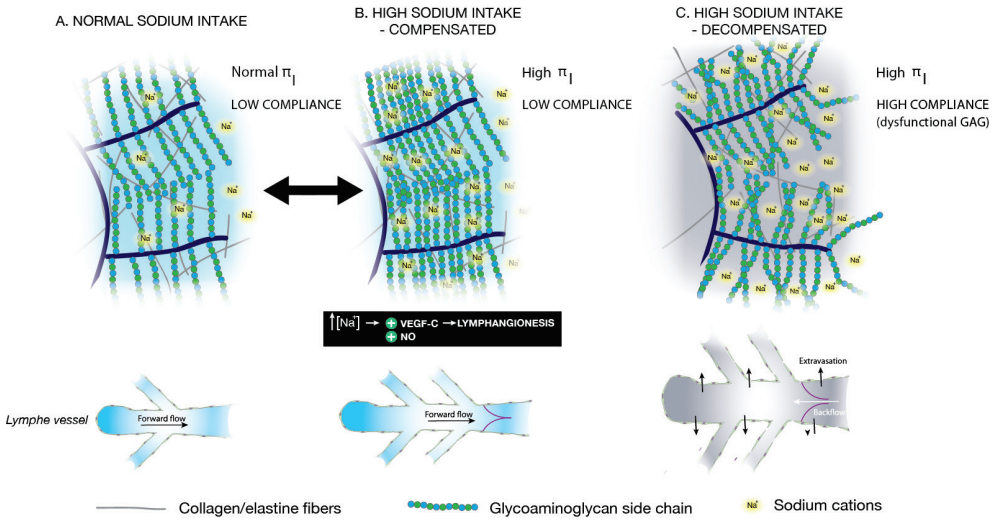

Figure 2. The interstitium. *A) The interstitium consists of a network of proteoglycans and collagen/elastin fibers, which determine its compliance. Interstitial fluid is formed by transcapillary filtration of plasma fluid and subsequently drained by many lymphatic vessels B) High dietary sodium intake leads to an interstitium with a large GAG-network which can accumulate a higher concentration of sodium (and proteins) and thus creating a higher interstitial oncotic pressure. A high interstitial concentration of sodium acts as a positive stimulus for lymphangiogenesis through tissue VEGF-C and NO production. Because of the low interstitial compliance and increased lymphatic capacity, interstitial fluid is efficiently drained and there is no interstitial fluid accumulation nevertheless interstitial oncotic pressure is high (compensated state) C) When the conformation of the GAGs is altered, the GAG network becomes dysfunctional and the interstitium enters an high-compliance state. Furthermore, high interstitial oncotic pressure (as in high salt intake) favors transcapillary filtration of interstitial fluid. Additionally, lymphatic drainage becomes less efficient due to widening of lymphatic vessels and disturbance of propagation coordination, leading to lymph extravasation. Therefore, in HF, interstitial fluid can accumulate (decompensated state) even in patients with mildly elevated venous pressures.*

Interstitial fluid transport is regulated by interstitial sodium microenvironments

As stipulated previously, GAGs create a high osmotic pressure microenvironment (34). Therefore, individuals with a more dense interstitial GAG network—and consequently with a higher interstitial oncotic pressure (π_I)—will have more filtration of plasma fluid over the capillary membrane into the interstitium. However, the limited elastic properties (and thus low compliance) of the interstitial GAG network prevent fluid accumulation (43, 44). Indeed, small increases in interstitial fluid content lead to important increases in interstitial tensile stress. This forces interstitial fluid into the gaping lymphatic vessels. As fluid quickly drains into the systemic circulation, interstitial hydrostatic pressure remains low and oncotic

pressure remains high (*compensated state*) (Figure 2, B). Moreover, subcutaneous tissue macrophages can sense *high sodium concentrations* and react by expressing tonicity enhancer binding protein (TonEBP)(40), a transcription factor regulating the expression of osmoprotective genes in response to osmotic stress. Vascular endothelial growth factor C (VEGF-C), a potent inducer of lymphatic vessel formation and endothelial nitric oxide synthase (eNOS) expression, is 1 of the genes induced by TonEBP, further stimulating lymphangiogenesis (45, 46). Indeed, higher VEGF-C levels and robust lymphatic vessel hyperplasia in the dermal interstitium have been found in response to high-salt feeding (47, 48).

Interstitial edema formation depends on interstitial matrix composition

Interstitial fluid accumulates when the rate of transudation from capillaries into the interstitium exceeds the rate at which the lymphatic system can efficiently drain the fluid. Venous pressure, more than arterial pressure, increases capillary hydrostatic pressure (24). Therefore, increased central venous pressure (CVP) and pulmonary capillary wedge pressure (PCWP) in HF promote interstitial fluid accumulation. In response, lymphatic capacity gradually increases, parallel to a rise in venous pressure, and only in higher ranges of venous pressures is the return of lymph to the great veins impeded (49, 50). However, while decompensated HF patients always present with elevated filling pressures (19), the occurrence of pulmonary and peripheral edema correlate poorly with PCWP and CVP, respectively (19, 51, 52). Other factors in addition to increased capillary pressure must play a more important role in determining the occurrence of edema.

Indeed, as described by the Starling equation, high *interstitial oncotic (*π_I) pressures and low *interstitial hydrostatic* pressures promote transudation of plasma fluid into the interstitium, while *low interstitial compliance* opposes fluid accumulation. A prolonged, excessively high sodium concentration leads to a dense interstitial GAG network with the accumulation of sodium cations (high π_I), as well as altered conformation of the GAG macromolecules, creating a *dysfunctional GAG network.* This may result in decreased tensile stress, and thus a high compliance state of the interstitial matrix (Figure 2, C). The combination of high interstitial oncotic pressure and high compliance facilitates fluid transudation (44). Importantly, spironolactone stabilizes altered GAGs, suggesting that unrestrained neurohumoral stimulation in HF further contributes to dysfunction of the GAG network (53, 54). Moreover, lymphatic vessel integrity is altered as lymph vessels start to widen, leading to leakage of lymph into the interstitium (Figure 2, C)(50). Thus, when

interstitial GAG networks become dysfunctional, even mildly elevated venous pressures in heart failure might lead to pulmonary congestion and peripheral edema *(decompensation).*

Sodium and endothelial function

The endothelial GAG network controls endothelial function

The eGC, the inner fragile layer of the endothelium—a highly specialized variant of interstitium —comprises a network of membrane-bound and different types of soluble proteoglycans (mostly heparin sulfate) and glycoproteins, which are connected to the endothelial cell membrane through adhesion molecules. A dynamic equilibrium exists between the eGC and flowing blood, which continuously affects the composition and thickness of the eGC (53, 55). Both endothelium- and plasma-derived soluble molecules (e.g., albumin, proteins, ions) interact with this mesh (Figure 3)(56).

The eGC has multiple vasoprotective functions, and shields the underlying apical side of the endothelium from the plasma. It reduces vascular permeability, restricts molecules from reaching the endothelium and prevents interaction of platelets and leucocytes with endothelial cell adhesion molecules (57, 58). Moreover, the *endothelial GAG network* acts as a sodium buffer by binding positively charged sodium cations (59). Sodium reversibly binds and dissociates from eGC binding sites. As a result, the eGC buffer allows gradual passage of sodium from the blood into the space between the eGC and endothelium (60). Sodium can subsequently enter the endothelial cell through apical endothelial Na^+ channels (EnNaC, almost identical to epithelial Na^+ channels). Next, sodium/potassium (Na^+/K^+) ATPase pumps at the basolateral side quickly try to restore cell homeostasis, by creating a transcellular passage for sodium into the interstitium (24). However, most sodium is transported between endothelial cells along its electrochemical gradient via the paracellular pathway. Importantly, the eGC also acts as a mechanotransducer, transmitting shear stress signals into specific cell signaling processes in the endothelial cell, and influencing nitrous oxide (NO) production and cytoskeletal reorganization (55, 61).

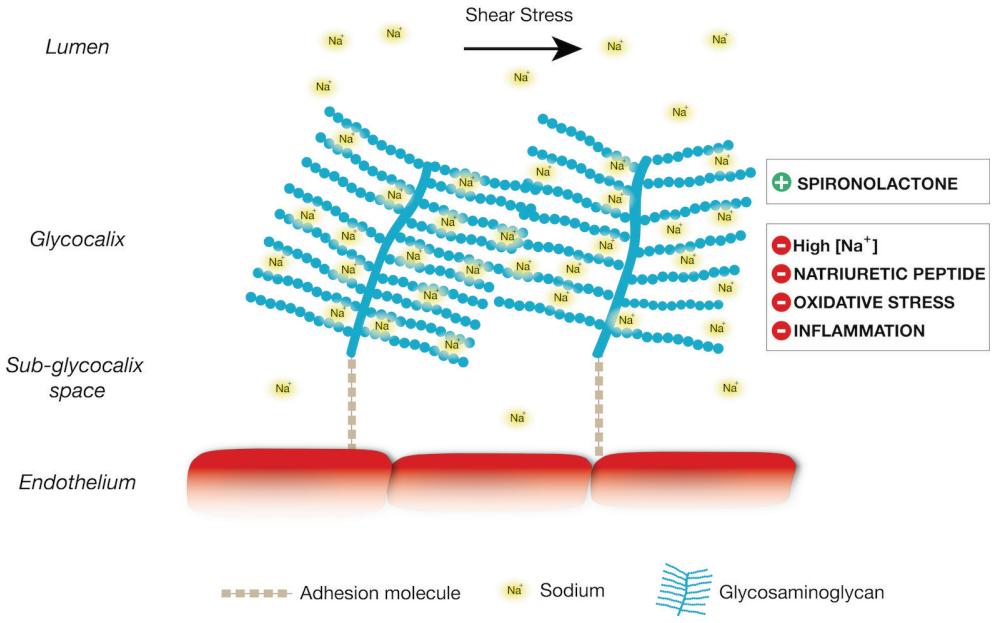

Figure 3. The endothelial glycocalyx (eGC). *The eGC is a network of glycosaminoglycans, connected to the underlying endothelium by adhesion molecules. It acts as a mechanotransducer and shields the underlying epithelium from blood cells. Moreover, due to the net negative charge, positively charged molecules like sodium and plasma-derived proteins are concentrated inside the GAG network creating a buffer, and a high internal oncotic pressure. Inflammation, oxidative stress, natriuretic peptides and high sodium concentration all will shed the eGC, while spironolactone has protective effects.*

High intraendothelial sodium concentration reduces nitric oxide production

Sodium uptake in the endothelial cell is promoted if the abundance of EnNaC in the apical membrane increases and/or EnNaC channel activity is stimulated. This occurs when the plasma sodium concentration increases (Figure 4)(62). Furthermore, high concentrations of aldosterone, (as observed in HF) trigger rapid membrane insertion of preformed EnNaC complexes residing in vesicles just beneath the plasma membrane (63). As a consequence, sodium uptake into endothelial cells is stimulated in HF patients, especially those on high-salt diets. Importantly, even minor elevations in sodium influx into the endothelial cell through EnNaCs have large consequences for endothelial stiffness and NO production (64, 65). Recent atomic force microscopy studies have observed that the endothelial cell stiffens within minutes of an acute elevation of intraendothelial sodium concentrations (62). This acute endothelial cell reaction is mediated by interactions between EnNaC and cytoskeletal proteins (actin) in the endothelial cell submembraneous cortical cytoskeleton (Figure 4)(66).

Sodium entry via EnNaCs also reduces endothelial nitric oxide synthase (eNOS) activity via the PI3K/AKT signaling pathway (67-69). Additionally, sodium up-regulates the endogenous inhibitor of NOS, asymmetrical dimethyl-L-arginine. Both result in decreased nitric oxide (NO) levels (a hallmark of endothelial dysfunction) and impair relaxation of the smooth muscle cells surrounding the vessels (62, 70). This plays an important role in endothelial dysfunction, on top of the loss of mechanotransduction of shear stress when the eGC is diminished.

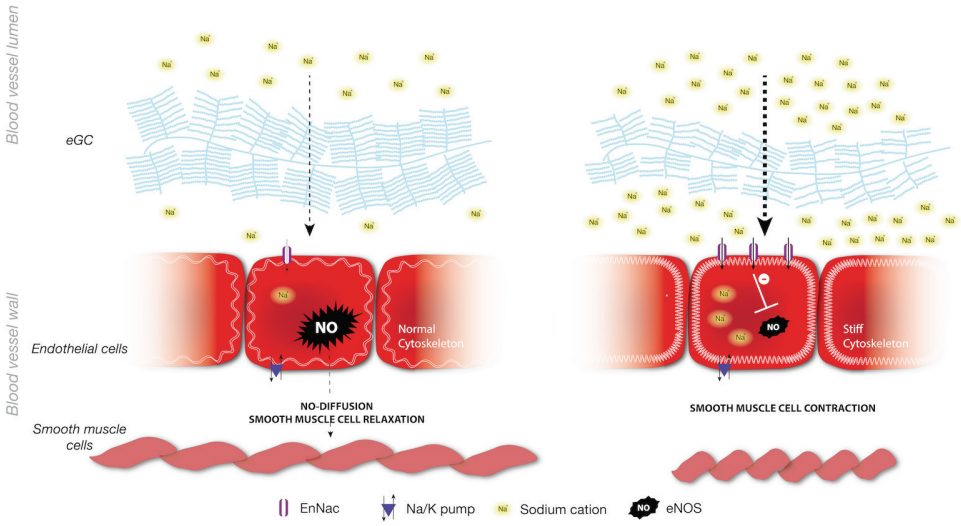

Figure 4. Endothelial dysfunction. *Damaging of the eGC leads to increased vascular permeability (dotted arrow), diminished sodium buffer capacity and disturbed mechanotransduction in response to shear stress. High sodium concentrations and high aldosterone concentration promote the abundance of EnNaCs at the apical membrane of endothelial cells. Subsequently, sodium activates EnNaCs, altering endothelial cytoskeleton organization, and stiffening the endothelial cell. Moreover, high EnNaC activity and disturbed mechanotransduction will impair NO production – a characteristic of endothelial dysfunction - influencing smooth muscle cell contraction.*

Sodium and natriuretic peptides disrupt the eGC

Prolonged plasma sodium concentrations in the high physiological range (>140 mmol/L) damage the eGC. In an original in vitro experiment, Oberleithner and coauthors showed that sodium overload changes the negatively charged sulfate residues in the eGC, resulting in eGC dysfunction (53). Interestingly, spironolactone prevented these harmful effects of sodium on the eGC (53). Furthermore, it is well known the eGC can be severely damaged by inflammation, ischemia/reperfusion, oxidative stress, excessive shear stress and enzymatic degradation, all of which are common in heart failure (55, 71). Intriguingly,

several animal studies have demonstrated that natriuretic peptide (ANP, BNP and CNP) disrupt the eGC (72, 73). In an elegant in vivo model, physiological doses of natriuretic peptides were observed to lead to shedding of the eGC, as assessed by venous washout of glycocalyx constituents (syndecan [an endothelial proteoglycan] and heparin sulfate), as well as morphologically confirmed electron microscopic changes in eGC integrity (73).

Oberleithner further demonstrated that sodium overload leads to increased intracellular endothelial sodium concentrations. It is speculated that sodium will not be buffered when the eGC is damaged and—instead of a *gradual* presentation to the underlying endothelium—large amounts of sodium cations reach the apical side of the endothelium and intercellular clefts at once. This enhances the activity of EnNaCs, altering endothelial mechanical properties and function, as well as promoting paracellular sodium transport to the interstitial space, contributing to interstitial fluid accumulation.

A dysfunctional eGC results in vascular dysfunction

In vitro experiments confirmed that inadequate responses to shear stress variations and impaired NO production are noticed when the eGC is disrupted (74). It is well known that, compared with healthy subjects, endothelium-dependent NO-mediated vasodilation is impaired in skeletal muscle, and in the coronary and pulmonary circulations of patients with chronic HF (75-77). Overall, the lack of NO increases vascular smooth muscle tone and consequently increases vascular resistance (78). Increased arteriolar resistance results in increased cardiac afterload, which often characterizes ADHF. In this respect, it is interesting to note that multiple studies have observed that arterial stiffening significantly improves with dietary salt reduction (79, 80). Furthermore, in vivo experiments showed that high salt intake and deficient NO production also leads to a higher tone in the venous side of the vasculature (81-84). Importantly, the largest part of total blood volume—about three-fourths—resides in veins and venules (24). Similar to autonomic regulated venous constriction (85), a dysfunctional eGC might also contribute to the shift of fluid from the venous reservoir into the effective circulatory volume. Finally, when the eGC is disrupted, vascular permeability increases, and plasma fluid extravasation into the interstitium of different tissues is no longer impeded (86, 87).

In conclusion, a dysfunctional eGC contributes to the increased cardiac filling pressures in HF patients, which may be an important contributor to decompensation, as it consistently precedes ADHF admissions (88, 89).

TARGETING INTERSTITIAL SODIUM IN HF

Current strategies assume enhanced sodium excretion with diuretics or antagonizing neurohumoral up-regulation to effectively achieve sodium homeostasis at the level of the nephron. However, mortality and rehospitalization rates for HF remain tremendously high (15). As the interstitial compartment also plays an important role in the body's sodium and fluid homeostasis, further understanding of this new "compartment" is of great interest. Moreover, preservation and restoration of normal GAG function in the interstitium, as well as in the eGC, could be interesting new strategies in HF management.

First, identifying dysfunctional interstitial GAG networks and a disrupted eGC may predict HF patients at a higher risk for decompensation. Because persistent signs of sodium and fluid overload in HF are important predictors of mortality and HF rehospitalization, interstitial sodium content might be a good indicator to guide therapy and a new cardiovascular risk factor (90, 91). A recently developed imaging technique, $^{23}Na^{+}$ magnetic resonance imaging, makes it possible to assess the sodium content of different tissues or the whole body and to monitor its evolution during therapy (26). Furthermore, there are currently several methods to determine eGC integrity such as measurements of products shed in plasma (e.g., syndecan or heparan sulfate) or visualization of the eGC with sidestream dark field imaging (videomicroscopy). This technique makes it possible to directly visualize the microcirculation and to detect changes in glycocalyx volume on the basis of in vivo recordings of the sublingual microvasculature (Figure 5)(92).

Secondly, because the eGC plays an important protective role in the maintenance of a normal endothelial function and vascular permeability, stabilizing or restoring this specific GAG network is an interesting new therapeutic target. Preliminary data have shown that in the acute inflammatory and proteolytic situation of a myocardial infarction, hydrocortisone might help to sustain vascular barrier function and, possibly, to abrogate damage to the glycocalyx, (93, 94). A potential area of further research is to determine whether this strategy prevents eGC damage when high levels of natriuretic peptides circulate in ADHF. *Sulodexide* is an old drug that is the subject of renewed interest, especially in the fields of nephrology and vascular medicine. It is a mixture of naturally occurring GAG components (20% dermatan sulfate, 80% heparan sulfate) that can be given orally or intravenously, and was originally used as an anticoagulant. After modification and reduction of its anticoagulant activity, sulodexide also restores the eGC (95, 96). Various mechanisms

could be responsible, such as its anti-inflammatory effects, promotion of synthesis and sulfation of endogenous GAGs and proteoglycans, and antiproliferative properties (97, 98).

Dietary sodium reduction is currently a simple (and probably the most important) way to prevent endothelial dysfunction and interstitial sodium accumulation causing GAG dysfunction. Dietary intake is associated with a higher cardiovascular mortality and more ADHF events in stable HF patients (99, 100). Furthermore, a reduction in sodium intake can lead to a significant decrease in plasma sodium (1.5 to 3.0 mmol/L), and possibly restore dysfunctional GAG networks (101). In this regard, a meticulous change in dietary sodium intake alone can reverse vascular endothelial dysfunction and improve vascular compliance (102, 103).

When edema is present, current applied therapies in ADHF—loop diuretics and ultrafiltration—target free sodium cations and water in the plasma compartment. It is currently not clear how they influence interstitial sodium and GAGs. However, spironolactone has proven beneficial effects that extend beyond its natriuretic effect. Kopp et al. observed that spironolactone induced a large reduction in tissue sodium in hypertensive subjects with high aldosterone levels (38). This recommendation could seem futile because mineralocorticoid receptor antagonists (MRAs) are currently part of general HF management. However, despite their widely demonstrated beneficial effects in HF with reduced ejection fraction (HFREF) patients, MRAs are significantly underused, especially during ADHF, when further mobilization of interstitial sodium may be essential (104, 105). Spironolactone also protects the eGC and influences downstream negative endothelial consequences of sodium overload, as it diminishes EnNaC surface abundance and increases endothelial NO production in vitro (53, 106).

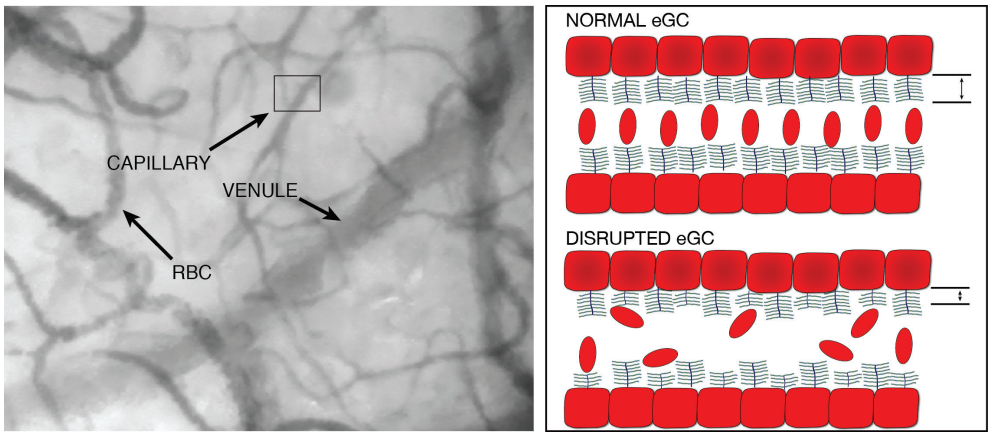

Figure 5. Side Dark Field Videomicroscopy of the Microcirculation. *Real time images of the sublingual microvasculature can be obtained with a handheld videomicroscope (left panel). Moving red blood cells can be directly visualized in arterioles, capillaries and venules. Differentiation is based on vessel width and flow direction. Post hoc soft ware analyses make it possible to assess the integrity of the endothelial glycocalyx. These analyses are based on the mean distance between RBC and vessel wall and the appearance of the flow of RBC in the capillaries (aligned and homogenously distributed along the capillary when the eGC functions properly versus inhomogeneously distributed when the eGC is disrupted) (right panel) (92, 107, 108)**.*

CONCLUSIONS

The interstitium plays an important role in sodium and fluid homeostasis. Sodium is distributed in the body as free cations and bound to networks of negatively charged biopolymers, GAGs, in the interstitium of different tissues. These interstitial GAG networks function as sodium buffers, regulating interstitial fluid accumulation, lymphatic vessel formation, and endothelial function. Chronic sodium overload and neurohumoral up-regulation in HF cause dysfunction of interstitial GAG networks, resulting in increased vascular resistance and permeability, as well as edema. Additional studies are needed to assess if interstitial sodium content is more than just an amenable cardiovascular risk factor. Appraisal of this sodium compartment may provide new therapeutic strategies targeting interstitial network dysfunction and eGC integrity, thereby reducing the burden of HF.

CHAPTER 2

Dermal interstitial alterations in heart failure patients with reduced ejection fraction: A potential contributor to volume overload

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Submitted

ABSTRACT

Background: Large networks of interstitial glycosaminoglycans (GAGs) help to regulate water and electrolyte homeostasis. The relation between dermal interstitial alterations and the occurrence of edema in heart failure patients with reduced ejection fraction (HFrEF) is unknown.

Objectives: The objectives of this study are to demonstrate in HFrEF patients that 1) interstitial GAG density is increased, 2) changes in the interstitial GAG network are associated with interstitial fluid accumulation and, 3) there is a link between the interstitial GAG network and the renin-angiotensin-aldosterone system (RAAS).

Methods: Two punch biopsies of the skin were obtained in healthy subjects (n=18) and HFrEF patients (n=29). Alcian blue staining and immunostaining for the angiotensin II type 1 receptor was performed. After obtaining tissue water content (TWC), total interstitial GAG (Uronic Acid (UA)) and sulphated GAG (sGAG) were quantified. A venous blood sample, clinical examination and echocardiography were simultaneously obtained.

Results: A significantly higher interstitial GAG content was observed in HFrEF patients compared to healthy subjects (UA: 13.0±4.2 vs 9.6±1.6 μg/mg; p=0.002; sGAG 15.7±5.9 vs 10.1±1.2 μg/mg; p<0.001). Only in HFrEF patients, (s)GAG density was strongly associated with TWC and peripheral edema (UA: R^2=0.5,p<0.001 and sGAG R^2=0.7;<0.001). Expression of the angiotensin II type 1 receptor was found on dermal cells, while use of ACE-inhbitors/ARB was associated with significantly lower levels of interstitial GAGs in HFrEF patients.

Conclusion: Interstitial GAG concentration is significantly increased in HFrEF patients compared to healthy subjects, and correlated with tissue water content and clinical signs of volume overload. A better appreciation of the interstitial compartment might improve current management of volume overload in HF.

INTRODUCTION

Congestive heart failure (HF) is characterized by signs and symptoms of fluid overload. Secondary to neurohumoral upregulation, the kidneys are not capable of adjusting sodium excretion to intake in HF which leads to sodium and fluid accumulation. Generally it is assumed that edema occurs when Starling forces of the capillary membrane favor transudation into the interstitial compartment exceeding lymphatic drainage (10). However, while decompensated HF patients always present with elevated filling pressures, the occurrence of pulmonary and peripheral edema is poorly correlated with cardiac filling pressures (11, 12). Therefore, other factors than increased capillary hydrostatic pressure might also determine the occurrence of extravascular edema.

Recent insights suggest that large networks of glycosaminoglycans (GAGs) in the interstitium of different tissues may influence sodium and water homeostasis (109). In normal circumstances, GAG networks can buffer a large amount of sodium (Na^+) ions, yet keeping tissue water content stable. We recently hypothesized that in HFrEF, total body sodium accumulation and neurohumoral alterations might increase GAG density and sulphation, ultimately contributing to fluid accumulation (109).

The objectives of this study are threefold. First, to demonstrate that interstitial GAG density and sulphation of GAGs are increased in HFrEF patients compared to healthy subjects. Second, to demonstrate that interstitial fluid accumulation is associated with changes of the interstitial GAG network and the clinical presentation of edema in HF patients. Third, that the renin-angiotensin-aldosterone system (RAAS) affects structure and function of these networks.

METHODS

This study was carried out in a single tertiary care center (Ziekenhuis Oost-Limburg, Genk, Belgium). Inclusion was between March 15, 2016 and June 15, 2016. The study complied with the Declaration of Helsinki and the institutional review board approved the study protocol. All subjects provided written informed consent before any study-specific intervention was performed.

Study population

Patients were eligible for study inclusion if ≥18 years of age and able to give informed consent. Per cohort consecutively consented patients were included. *Healthy volunteers*

were recruited through general announcements and had 1) no history of cardiac or renal disease; 2) a normal clinical examination; 3) normal cardiac function on transthoracic echocardiography. Patients with HFrEF were screened in the outpatient HF clinic and had a prior diagnosis of heart failure based on the combination of signs and symptoms of heart failure with evidence of impaired left ventricular ejection fraction ≤40% and an elevated NT-proBNP > 125 ng/L. Exclusion criteria were 1) administration of intravenous diuretics within 1 week before study inclusion; 2) concurrent diagnosis of an acute coronary syndrome; 3) renal replacement therapy; and 4) any change in neurohumoral blockade 3 months before inclusion. Exclusion criteria for both healthy subjects and HFrEF patients were 1) a history of rheumatologic or inflammatory disease warranting systemic anti-inflammatory drugs; 2) current or prior skin disease such as atopic dermatitis; 3) treatment with local applied retinoids or steroids which might affect skin homeostasis; 4) use of long-stretch bandages on the lower extremity and 5) prolonged time in supine position such as bedridden patients.

Study design

After informed consent, subjects were placed in a recumbent position for an adaptation period of at least 60 minutes. Subsequently, a brief history and clinical examination was carried out and a transthoracic echocardiography was obtained in the left decubitus position. Afterwards two skin biopsies of the lower extremity were taken while the subject remained in a semi-supine position. Finally, a venous blood sample was obtained.

Presence of clinical signs of peripheral edema

The presence of peripheral edema was graded on a scale of 0 to 3 as follows: 0 = no visual edema; 1 = trace of edema meaning slight pitting after pressure (2 millimeter or less), no visible distortion of the lower extremity, disappears rapidly; 2 = pitting edema (2-4 millimeter), extremity looks swollen, lasts longer than a few seconds to rebound; 3 = pit is 6-8mm after local pressure, lasts more than one minute to rebound, extremity is grossly distorted.

Human skin biopsies

Two skin specimens were obtained by punch biopsy (diameter 4 mm) of the lower leg (medial side of calf 20-25 cm above footpad) after local topical anesthesia (2 gram of hydrophilic crème 5% lidocaine/prilocaine during 10 minutes). This area of the lower extremity was chosen based on the typical occurrence of peripheral edema as well as a

relatively low risk for damaging vascular and nerve structures. Biopsies were immediately transported and processed.

Histology and immunocytochemistry

Intradermal GAG content was illustrated by means of an Alcian Blue staining. The expression of Angiotensin II type 1 receptor was determined by means of immunohistochemistry (see supplemental Materials and Methods).

Glycosaminoglycan and tissue water quantification

After determining fresh weight (FW), skin biopsies were lyophilized (VirTis Freezemobile) until a constant dry weight (DW) was reached. Tissue water content (TWC) was calculated by subtraction of dry weight from fresh weight (TW=FW-DW; ml/mg). Subsequently, samples were defatted and net dry defatted weight (DDW; mg) was calculated. A GAG molecule is a linear polymer of disaccharide units (each composed of a uronic acid (UA) and hexosamine) of variable length that is modified by sulphation. Total uronic acid (UA) content was quantified by the carbazole reaction (110) (Supplemental material). The UA content stands for the total interstitial GAG content (111). Sulphated GAG (sGAG) content was quantified using the Blyscan Sulfated Glycosaminoglycan Assay Kit (Biocolor Ltd., Belfast, UK). sGAG stands for the total amount of sulphate groups within the interstitial GAG network. UA and sGAG are reported as μg per mg DDW (μg/mg). The degree of sulphation was calculated as the ratio of sGAG/UA. For all quantifications 2 replicates were obtained and the average value was reported. The variability between replicates for sGAG and UA measurements were respectively 1.7±0.6 and 2.5±0.9%.

Laboratory and echocardiography measurements

Venous blood samples were obtained with the patient in the recumbent position after an adaptation period of at least 30 minutes. Plasma NT-proBNP (Cobas proBNP II, Roche, Rotkreuz, Switzerland). PRA (RIAZEN immunoassay, ZenTech, Liège, Belgium), Comprehensive 2-dimensional echocardiography examinations were performed with a commercially available system (Philips Healthcare, iE33). Images were acquired in the left lateral decubitus position and digitally stored in the cine loops in DICOM format. A single investigator analyzed the images offline. All reported measurements were averaged from 3 consecutive cycles and assessed as recommended by the American society of Echocardiography (112).

Statistical analyses

Continuous variables are expressed as mean ± standard deviation, if normally distributed, or otherwise by median [interquartile range]. Normality was assessed by the Shapiro-Wilk statistic. Categorical data are expressed as percentages and compared with the Pearson χ^2-test. Student's t test or the Wilcoxon-Mann-Whitney test were used as indicated. To establish determinants of GAG density and sGAG density in healthy subjects and HFrEF patients, univariate regression modelling was performed. Variables with a significant univariate association (p-value <0.100) were entered in a standard multivariate regression model. Statistical significance was always set at a 2-tailed probability level of <0.05. All statistics were performed using SAS JMP Pro (version 11.2 for Windows).

RESULTS

Study population

Baseline characteristics of healthy control patients (n=18) and heart failure patients (n=29) are presented in Table 1. Healthy control patients are on average 38±13 years, have normal laboratory and echocardiographic measurements.

Heart failure patients are on average 71±9 years old, are predominantly male, have a LVEF of 33±10%. Thirteen out of 29 HFrEF patients had no peripheral edema (grade 0) versus 16 patients with peripheral edema of the lower extremities (grade 1 n=2; grade 2 n=7 and grade 3 n=7).

Interstitial GAG content and expression of Angiotensin II type 1 receptor

In order to illustrate intradermal GAG content in each sample, an Alcian Blue staining was performed. Both healthy subjects and HFrEF patients showed a strong staining throughout the epidermis and dermis, with a heterogeneous staining pattern within the different dermal layers (Figure 1). Strong expression of Angiotensin II type 1 receptor was observed in all endothelial cells, vascular smooth muscle cells, keratinocytes and many intra-dermal cells of both healthy subjects and HFrEF patients (Figure 1).

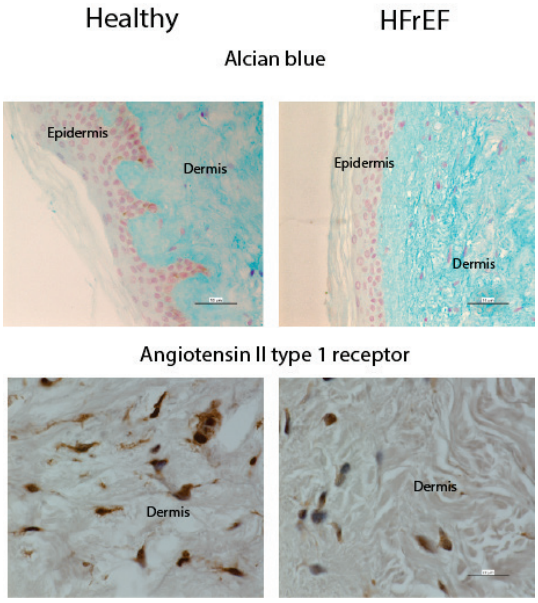

Figure 1. Immunohistochemical staining of dermal glycosaminoglycans and Angiotensin II type 1 receptor in healthy subjects and HFrEF patients

Interstitial GAG quantification

Both significantly higher levels of total GAG content (UA) and sGAGs were observed in HFrEF patients compared to healthy controls (UA: 13.0±4.2 vs 9.6±1.6 μg/mg; p=0.002 and sGAG 15.7±5.9 vs 10.1±1.2 μg/mg; p<0.001) (Table 1 and Figure 2). Moreover, the degree of GAG sulphation per dissacharide, was significantly higher in HFrEF patients compared to healthy controls (1.23±0.23 vs 1.07±0.15; p=0.008). Within the cohort of HFrEF patients, UA and sGAG concentration were significantly higher in patients with clinical signs of peripheral edema versus HFrEF patients without clinical signs of edema (Figure 2). There was no significant difference in the degree of sulphation per dissacharide in patients with versus without edema.

	Healthy n=18	**Heart failure n=29**	**p-value**
Baseline demographics			
- Age (years)	38±13	71±9	<0.001
- Male gender	88%	83%	0.223
- Body mass index (kg/m^2)	23.7±2.1	27.2±3.5	0.007

- Grade Pitting edema edema 0/1/2/3 (n)	20/0/0/0	13/2/7/7	<0.001
- NYHA class I/II/III/IV	95%/5%/0/0	0%/33%/50%/17%	<0.001
- ACE-i/ARB use	0%	77%	<0.001
- Beta-blocker use	0%	97%	<0.001
- Spironolactone use	0%	87%	<0.001
- Loop diuretic use	0%	57%	<0.001
Laboratory results			
- Hemoglobin (g/dl)	14.5±1.1	12.8±1.7	0.004
- eGFR (ml/min/1.73 m^2)	93.6±18.2	46.6±20.0	<0.001
- serum sodium (mmol/L)	141±2	137±3	<0.001
- NT-proBNP (ng/L)	29[18;81]	3342[1224;8104]	<0.001
- PRA (ng/ml/h)	2.2[1.7;3.1]	12.7[2.9;44.1]	<0.001
Echocardiographic results			
- LVEF (%)	59±7	33±10	<0.001
- LVEDV (ml)	108±36	150±64	0.125
- LAV (ml)	43±21	75±38	0.006
- E/E'	8±2	21±10	0.003
Skin biopsy			
- TWC (ml/mg)	3.4±0.6	5.5±2.6	0.001
- sGAG (ug/mg)	10.1±1.2	15.7±5.9	<0.001
- UA (ug/mg)	9.6±1.6	13.0±4.2	0.002
- sGAG/UA	1.07±0.15	1.23±0.23	0.008

Table 1. Baseline characteristics of healthy control subjects and patients with Heart failure and reduced ejection fraction. *eGFR: estimated glomerular filtration fraction calculated with the CKD-EPI formula, LAV (left atrial volume); LVEF: left ventricular ejection fraction, LVEDV: left ventricular end diastolic volume, NT-proBNP: N-terminal pro hormone of brain natriuretic peptide, NYHA: New York Heart Association class, PRA: plasma renin activity; TWC: tissue water content, sGAG: sulfated glycosaminoglycan, UA: uronic acid.*

Tissue water content and relation with GAG-density and sulphation

TWC of skin was significantly higher in HFrEF patients compared to healthy controls (5.5±2.8 vs 3.4±0.6 ml/mg; p=0.001). HFrEF patients with clinical edema had also significantly higher TWC compared to HFrEF patients without edema (6.8±3.0 vs 4.0±0.9; p=0.003). The correlation between TWC and UA in HFrEF patients is strong (R^2 = 0.53 p<0.0001) but absent in healthy subjects (R^2=0.02; p=0.810). The correlation between sGAG and TWC in HFrEF is strong (R^2 =0.69, p<0.0001) but poor in healthy subjects (R^2 =0.22, p=0.05) (Figure 3).

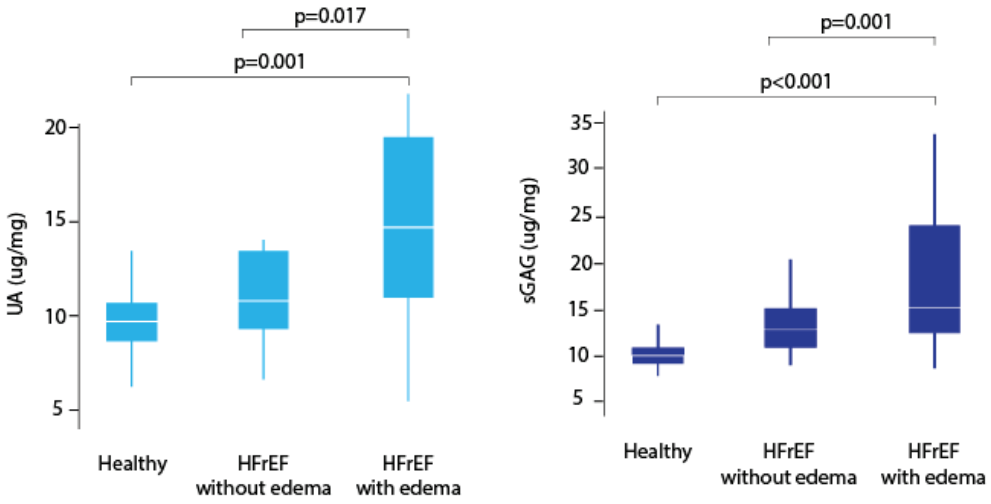

Figure 2. Total GAG density and GAG sulphation in skin biopsies of the lower extremity in healthy subjects and HFrEF patients with and without edema. *Total GAG density (left panel) and sGAG density (right panel) are significantly higher in HFrEF patients comparted to healthy controls. Moreover, GAG densities were also significantly higher in HFrEF patients with versus without clinical presence of peripheral edema. GAG: glycosaminoglycan; HFrEF: Heart failure with reduced ejection fraction; sGAG: sulphated glycosaminoglycan*

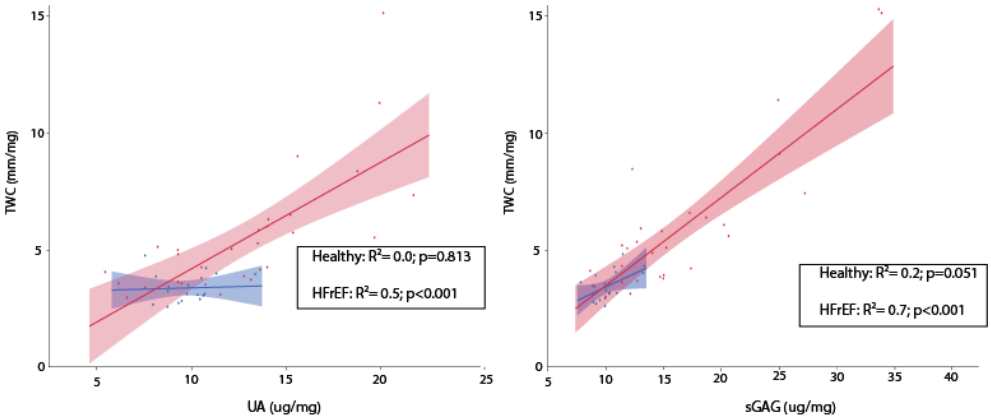

Figure 3. Relation between total interstitial GAG density (left panel) or sGAG density (right panel) and tissue water content in healthy subjects (blue) and HFrEF patients (red). *In healthy subjects TWC is rather stable over a range of GAG densities. In contrast in HFrEF patients there is a strong association between total GAG density or sGAG density and TWC.GAG: glycosaminoglycan; HFrEF: Heart failure with reduced ejection fraction; sGAG: sulphated glycosaminoglycan ; TWC: tissue water content*

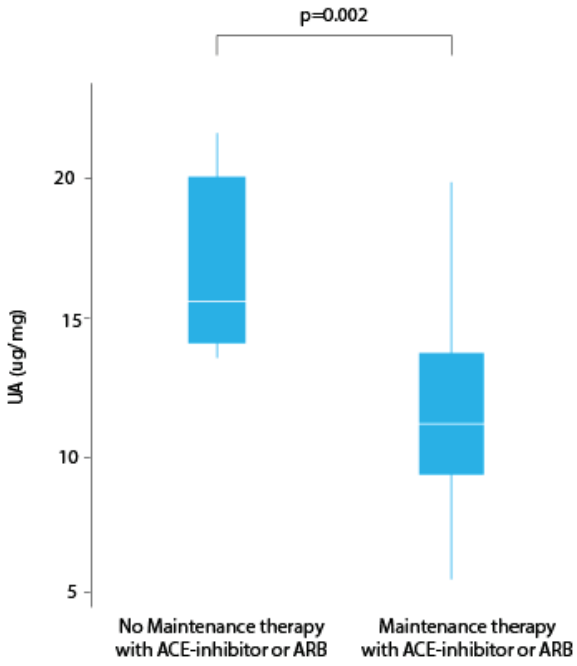

Figure 4. Difference in interstitial GAG density in patients on versus off ACE-inhibitors/ARB
ACE-inhibitor: angiotensin converting enzyme-inhibitor; ARB: angiotensin receptor blocker; GAG: glycosaminoglycan;UA: uronic acid

Factors associated with interstitial GAG density within the population of healthy volunteers and HFrEF patients

The association between total GAG density (UA) or sulphation (sGAG) and different clinical, echocardiographic and biochemical parameters was investigated with univariate and multivariate regression analysis (Supplemental material Table 1 to 4). In the cohort of healthy subjects, there was a significant relation between renal function and sGAG but no relation with any measured factor including age and gender, for UA and sGAG. In HFrEF subjects, a significant relation between UA and TWC, use of angiotensin converting enzyme (ACE)-inhibitor or angiotensin receptor blocker (ARB) and female gender was found. After multivariate regression analysis, there remained only a significant association between UA and TWC. Similarly, a significant relation between sGAG and age, TWC, use of ACE-inhibitor/ARB, MRA use and NTproBNP was present in HFrEF patients. After multivariate regression analysis, there remained only a significant association between sGAG and TWC.

Patients who did not take ACE-I or ARB had significantly higher interstitial GAG density and sulphation (respectively, 17.0±3.2 vs 11.9±3.8 μg/mg; p=0.003 and 21.1±7.9 vs 14.3±4.2 μg/mg; p=0.026) (Figure 4). These patients had similar left ventricular ejection fractions (36±9 vs 31±10%, p=0.357), but a significantly lower eGFR (30.9±17.3 vs 51.5±18.5 ml/min/1.73m^2, p=0.008) and higher NTproBNP levels (11.157 (3342;13669) vs 2.687(562;4903) ng/L, p=0.014). There was no relation observed between measured PRA and GAG-density or sulphation.

DISCUSSION

Congestive heart failure is characterized by signs and symptoms of fluid overload which negatively impacts morbidity and mortality (6). We investigated for the first time the interstitial GAG composition of skin in healthy persons and patients with HFrEF with and without edema. The main findings are: 1) Patients with HFrEF have higher interstitial GAG density and degree of sulphation compared to healthy controls. The highest interstitial GAG density is observed in patients with clinical presence of peripheral edema; 2) In contrast to healthy subjects where tissue water content is rather stable over a wide range of interstitial GAG density, a significant and strong relation between interstitial GAG density and tissue water content exists in HFrEF patients; 3) Dermal interstitial cells express the angiotensin II type 1 receptor, and HFrEF patients without intake of an ACE-inhibitor or ARB have significantly higher levels of interstitial GAGs. Therefore, our data suggest that local interstitial factors aside from Starling forces are associated with the occurrence of interstitial edema in HFrEF patients, and that the neurohumoral system is likely involved in structure and function of such interstitial networks.

The interstitium and GAG-networks

The interstitium or extracellular matrix determines structure and compliance of tissues while serving as a transport medium for nutrients and signaling molecules. The interstitium is composed of collagen, elastin and large networks of GAGs. GAGs are linear polymers of disaccharides (each composed of a uronic acid (UA) and hexosamine) with variable lengths. Due to their large amount of negative charges, GAG-networks can bind ("buffer") a large quantity of cations (mainly Na^+) and attract water, influencing tissue hydration. Furthermore, GAGs are modified by sulphation. The degree of sulphation varies from none to 3 sulfamino groups per dissacharide (27, 113). Sulphate groups strongly increase the negative charge density of GAGs and determine interactions with mediators (e.g. growth

factors) and positively charged ions (mainly sodium) (29, 40). Importantly, it has been observed that GAG-networks are dynamic and can adjust to different circumstances (114, 115).

Skin Interstitial GAG density and sulphation is increased in patients with HFrEF

Based on Alcian Blue staining it is apparent that the skin interstitium of healthy subjects and HFrEF patients is abundant with GAG-networks. Different triggers like an increase in total body sodium, inflammation, oxidative stress, local RAAS activation, known to promote GAG synthesis and the degree of GAG sulphation, are often present in HFrEF patients (21, 39, 109, 116-118). Indeed, the interstitium of patients with HFrEF contained a significantly higher density of total GAGs (UA) and sGAGs. Moreover, the degree of sulphation was significantly increased indicating that the amount of sulphate bounds *per dissacharide* also increased. Intriguingly, the highest total GAG density and sGAG was observed in the group of patients with clinical presence of peripheral edema. However, the degree of sulphation in HFrEF patients with versus without edema was non-significantly higher. The latter suggests that more GAG dissacharides were present (and thus also the total amount of sulphate bounds) but the amount of sulphate bounds *per dissacharide* did not further increase. However, at the moment it's unknown if the maximal degree of sulphation of such GAG networks can increase above what was observed in our study.

The relation between GAG density and tissue water and clinical presentation of edema

Under normal circumstances, extracellular fluid volume and tissue hydration is kept relatively stable. This is even possible despite important changes in total body sodium (21, 22). GAG networks can accumulate a high amount of Na^+ ions and can even increase this capacity under conditions of higher salt intake due to GAG synthesis and increased sulphation. We recently hypothesized that the process of interstitial GAG expansion may be a feedback mechanism when total body sodium increases such as in HF (109). By increasing GAG density and sulphate bounds, more sodium can be *buffered* in the interstitum without increasing total body water. Although this process creates a higher interstitial oncotic pressure, the normal low compliance of the interstitial GAG network creates a driving force for higher lymphatic drainage when fluid enters the interstitium (Figure 5, nr 1 and 2) (26, 38, 109). This hypothesis was supported in the current study by the fact that TWC in healthy subjects was relatively stable over a range of interstitial GAG density.

In contrast to healthy subjects, we observed a significant positive and strong relation between total GAG density and TWC in HFrEF patients, especially between sGAG and TWC. This indicates that the interstitial GAG content, particularly the total amount of negative sulphate bounds in the interstitium, strongly relates to accumulation of interstitial fluid. Since the change in degree of sulphation was very limited, the increase in sGAG is mainly the consequence of an absolute increase in GAG dissacharides. However, it remains unclear if the observed alterations in GAG structure are the cause or rather consequence of decompensation. Under influence of increased sulphation and more cations bound to the GAG network, a conformation-change can occur which might disrupt the low-compliant state of the interstitial network (4). This will result in less driving force for lymphatic drainage which together with a high interstitial oncotic pressure will promote interstitial fluid accumulation (Figure 5 nr 3) (109). Certainly, when intracapillary hydrostatic pressure is elevated (as during increased cardiac filling pressures), this increases the risk for interstitial edema formation. However, individual differences in the interstitial structure and function may be an important factor explaining the variety in clinical presentations of edema in HF patients over a wide range of central venous pressures (Figure 5) (19, 119).

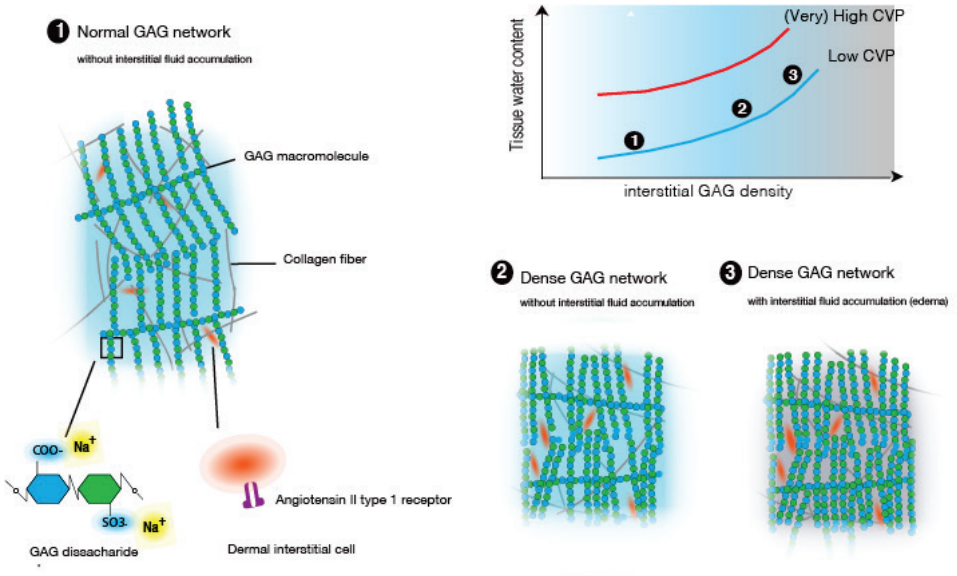

Figure 5. An increase in GAG density can lead to interstitial dysfunction and accumulation of interstitial fluid. *The interstitial GAG-networks regulate extracellular fluid volume and tissue hydration (nr 1). An increased GAG synthesis and sulphation is noticed in circumstances of high salt intake or neurohumoral upregulation (nr 2). Due to the low compliance of the interstitial GAG network, interstitial*

fluid accumulation will be prevented and tissue water content kept stable. A conformation-change of GAG-macromolecules due to an increased sulphation might disrupt GAG-network structure resulting in a high compliance-state promoting interstitial fluid accumulation (nr 3) (109). Interstitial structure and compliance may explain the variety in clinical presentation of edema in HF patients over a wide range of central venous pressure. (19, 119). CVP: central venous pressure ; GAG: glycosaminoglycan; Na+: sodium; SO3-: sulphate group.

The role of the renin-angiotensin-aldosterone system in interstitial structure and function

Immunohistochemical analyses in our study illustrated that the angiotensin II type 1 receptor was expressed on all endothelial cells and vascular smooth muscle cells of dermal capillaries. Furthermore, we observed a strong expression of the angiotensin II type 1 receptor on dermal cells. Previously, it has been reported that dermal fibroblasts and macrophages express this receptor (120-124). Also, it has been shown that the angiotensin II type 1 receptor can influence cell proliferation and synthesis of extracellular matrix proteins in various cell types such as cardiac myocytes and fibroblasts and cause adverse cardiac remodeling (125-130). In HF, an increase in skin interstitial GAG density and sulfate groups might be a compensatory pathway to maintain water and electrolyte homeostasis by expanding Na^+ storage capacity in conditions with high salt intake (109). Therefore, it may not be surprising that the RAAS system, which is responsible for total body water homeostasis, is involved in this process. ACE-inhibitors and ARB reduce proliferation of fibroblasts, collagen deposition and GAG release by cardiac fibroblasts (131, 132). Our data show that HFrEF patients who were not on maintenance therapy with blockers of the angiotensin II type 1 receptor or ACE-inhibitors had significantly higher levels of interstitial GAGs in their skin. Though these patients were considerably sicker (higher NTproBNP and lower renal function), our data suggest that the RAAS plays very likely a role in the interstitial structure and function.

STUDY LIMITATIONS

The current study has several limitations. First, this was a small, single-center pilot study indicating that results should be considered hypothesis-generating. Causality between GAG density and peripheral edema needs to be further studied. Second, serial biopsies in stable conditions and before vs after recompensation to better understand interstitial volume homeostasis were not obtained. Third, the control group was significantly younger than HFrEF patients. However, within the group of healthy controls and HFREF patients there was

no significant association with age, and in the literature rather a decrease in GAG density and tissue water content with increasing age is observed (111, 133). Fourth, we preferred to investigate dermal interstitium since the presence of peripheral edema can be clinically verified, dermal interstitium is easily accessible and with very low risk for serious complications. However, GAG-networks are also present in the interstitium of lung, myocardium, kidney, gut, etc. and a similar process may be involved in the pathophysiology of lung edema, renal dysfunction, gastro-enterologic malabsorption, myocardial dysfunction, etc (134, 135).

CONCLUSION

Interstitial GAG concentration is increased in HFrEF patients and correlates with tissue water content and clinical signs of volume overload. Therefore, a better appreciation of the interstitial compartment might improve current management of volume overload in HF.

SUPPLEMENTAL MATERIAL

	Beta	Standard error	p-value uni
age	-2.08	2.03	0.320
gender	0.19	0.33	0.567
BMI	0.49	0.33	0.160
Na+	0.24	0.30	0.446
PRA	0.09	0.16	0.573
eGFR	2.90	2.71	0.299

Table 1. Univariate analyses of predictors of interstitial GAG (UA) density within Healthy subjects. *BMI: body mass index, eGFR: estimated glomerular filtration rate; Na+: sodium; PRA: plasma renin activity*

	beta	Standard error	p-value uni
age	-2.80	2.70	0.315
gender	-0.55	0.47	0.240
BMI	0.49	0.45	0.297
Na+	-0.66	0.32	0.059
PRA	0.00	0.21	0.972
eGFR	7.02	3.29	0.049

Table 2. Univariate analyses of predictors of sGAG density in Healthy subjects *BMI: body mass index, eGFR: estimated glomerular filtration rate; Na+: sodium; PRA: plasma renin activity*

		Univariate		Multivariate
	beta	**Standard error**	**p-value uni**	**p-value multi**
age	0.49	2.37	0.838	
gender	-0.66	0.28	**0.018**	0.479
BMI	0.24	0.16	0.140	
TWC	0.41	0.09	**<0.001**	**0.037**
ACE-inhibitor/ARB use	0.36	0.15	**0.032**	0.758
BB use	0.66	0.60	0.268	
MRA use	0.07	0.13	0.609	
Systolic function	0.35	0.46	0.451	
E/E'	0.48	0.72	0.533	
TAPSE	0.44	0.39	0.295	
NT-proBNP	403.7	251.2	0.119	
Na+	-0.02	0.13	0.868	
PRA	-0.77	1.18	0.515	
eGFR	-1.54	0.839	0.078	0.146

Table 3. Univariate and multivariate analyses of predictors of interstitial GAG density (UA) within HFrEF patients *ACE-inhibitor: angiotensin converting enzyme inhibitor; ARB: angiotensin receptor blocker; BB: beta blocker; BMI: body mass index, eGFR: estimated glomerular filtration rate; Na+: sodium; MRA: mineralocorticoid receptor antagonist; Na+: sodium; NT-proBNP: N-terminal of the prohormone of brain natriuretic peptide; PRA: plasma renin activity; TAPSE: tricuspid annular plane systolic excursion; TWC: tissue water content*

	Univariate			Multivariate
	beta	**Standard error**	**p-value**	**p-value**
age	0.54	0.26	0.050	0.535
gender	-19.21	$18.48*10^4$	0.999	
BMI	0.12	0.12	0.329	
TWC	0.37	0.04	<0.0001	<0.0001
ACE-inhibor/ARB USE	0.21	0.09	0.021	0.660
BB use	7.94	$41.42*10^4$	1.000	
MRA use	0.15	0.09	0.080	0.611
Systolic function	0.13	0.32	0.695	
E/E'	0.29	0.62	0.662	
TAPSE	0.31	0.41	0.486	
NT-proBNP	328.46	183.67	0.085	0.363
Na+	-0.00	0.09	0.968	
PRA	-0.42	0.87	0.632	
eGFR	-0.85	0.63	0.190	

Table 4. Univariate and multivariate analyses of predictors of degree of sGAG density within HFrEF patients. *ACE-inhibitor: angiotensin converting enzyme inhibitor; ARB: angiotensin receptor blocker; BB: beta blocker; BMI: body mass index, eGFR: estimated glomerular filtration rate; Na+: sodium; MRA: mineralocorticoid receptor antagonist; Na+: sodium; NT-proBNP: N-terminal of the prohormone of brain natriuretic peptide; PRA: plasma renin activity; TAPSE: tricuspid annular plane systolic excursion; TWC: tissue water content*

Histology and immunocytochemistry

Of each patient and healthy subject, one skin biopsy was transferred immediately after collection into 10% neutral buffered formalin solution and stored at room temperature overnight. Intradermal GAG content was illustrated by means of an Alcian Blue staining. The expression of Angiotensin II type 1 receptor was determined by means of immunohistochemistry.

Alcian Blue staining

Following deparaffinization and rehydration, serial tissue sections (5 µm) were incubated for 30 min in an Alcian Blue staining solution (pH 2.5) in 3% acetic acid. After 10 min of washing with tap water, samples were counterstained with Nuclear Fast Red for 10 min after which they were rinsed very briefly with distilled water. Samples were dehydrated and mounted with DePeX mounting medium (VWR, Leuven, Belgium). Representative pictures were taken with a Nikon Eclipse 80i microscope equipped with a DS-5 M digital camera.

Immunohistochemical staining for Angiotensin II type 1 receptor

In order to demonstrate the presence of Angiotensin II type 1 receptor in human skin samples, an immunohistochemical staining was performed. Following deparaffinization and rehydration of serial tissue sections (5 µm), heat-mediated antigen retrieval was performed in 1x Target Retrieval Solution (Dako, Agilent Technologies, Heverlee, Belgium). Endogenous peroxidase activity was blocked for 30 min. by means of a commercially available peroxidase block (Dako). Following extensive washing with phosphate-buffered saline (PBS), all samples were incubated for 60 min with 3% bovine serum albumin (BSA) in PBS in order to block aspecific binding sites. Afterwards, tissue sections were incubated overnight with a 1:400 dilution of the rabbit polyclonal antibody against human angiotensin II receptor type 1 (ab124505, Abcam, Cambridge, UK) in 0.3% BSA at 4°C. In the negative control condition, primary antibodies were omitted and sections were incubated overnight with 0.3% BSA. After extensive washing with PBS, the samples were incubated for 45 min with a 1:170 dilution of the HRP-labeled swine anti-rabbit secondary antibody (Dako) in PBS at room temperature. The staining was visualized using a 3,3' diaminobenzidine (DAB) solution according to the manufacturer's instructions (DAB Envision System kit, Dako). Following counterstaining with Mayer's hematoxylin for 8 min, samples were dehydrated and mounted with DePeX mounting medium (VWR, Leuven, Belgium). Representative

pictures were taken with a Nikon Eclipse 80i microscope equipped with a DS-5 M digital camera.

Glycosaminoglycan and tissue water quantification

After determining fresh weight (FW), the second skin biopsy was stored at -80°C. After collection of all samples, skin biopsies were lyophilized (VirTis Freezemobile) until a constant dry weight (DW) was reached. Tissue water content (TWC) was calculated by subtraction of dry weight from fresh weight (TW=FW-DW; ml/mg). Subsequently, samples were defatted at room temperature in chloroform/methanol for 24 hours. After 3 hours of lyophilizing, net dry defatted weight (DDW; mg) was calculated. Skin biopsies were digested with pronase overnight at 55°C. Total uronic acid (UA) content was quantified by the carbazole reaction (110). Sulphated GAG (sGAG) content was quantified using the Blyscan Sulfated Glycosaminoglycan Assay Kit (Biocolor Ltd., Belfast, UK).

Uronic acid (Carbazole assay)

A volume of 150 µl of pronase E degraded sample was precipitated in 90% ethanol at -20°C overnight. After centrifugation at 12000 g and 4°C for 15 minutes, the pellet was resuspended in 90 µl Milli-Q water. Forty µl of GAG sample in duplicate were added to pyrex disposable culture tubes (Corning Inc.) in a refrigerated chamber. Then, 200 µl of ice-cold solution of 25 mM sodium tetraborate in 98% sulfuric acid were added and vortexed. To make this solution, sodium tetraborate was first dissolved in hot water, followed by addition of ice-cold sulfuric acid. After heating at 100°C for 15 minutes and 10 minutes cooling at 4°C, 8 µl of 0,125 w/v% carbazole in absolute ethanol was added. The tubes were reheated at 100°C for 15 minutes and again 10 minutes cooled in the refrigerated chamber. Two hundred microliters were pipetted into the 96-well plate and the absorbance was read at 540 nm. The concentration of uronic acids was determined using an eleven points calibration curve of either dermatan sulfate standard (C3788, Sigma-Aldrich) for patient's skin samples.

CHAPTER 3

Endovascular Shedding Markers in Patients with Heart Failure with Reduced ejection fraction

Results from a single-center exploratory study

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Submitted

ABSTRACT

Background: Increased endothelial glycocalyx degradation has previously been associated with tissue edema formation, endothelial dysfunction, renal impairment and mortality in patients with cardiovascular disease.

Aims: to explore the role of glycocalyx shedding markers; hyaluronic acid (HA) and syndecan-1, in patients with heart failure with reduced ejection fraction (HFrEF), and to study their potential association with other HF-related variables and outcome.

Methods: In 123 patients with HFrEF a medical history, clinical investigation and venous blood sample for shedding markers and other HF-related biomarkers were obtained. Cut-off values of normal were derived from a cohort of normal subjects (n=30). The study end point was a composite of all-cause mortality and hospitalization for HF. HFrEF patients were prospectively followed up till 2 years.

Results: The cut-off value of normal for plasma values of HA was 50.2 ng/ml and for Syndecan-1 was 365.4 ng/ml. Median HA levels and syndecan-1 levels in HFrEF patients were respectively 29.4(10.7;61.6) ng/ml and 48.5(33.6;80.8) ng/ml. Overall, HA-levels were significantly higher in HFrEF patients compared to healthy subjects but only 31% of HFrEF patients had HA levels above the cutoff. There was no significant difference among HFrEF patients and healthy subjects regarding syndecan-1 levels. HFrEF patients with elevated HA- levels had a significantly worse outcome (log rank=0.01) which remained significant after correction for established risk factors (HR 2.53 (1.13-5.69); p=0.024). There was no significant relation between levels of shedding markers and neurohumoral activation (plasma renin activity, serum aldosterone, NT-proBNP), myocardial injury (HS-trop), inflammation (CRP) or other baseline characteristics.

Conclusion: The glycocalyx shedding marker HA is significantly elevated in a subgroup of HFrEF patients and an independent predictor for worse clinical outcome independent of other established risk factors and HF related processes. There was no significant difference between syndecan-1 levels in HFrEF patients and normal subjects.

INTRODUCTION

The endothelial glycoclayx is the inner fragile layer of the endothelium and composed of a network of membrane-bound and different types of glycosaminoglycans (GAG) and proteoglycans (55) (Figure 1). The glycocalyx has multiple vasoprotective functions: it reduces vascular permeability, acts as a mechano-transducer of shear stress and prevents interaction of platelets and leucocytes with endothelial cell adhesion molecules (57). Moreover, recent evidence suggests that the endothelial GAG network acts as a sodium buffer by binding positively charged Na+ cations (59).

Various conditions can lead to disruption of this endothelial barrier such as ischemia and hypoxia (136-139), oxidative stress and inflammation (140-147), hyperglycemia (148), volume and salt overload (149), etc. It has already been demonstrated that a dysfunctional glycocalyx is involved in the process of atherosclerosis, endothelial dysfunction, tissue edema, renal dysfunction and related to increased cardiovascular events in different patient populations (71, 92, 137, 150-155). Therefore, there is growing interest to further study glycocalyx integrity. However, in vitro and in vivo structural and/or functional assessment of the glycocalyx remains cumbersome (156-158). Currently, the best way to investigate endothelial glycocalyx integrity is through the presence of glycocalyx shedding markers in plasma. Hyaluronic acid and syndecan-1 are the main constituents of the endothelial glycocalyx and elevated serum levels indicate degradation (figure 1) (159).

We recently hypothesized that increased glycocalyx shedding might also be present in heart failure (HF) and relate to prognosis (109). The clinical value of shedding markers as a prognosticator in HFrEF has not been established yet. The objectives of this exploratory study are three-fold: 1) to study if levels of HA and syndecan-1 are increased in HFrEF patients or in specific subgroups reflecting glycocalyx shedding ; 2) to investigate whether glycocalyx shedding is related to other processes present in HF; and 3) to study the prognostic value of these markers.

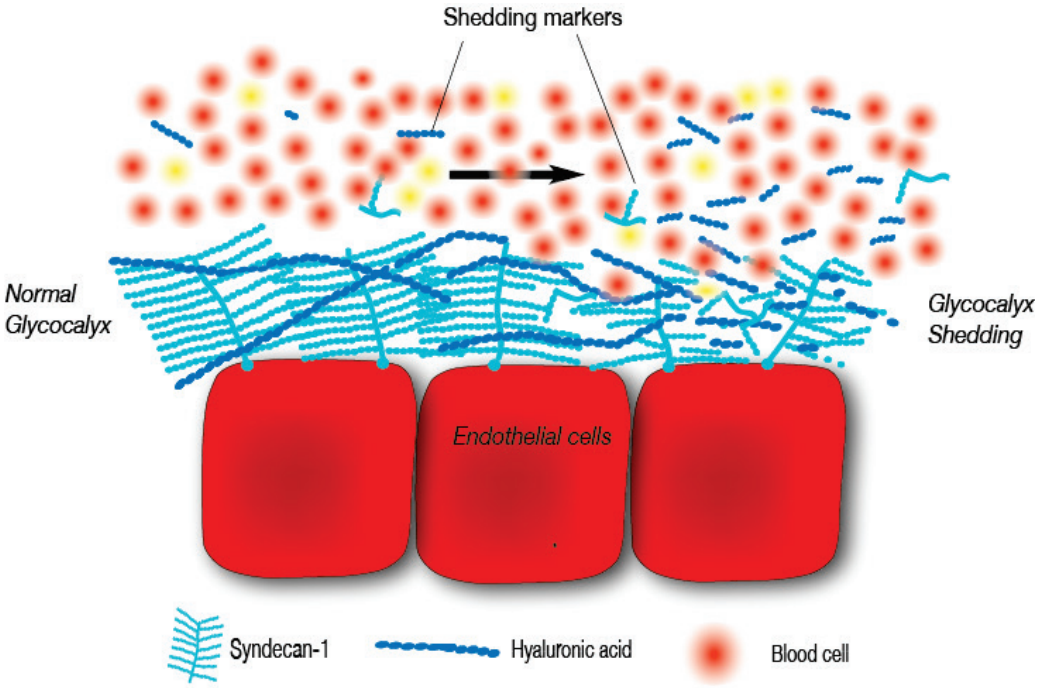


Figure 1.The endothelial Glycocalyx

METHODS

This study was carried out in a tertiary care center (Ziekenhuis Oost-Limburg, Genk, Belgium). The study complies with the Declaration of Helsinki and the institutional review board approved the study protocol.

Patient population

The current study cohort is the result of a pooled analysis of 3 investigator-initiated prospective studies in HFrEF patients with overlapping baseline and clinical outcome data, and availability of a venous blood sample which was obtained, processed and stored in similar conditions. All subjects were recruited in a single tertiary care center (Ziekenhuis Oost-Limburg, ZOL Genk) between January 2013 and May 2016. In each cohort, consecutive patients were included. Subjects were eligible for study inclusion if ≥18 years of

age and able to give informed consent. All subjects had a prior diagnosis of heart failure with evidence of impaired left ventricular ejection fraction ≤40%. Exclusion criteria were: 1) renal replacement therapy or severe renal dysfunction with an estimated glomerular filtration rate ≤15 ml/min/1.73m^2 determined by the Chronic Kidney Disease Epidemiology Collaboration equation; (160) 2) administration of intravenous medication within 1 month of inclusion; 3) concurrent diagnosis of an acute coronary syndrome; or 4) concurrent diagnosis of an infectious or inflammatory disease

Data collection

All subjects were screened by 2 heart failure specialist (W.M. and M.D.) at the outpatient clinic or emergency room. After completion of informed consent all patients underwent collection of detailed baseline characteristics including severity of HF (NYHA-functional class), registration of comorbidities, baseline medication, clinical parameters and a clinical examination for signs of decompensation. Based on a clinical congestion score (table 1 supplemental material), patients were classified as stable heart failure patients if the clinical decongestion score was ≤1. In contrast, patients were classified as decompensated if having a clinical congestion score of >1 leading to a change in maintenance dose of loop diuretics and/or oral vasodilators or admission to the hospital.

Biochemical analysis

All blood samples were obtained after a period of at least 15 minutes in the semi-supine position and before admission to the hospital or administration of new medication. Samples were immediately processed and stored at -80°C until analysis was performed. Plasma N-terminal of the prohormone of B-type natriuretic peptide (NT-proBNP) levels were measured by the Roche Diagnostics Assay (Roche, Rotkreuz, Switzerland). Plasma renin activity (PRA) was determined using the Gamma-coat*radio immunoassay (DiaSorin, Sallugia, Italy). Plasma aldosterone levels were assessed by the Aldosterone Maia radioimmunoassay (Adaltis, Rome, Italy). Estimated glomerular filtration rate was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula (161). Levels of hyaluronic acid were measured using a commercially available ELISA-kit (Cisbio Bioassays HYAL-US). Reported intra- and inter-assay coefficients of variation are <3% and <7% respectively. Levels of Syndecan-1 were measured using a commercially available ELISA-kit (Cusabio

Human Syndecan-1). Reported intra- and inter-assays coefficient of variations (CV) are respectively <8 and <10% while measerd intra-assay were 2.1% and 13.4%.

End point

The study end point was defined as the combined end point of all-cause mortality and heart failure readmissions (defined as hospitalizations due to signs or symptoms of congestion or low cardiac output that warranted treatment with parenteral drugs). Vital status and hospitalizations were retrieved from the hospital medical electronic records which is linked to the national death registry. From the day of inclusion, all events were prospectively registered up till 2 years.

Statistical analysis

Continuous variables are expressed as mean±standard deviation if normally distributed, or otherwise as median [interquartile range]. Normality was assessed by the Shapiro-Wilk statistic. The unpaired student's t-test and Mann-Whitney test were used when appropriate. Categorical data were expressed as percentages and compared with the Pearson χ^2-test. To establish clinical determinants of doubling of shedding markers and its relation to other markers, multiple linear regression models were constructed after a 2-log transformation. Variables with a significant univariate association (<0,10) were entered in a stepwise forward multivariate model based on the strength of their univariate association. Unadjusted time-to-event comparisons between HFrEF patients with HA levels below or above the cutoff of normal were conducted using Kaplan-Meier survival estimates and log-rank test. For adjusted analyses, a Cox proportional hazards regression model was used to estimate hazard ratios with corresponding 95% confidence interval. The adjusted hazard ratios was corrected for established risk factors. Statistical significance was always set at a 2-tailed probability level of <0.05. All statistics were performed using SAS JMP Pro (version 11.2 for Windows).

Cut-off of normal value of shedding products

Normal values for HA and syndecan-1 were obtained in a cohort of 30 healthy subjects of which venous blood samples were simultaneously obtained, processed and analyzed with samples of HFrEF patients (characteristics of healthy subjects are presented in Table 2 of

the supplemental material). Cut-off values were determined based on the Robust method as recommended by the Clinical and Laboratory Standards Institute (162, 163).

RESULTS

Cut-off values of normal for HA and syndecan-1

Baseline characteristics of the cohort of healthy subjects (n=30, mean age 27±10) are presented in the supplemental material. The median plasma level of HA was 18.9(12.1;29.7) ng/ml and the median value for plasma syndecan-1 was 54.5 (32.2;130.3). The cut-off value of normal (the upper limit of normal) for HA was 50.2 ng/ml and 365.4 ng/ml for syndecan-1.

Characteristics of HFrEF patients

HFrEF patients were on average 66±13 years old with a mean left ventricular ejection fraction of 28±10%. Fourthy four % of HFrEF patients were clinically stable while 56% were decompensated. Baseline characteristics for all HFrEF patients are presented in Table 1 and baseline characteristics of stable versus decompensated HFrEF patients are presented in Table 3 of the supplemental material.

Variable	HFrEF patients
N	123
age	66±13
male gender	84%
ischemic etiology	67%
BMI	29±5
LVEF%	28±10
Decompensation	
no	44%(n=54)
yes	56% (=69)
NYHA class	
I-II	46%
III-IV	54%
Systolic BP	125±21
Medical history	
- MI	67%
- Hypertension	41%
- AF	38%
- DM	29%

Laboratory values	
- Hb (g/dl)	13.1±1.8
- Sodium (mmol/L)	138±3
- CRP (mg/dl)	3.1(1.2;8.4)
- Creatinine (mg/dl)	1.5±0.7
- BUN	65±37
- eGFR ml/min/1.73m2	57±24
- NTproBNP ng/L	2345(951;6263)
- PRA	3.9(1.1;12.7)
- Serum aldosterone (ng/L)	193(139;358)
- HS-trop	22(12;40)
Plasma levels of shedding products	
- Syndecan ng/ml	48.5(33.6;80.8)
>cutoff	0%
- Hyaluronic Acid ng/ml	29.4(10.7;61.6)
>cutoff	31%
Maintenance therapy at inclusion	
- ACE/ARB use	**68%**
- BB use	**85%**
- MRA use	**67%**
- Loop diuretic use	**59%**
- Hydralazine/Nitrate use	**21%**

Table 1. Baseline Characteristics of HFrEF patients. *ACE: angiotensin converting enzyme inhibitor;ARB: angiotensin receptor blocker, BMI: body mass index; BP: blood pressure; BUN: blood urea nitrogen; COPD: chornic obstructive pulmonary disease; CRP: C-reactive protein; LVEF: left ventricular ejection fraction; MRA: mineralocorticoid receptor antagonist; NYHA: New york Heart association-class; NT-proBNP: N-terminal of the pro-hormone of brain natriuretic peptide; PRA: plasma renin activity.*

Shedding markers in HFrEF patients

Median HA level in HFrEF patients was 29.4(10.7;61.6). Overall, the cohort of HFrEF patients had significantly higher HA levels compared to normal subjects. Of this cohort, 31% had an elevated HA-level. Decompensated HFrEF patients had significantly higher levels than stable HFrEF patients (29.4(11.6;92.2) vs 26.5(37.2) ng/ml; p=0.024). 20% of stable HFrEF patients had elevated HA-levels versus 39% of decompensated patients.

The median syndecan-1 levels in HFrEF patients was 48.5(33.6;80.8) ng/ml. There was no significant difference between syndecan-1 levels of healthy subjects and HFrEF patients or

between stable HFrEF patients and decompensated HFrEF patients (Figure 2). None of the HFrEF patients exceeded the cut-off value of normal for plasma levels of syndecan-1.

Association between shedding markers and other variables

To assess whether shedding markers were associated with different processes or variables in HFrEF such as neurohumoral activation (PRA and serum aldosterone), natriuretic peptide activation (NT-proBNP), inflammation (CRP), myocardial injury (HS-trop), renal dysfunction (blood urea nitrogen(BUN) and eGFR), and other baseline characteristics; a multivariable regression analysis was performed (Table 4 of the supplemental material). There was a positive association found between doubling of HA (log transformation) and age in HFrEF patients. However, this correlation was poor (R^2=0.05,p=0.018) and was non-significant between absolute values of HA and age (R^2=0.03 ;p=0.070). No significant association could be observed between Syndecan-1 and any clinical variable tested.

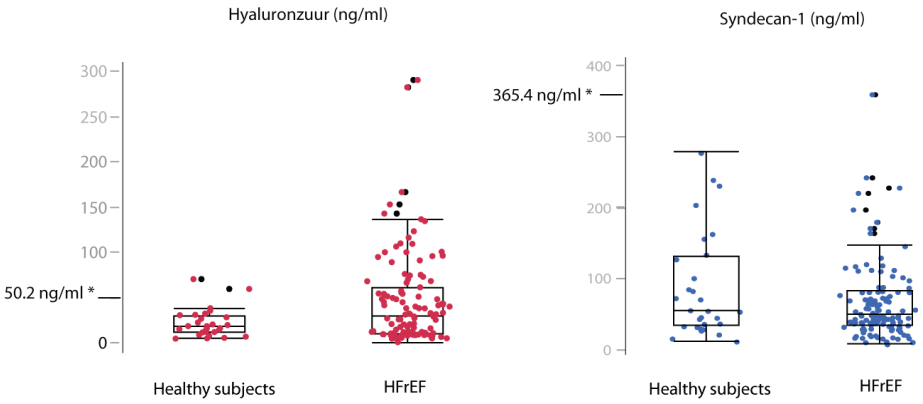
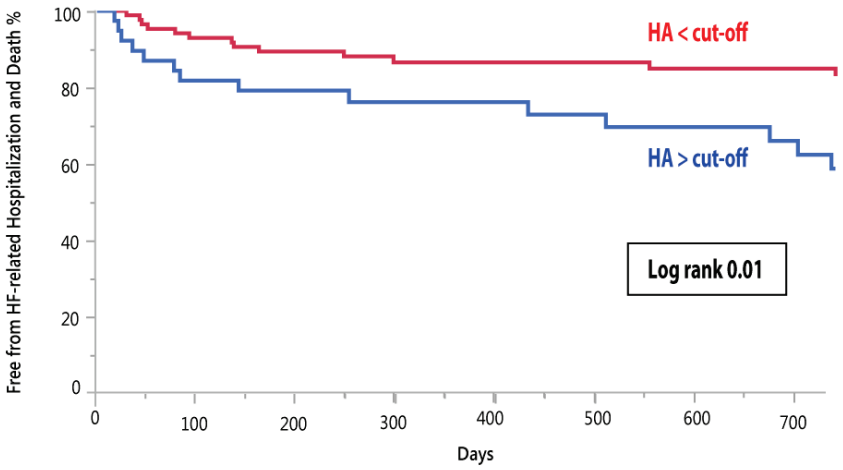


Figure 2. Plasma values of shedding products in Healthy subjects and HFrEF patients.

HA and Clinical Outcome in HFrEF patients

During a mean follow up of 16±8 months a total of 40 events occurred of which 20 deaths and 20 HF -associated hospitalizations. Figure 3 illustrates Kaplan-Meier curves for the combined endpoint in HFrEF patients with normal versus elevated HA-levels (characteristics of both groups are presented in Table 2). Elevated HA levels showed a significant increase for the combined end point after adjusting for baseline differences (age, CRP, ACE-inhibitor/ARB use, decompensated state) (hazard ratio (HR) 2.76; 95% confidence interval (CI) 1.68-6.79; p=0.021) or established outcome related variables (age>75years, medical

history of myocardial infarction, NT-proBNP level and renal function<60 ml/min/1.73m2) (hazard ratio (HR) 3.32; 95% confidence interval (CI) 1.36-8.18; p=0.009) (Table 3). There was no significant relation found between syndecan-1 and outcome in HFrEF patients.



Nr at risk	Baseline	1 month	6 months	12 months	18 months	24 months
Red=<cutoff	85	84	76	57	54	43
Blue>cutoff	38	35	30	23	20	17

Figure 3. Kaplan-Meier curves for combined endpoint in HFrEF patients stratified by HA value below versus above the cut-off of normal

	HFrEF with normal HA-levels (<50.2 ng/ml)	HFrEF with Elevated HA-levels (>50.2 ng/ml)	p-value
n	85	38	
Age	64±11	69±14	0.044
male gender	81%	89%	0.249
ischemic etiology	67%	66%	0.442
BMI	29±5	28±5	0.438
LVEF	29±10	27±10	0.266
Decompensation			0.003
No	50%	29%	
yes	50%	71%	
NYHA class	54%	29%	0.137
I-II	46%	71%	
III-IV	126±21	125±22	
Systolic BP			0.850
Medical history	67%	68%	
- MI	47%	29%	0.882
- Hypertension	38%	38%	0.060
- AF	27%	34%	0.984
- DM			0.421
Labarotory values			
- Hemoglobin (g/dl)	13.1±1.7	12.9±1.9	0.376
- Na mmol/L	138±3	138.3	0.845
- CRP	3.0(1.2;6.3)	4.0(1.6;23.0)	0.009
- Trop	19(10;35)	32(20;60)	0.779
- BUN	65±34	66±43	0.885
- eGFR ml/min/1.73m2	58.0±24.0	55.6±25.1	0.614
- NTproBNP ng/L	1911(605;4515)	3558(1417;7535)	0.240
- PRA	4.5(1.3;13.9)	3.1(0.9;9.5)	0.060
- Serum aldosterone (ng/L)	194(143;428)	176(133;272)	0.128
Plasma levels of shedding products			
- Syndecan ng/ml	47.8(29.6;81.0)	49.7(38;73.5)	0.387
- Hyaluronic Acid ng/ml	12.5(9.1;31.7)	95.4(95.5;67.8)	<0.001
Maintenance therapy at inclusion			
- ACE/ARB use	75%	52%	0.014
- BB use	87%	79%	0.250
- MRA use	72%	59%	0.129
- Loop diuretic use	62%	52%	0.311
- Hydralazine/Nitrate use	21%	20%	0.910

Table 2. Characteristics of HFrEF patients with normal levels of HA (< 50.2 ng/ml) or elevated levels. *ACE: angiotensin converting enzyme inhibitor;ARB: angiotensin receptor blocker, BMI: body mass index; BP: blood pressure; BUN: blood urea nitrogen; COPD: chornic obstructive pulmonary disease; CRP: C-reactive protein; LVEF: left ventricular ejection fraction; MRA: mineralocorticoid receptor antagonist; NYHA: New york Heart association-class; NT-proBNP: N-terminal of the pro-hormone of brain natriuretic peptide; PRA: plasma renin activity.*

Unadjusted Hazard ratio

Variable	HR	95% CI	p-value
Elevated HA	2.63	1.23-5.68	0.013

Adjusted Hazard ratio (1)

Variable	HR	95% CI	p-value
Elevated HA	2.76	1.68-6.79	**0.021**
No ACE-inhibitor/ARB use	1.67	0.69-4.12	0.252
Decompensated	1.55	0.60-4.51	0.374
CRP (mg/dl)	0.97	0.93-1.00	0.202
Age> 75 years	1.47	0.60-3.49	0.385

Adjusted Hazard ratio (2)

Variable	HR	95% CI	p-value
eGFR<60 ml/min/1.73m2	4.41	1.68-12.96	**0.002**
Elevated HA	2.52	1.13-5.69	**0.024**
History of myocardial infarction	2.53	0.95-8.78	0.065
NT-proBNP (ng/L)	1.21	0.17-6.47	0.832
Age>75 years	1.09	0.46-2.56	0.836

Table 3. Unadjusted and Adjusted hazard ratio for elevated HA levels regarding the combined endpoint of all-cause mortality and HF associated hospitalization. *95%CI: 95% confidence interval, eGFR: estimated glomerular filtration function; HA: hyaluronic acid (elevated = >52.2 ng/ml), HR: Hazard ratio; NT-proBNP: N-terminal of the pro hormone of brain natriuretic peptide (ng/L). Hazard ratio (1) was adjusted for variables which significantly differed between HFrEF patients with elevated versus normal HA-levels. Hazard ratio(2) was adjusted for established risk factors in HF.*

DISCUSSION

This study aimed to explore the presence and prognostic value of glycocalyx shedding markers in HFrEF. Our main observations are that 1) Elevated HA-levels are present in 31% of HFrEF patients in our cohort. There was no difference in syndecan-1 levels between healthy subjects and HFrEF patients 2) increased HA is a specific and independent predictor for clinical outcome in patients with HFREF. 3) Shedding markers are not associated with baseline characteristics or any other HF-related processes in HFrEF patients and seem to represent an independent pathologic process in HF.

Shedding products in HFrEF patients

The major constituents of the glycocalyx are hyaluronic acid (or hyaluronan), and syndecan-1 (Figure 1) (164). Data on HA levels in HF are extremely sparse. One small Chinese study observed higher levels of HA in higher NYHA classes of congestive HF compared to NYHA-class 1 (165). We found that levels of HA are significantly higher in patients with HF compared to healthy controls. However, of the total cohort , only 31% of patients had elevated HA-levels. Conditions responsible for glycocalyx shedding such as ischaemia, hypoxia, inflammation or infection, oxidative stress, volume and salt overload are frequently present in HFrEF patients, especially during decompensation. Moreover, previous experiments demonstrated that activation of the natriuretic peptide system also induces glycocalyx shedding (72, 73). We observed elevated levels in 39% of decompensated patients versus 20% of stable HFrEF patients ($p<0.05$).

We could not find a significant difference in levels of syndecan-1 between stable HFrEF and decompensated HF or even with healthy subjects. To our knowledge there are no studies comparing levels of syndecan-1 in patients with stable versus decompensated HFrEF or HFrEF and a normal control population. We found a wide distribution of syndecan-1 in healthy subjects (54.5 (32.2;130.3) ng/ml) which was more profound than found in other groups of healthy controls, and is likely responsible for the lack in significant differences or a meaning-full cut-off value (136, 166). Moreover, but further discussed below, no association between syndecan-1 levels and outcome could be found.

HA may be a better biomarker for glycocalyx shedding than syndecan-1 for several reasons. HA is a long polymer of hundreds of disaccharide units that carry strong negative charges. However, in contrast to other GAGs, HA is not bound to a core protein. Most of the HA in

the vasculature is incorportated in the endothelial glycocalyx (167). Tissue half-life of HA can range between 0.5 and 3 days (168). In contrast, syndecan-1 is a proteoglycan which is composed of a core protein with covalently bound GAG-chains. There are many cell-matrix interactions between syndecan-1 and surrounding structures. Its half-life is estimated around 6 hours due to rapid metabolization (169).

Associated factors with shedding markers

We found a significant relation between doubling of levels of HA and age. However, this association was poor and was non-existent for absolute values of HA and age. Indeed, increasing levels of HA with age is observed. After adjusting for age, the association between elevated HA levels and outcome remained significant.

Although previous studies reported glycocalyx shedding in various conditions such as hypoxia, volume and salt overload, activation of neurohumoral systems, no significant relationship could be established between HA or syndecan-1 and individual variabes. This is most likely due to the fact that glycocalyx shedding in HFrEF is multifactorial. Moreover, the absence of a significant correlation with frequently used cardiovascular biomarkers (NT-proBNP, HS-Trop, PRA etc.) supports our hypothesis that elevated levels of shedding products represent an independent pathophysiological process in HF patients.

Outcome

After adjusting for established HF risk factors in a multivariate cox model, only renal dysfunction (eGFR<60 ml/min/1.73m2) and elevated levels of HA remained significantly associated with outcome. These exploratory data indicate that further research in the area of glycocalyx shedding in HFrEF patients is more than justifiable.

Previous data on the prognostic value of syndecan-1 in HF patients is conflicting. Patients with acute decompensated HF and higher levels of syndecan-1 (>125 ng/ml) have a higher risk of 6-month mortality and renal dysfunction during hospitalization (154). Another study of 2033 patients with acute HF after being stabilized during hospital stay evaluated the diagnostic accuracy of 44 biomarkers including syndecan-1 for heart failure. Syndecan-1 was significantly correlated with 30-day and 180-day mortality (170). However, in other studies the relationship between syndecan-1 and mortality was only present in patients with

HF and preserved ejection fraction but not in HFrEF patients (171). We found no relation between syndecan-1 levels and outcome in HFrEF patients.

FUTURE PERSPECTIVES

Degradation of the endothelial glycocalyx is currently being associated with a growing number of cardiovascular diseases. Investigating the role of the endothelial glycocalyx in HF is promising due to its pathophysiological role in microvascular and endothelial function. The prevalence of HF is increasing and almost no new treatment strategies are discovered in the last two decades. Clinical studies indicating that protection of the endothelial glycocalyx from degradation benefits clinical outcome are lacking. Pharmacological agents such as inhibitors of inflammation, antithrombin, inhibitors of metalloproteases but also albumin and sulodexide, a preparation delivering precursors of glycocalyx constituents, display the potential to attenuate shedding of the glycocalyx .

STUDY LIMITATIONS

Although the strong statistical difference in HA and its prognostic relation, this was a small and single-center trial with inherent obvious limitations. Our findings should be considered hypothesis-generating and interpret as an exploratory study for further research in this domain. Due to the lack of a standardized in vivo investigation technique, glycocalyx structure and function was only indirectly studied by plasma-levels of shedding products.

CONCLUSION

This single-center exploratory study demonstrates that the glycocalyx shedding marker HA is significantly higher in HFrEF patients compared to healthy subjects and elevated in a subset of HFrEF patients. No significant difference could be found between HFrEF patients and healthy subjects for syndecan-1. Levels of shedding markers were not directly associated with neurohumoral activation, renal function or other variables. Importantly, elevated HA level is a specific and independent predictor for clinical outcome in patients with HFREF. Further trials regarding the role and prognostic importance of the endothelial glycocalyx are warranted.

SUPPLEMENTAL MATERIAL

Sign or symptom of decongestion	points
Pitting edema lower extremity> 1+	**1**
Elevated jugular venous pressure	**1**
ascites	**1**
Lung edema or congestion on chest X-ray	**1**
Orhtopnea	**1**
E/E' >15	**1**
NT-proBNP	
- >300 ng/ml	**1**
- >1000 ng/ml	**2**
Pulmonary capillary wedge pressure >12 measured during right heart catetherization	**2**

Table 1: Decompensation score *(≤1 was defined as stable and >1 was defined as decompensated)*

	Healthy subjects
n	30
age	27±10
male gender	60%
ischemic etiology	0%
BMI	26±4
LVEF	60±5
Decompensation	
No	0%
yes	100%
NYHA class	
I-II	100%
III-IV	0%
Systolic BP	126±21
Medical history	
- MI	0%
- Hypertension	0%
- AF	0%
- DM	0%

Labarotory values	
- Hemoglobin (g/dl)	14.2±1.3
- Na mmol/L	141±2
- CRP	1.8(0.7;2.4)
- Creatinine	0.9±0.2
- BUN	29±7
- eGFR ml/min/1.73m2	99±19
- NTproBNP ng/L	38(18.0;90.3)
- PRA	1.8(0.8;3.1)
- Serum aldactone (ng/L)	177.5(145.5;241.0)
Plasma levels of shedding products	
- Syndecan ng/ml	54.5(32.2;130.3)
- Hyaluronic Acid ng/ml	18.9(12.1;29.7)
Maintenance therapy at inclusion	
- ACE/ARB use	0
- BB use	0
- MRA use	0
- Loop diuretic use	0
- Hydralazine/Nitrate use	0

Table 2: Characteristics of Healthy Subjects. *Values are standardized β coefficients, ACE: angiotensin converting enzyme inhibitor;ARB: angiotensin receptor blocker, BMI: body mass index; BP: blood pressure; BUN: blood urea nitrogen; COPD: chornic obstructive pulmonary disease; CRP: C-reactive protein; LVEF: left ventricular ejection fraction; MRA: mineralocorticoid receptor antagonist; NYHA: New york Heart association-class; NT-proBNP: N-terminal of the pro-hormone of brain natriuretic peptide; PRA: plasma renin activity.*

	Stable HFrEF	Decompensated HFrEF	p-value
n	54	69	
age	66±11	68±13	0.119
male gender	91%	78%	0.063
ischemic etiology	78%	59%	0.031
BMI	28±4	28±5	0.737
LVEF%	32±7	25±11	0.001
NYHA class			<0.001
I-II	83%	19%	
III-IV	17%	81%	
Systolic BP	121±16	128±23	0.131
Medical history			
- MI	78%	59%	0.031
- Hypertension	43%	41%	0.822
- AF	23%	46%	0.017
- DM	26%	32%	0.471
Labarotory values			
- Hb (g/dl)	13.5±1.3	12.7±2.0	0.010
- Sodium (mmol/L)	138±3	138±3	0.476
- CRP (mg/dl)	1.7(0.9;3.6)	4.8(2.5±14.8)	<0.001
- Creatinine (mg/dl)	1.5±0.7	1.5±0.7	0.854
- BUN	59±26	70±43	0.098
- eGFR ml/min/1.73m2	59±25	56±24	0.393
- NTproBNP ng/L			
- PRA	1022(380;2100)	4383(2230;11087)	<0.001
- Serum aldosterone (ng/L)	11.0(3.7;34.5)	1.7(0.8;5.7)	<0.001
- HS-trop	206.5(138.8;378.0)	180(139.0;293.0)	0.325
	13(8;20)	32(20;62)	0.124
Plasma levels of shedding products			
- Syndecan ng/ml	26.5(8.8;46.0)	29.4(11.6;92.2)	0.771
>cutoff	0%	0%	
- Hyaluronic Acid ng/ml	45.8(30.4;71.9)	50.2(35.3;81.0)	0.024
>cutoff	20%	39%	
Maintenance therapy at inclusion			
- ACE/ARB use	89%	52%	<0.001
- BB use	96%	75%	<0.001
- MRA use	85%	54%	<0.001
- Loop diuretic use	57%	61%	0.698
- Hydralazine/Nitrate use	0%	21%	<0.001

Table 3: Baseline Characteristics of stable versus decompensated HFrEF patients *ACE: angiotensin converting enzyme inhibitor;ARB: angiotensin receptor blocker, BMI: body mass index; BP: blood pressure; BUN: blood urea nitrogen; COPD: chornic obstructive pulmonary disease; CRP: C-reactive protein; LVEF: left ventricular ejection fraction; MRA: mineralocorticoid receptor antagonist; NYHA: New york Heart association-class; NT-proBNP: N-terminal of the pro-hormone of brain natriuretic peptide; PRA: plasma renin activity.*

Variables	HA (ng/ml)			Syndecan ng/ml		
	Univariate β	p	Multivariate p	Univariate β	p	Multivariate p
Demographics and clinical signs						
- Age						
- Male sex	0.24	**0.018**	**0.025**	7.20	0.152	
- BMI (kg/m2)	0.00	0.977		-17.2	0.183	
- Systolic BP	0.02	0.831		-5.42	0.293	
- LVEF%	0.02	0.839		2.133	0.637	
- NYHA class	-0.11	0.269		3.43	0.473	
- Decompensation	0.20	**0.057**	0.607	4.67	0.335	
	0.40	**0.004**	0.406	2.81	0.771	
Medical History						
- Myocardial infaction	0.20	0.363		0.97	0.924	
- Hypertension						
- Atrial fibrillation	0.00	0.987		-10.28	0.289	
- Diabetes	0.08	0.725		18.28	**0.095**	0.149
	0.19	0.385		-16.81	0.108	

Laboratory values						
- Hemoglobin (g/dl)	-0.09	0.377		-4.32	0.371	
- Sodium (mmol/L)						
- NT-proBNP (ng/L)	0.00	0.476		1.03	**0.077**	0.992
- Trop (ng/ml)						
- CRP (mg/dl)	0.15	0.130		3.16	0.511	
- eGFR (ml/min/1.73m2)						
- BUN (mg/dl)	-0.02	0.843		-1.10	0.793	
- PRA (	0.29	**0.030**	0.158	-1.11	0.817	
- Serum aldosterone (ng/L)	-0.18	**0.063**	0.674	-8.80	**0.063**	0.331
	0.08	0.410		5.81	0.225	
	-0.15	0.122		-1.58	0.733	
	-0.12	0.231		1.50	0.757	
Maintenance therapy						
- ACE-inhibitor or ARB	-0.52	**0.016**	0.208	-6.14	0.550	
- β-Blocker						
- Diuretic	-0.06	0.820		22.71	**0.084**	0.120
- MRA	-0.20	0.340		10.57	0.276	
	-0.49	0.022	0.138	10.54	0.301	

Table 4 Clinical variables associated with (doubling of) shedding markers HA and syndecan-1 in HFrEF patients? *Values are standardized β coefficients, ACE: angiotensin converting enzyme inhibitor;ARB: angiotensin receptor blocker, BMI: body mass index; BP: blood pressure; BUN: blood urea nitrogen; COPD: chornic obstructive pulmonary disease; CRP: C-reactive protein; LVEF: left ventricular ejection fraction; MRA: mineralocorticoid receptor antagonist; NYHA: New york Heart association-class; NT-proBNP: N-terminal of the pro-hormone of brain natriuretic peptide; PRA: plasma renin activity.*

PART II

Volume handling in heart failure

OBJECTIVE | To investigate homeostasis of intravascular volume and identify the contribution of intravascular volume changes to the hemodynamic and renal dysregulations in Heart Failure

CHAPTER 4

Plasma volume is normal but heterogeneously distributed and true anemia is highly prevalent in patients with stable heart failure

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ABSTRACT

Background: Intravascular volume overload and depletion as well as anemia are associated with increased hospital admissions and mortality in patients with heart failure. This study aimed to accurately measure plasma volume and red cell mass (RCM) in stable patients with chronic heart failure with reduced ejection fraction (HFrEF) and gain more insight into plasma volume regulation and anemia in stable conditions of HFrEF.

Methods and Results: Plasma volume and RCM measurement based on ^{99}Tc-labeled red blood cells, venous blood samples and clinical parameters were obtained in 24 stable HFrEF patients under optimal medical therapy. Measured plasma volume values were compared with predicted values based on body surface area. Plasma volume was on average normal (99,98% of predicted) but heterogeneously distributed (variations of 81% up to 133%). Neurohumoral activation and medication use were not associated with plasma volume status. Furthermore, anemia based on actual measurement of RCM was present in up to 75% of subjects, but exceptional dilutional.

Conclusion: In stable chronic HFrEF patients under optimal medical therapy, plasma volume is overall normal but heterogeneously distributed. Anticipated factors such as neurohumoral activation and heart failure medication were not associated with plasma volume. Furthermore, anemia is more common than assessed by hemoglobin.

INTRODUCTION

Much of the art of heart failure medicine consists of estimating volume status in patients, thus assessing whether they are *wet* or *dry.* Intravascular volume overload, resulting from increased sodium and water retention because of unrestrained neurohumoral activation, is considered to be an important cause of raised cardiac filling pressures in heart failure (i.e., congestion). Congestion may contribute to disease progression and relates to worse outcomes (91, 172-178). Furthermore, an expanded plasma volume may cause hemodilution, resulting in lower hematocrit levels. On the contrary, efficient *de*congestion and achieving of hemoconcentration during treatment in acute heart failure is associated with a reduced risk of mortality, even in the setting of worsening renal function (177, 179-181). Yet, intravascular volume depletion, resulting from strict adherence to a low-salt diet and overly aggressive use of diuretics, may compromise renal blood flow and further activate sodium- and water-retaining systems (182, 183). This indicates that prognosis in heart failure might be improved if therapy is guided to optimally balance plasma volume. However, little is known about plasma volume in stable patients with chronic heart failure and reduced ejection fraction (HFrEF). Therefore, the purpose of this study was to accurately measure plasma volume in stable chronic HFrEF patients who were optimally treated with neurohumoral blockers, and hence gain more insight into the determinants of plasma volume homeostasis in stable HFrEF. Additionally, blood volume measurements might also provide more insight in the presence of anemia in stable HFREF.

METHODS

Study design

This study was carried out in a single tertiary care center (Ziekenhuis Oost-Limburg, Genk, Belgium) between September 2014 and September 2015. The study complies with the Declaration of Helsinki and the institutional review board approved the study protocol. Written informed consent was obtained from every patient before any study-specific action was performed.

Study population

HFrEF patients were recruited from the outpatient clinic. Patients were eligible for inclusion if ≥18 years of age and able to give informed consent. Additionally, all of the following criteria had to be fulfilled: (1) a clinical diagnosis of HFrEF with evidence of impaired left ventricular ejection fraction (LVEF) ≤40% within 6 months before inclusion; (160) no hospital admission

for worsening heart failure as a primary or secondary diagnosis within the past 6 months; (3) stable New York Heart Association (NYHA) functional class <III (NYHA III is defined as unable to walk 50 meters or climb 1 flight of stairs without shortness of breath) for at least 6 months; (4) unchanged pharmacological treatment with renin-angiotensin system inhibitors, beta-adrenergic antagonists, mineralocorticoid receptor antagonists and diuretics for at least 3 months; (5) pharmacological treatment according to guideline recommendations including the maximally tolerated target dose for renin-angiotensin system inhibitors, beta-adrenergic antagonists and mineralocorticoid receptor antagonists; and (6) a daily maintenance dose of loop diuretics ≤2 mg bumetanide (equivalent to ≤80 mg furosemide). Exclusion criteria were: (1) renal replacement therapy or severe renal dysfunction with an estimated glomerular filtration rate ≤15 ml/min/1.73m^2 determined by the Chronic Kidney Disease Epidemiology Collaboration equation; (160) any clinical sign or symptom of volume overload (i.e. rales, orthopnea, jugular venous distention or ≥2+ edema); (3) clinical signs of low output failure (i.e., supine systolic blood pressure <100 mmHg) and (4) pregnancy.

Plasma and red cell mass analysis

Total blood volume was measured with a technetium (^{99}Tc)-labeled red blood cell technique, according to guidelines of the international committee for standardization in Hematology (184). Red cell mass (RCM) (indicating the total mass or volume of all red blood cells in the blood and not to be confused with the average mean corpuscular volume of a single red blood cell) and plasma volume were derived from the measured blood volume and venous hematocrit, corrected for trapped plasma and mean body hematocrit (185-187). Measured values were compared to predicted values for plasma and RCM, based on validated reference values in healthy subjects using body surface area and gender (188, 189).

In brief, patients were put in the supine position for 60 min. Subsequently, 2 catheters were placed in the forearm and blood was taken from the patient and labeled with ^{99}Tc in the nuclear lab. The resulting labeled blood (22.8±4.2 mCi) was reinjected into the patient. Afterwards, 5 mL of blood was collected at 10-min intervals for 30 min. Radioactivity was measured in an automated counter (Veenstra/COMECER, Joure – The Netherlands). Blood volume was calculated as the zero-time volume of distribution of the radio-labeled red blood cells obtained by semi-logarithmic extrapolation of values measured from the 3 samples.

Volumes are expressed as absolute measured values (L), indexed values for body surface area (L/m^2) and as percent deviation from predicted values (%). Anemia is defined by the

World Health Organization as a hemoglobin <13 g/dl for males or <12 g/dl for females (190, 191). Anemia defined by hemoglobin values was compared to anemia defined as an RCM<95% (true anemia) of the reference value (192, 193). A diagnosis of hemodilution was made in patients when hemoglobin was <13 or 12 g/dl and measured RCM was >95% of predicted (192).

Laboratory measurements

A venous blood sample was taken from each patient after an adaptation period of approximately 1 hour in the supine position. Fresh samples were immediately cooled and transported to the laboratory. Routine laboratory measurements, including N-terminal of the prohormone of B-type natriuretic peptide (NT-proBNP) were immediately performed. Samples for PRA and serum aldosterone were centrifuged and stored at -80° and analyzed in batch. All measurements were performed using commercially available kits according to the manufacturer's instructions. NT-proBNP levels were measured by the Roche Diagnostics Assay (Roche, Rotkreuz, Switzerland). Plasma renin activity (PRA) was determined using the Gamma-coat*radio immunoassay (DiaSorin, Sallugia, Italy). Plasma aldosterone levels were assessed by the Aldosterone Maia radioimmunoassay (Adaltis, Rome, Italy).

Transthoracic echocardiography

A two-dimensional echocardiographic exam was performed with a commercially available system (Philips Healthcare, iE33w Androver, MA). Images were acquired in the left lateral decubitus position. All reported echocardiography measurements were averaged from three consecutive cycles and assessed as recommended by the American Society of Echocardiography. Left ventricular ejection fraction was obtained by Simpson's biplane formula (194). Diastolic function was assessed in a standardized manner by use of the transmitral pulsed wave doppler signal, tissue velocity of the lateral and septal side of the mitral annulus and the isovolumetric contraction and relaxation time (195, 196). Diastolic dysfunction (DDF) was then categorized as normal, impaired relaxation, pseudonormal filling, restrictive filling (197). Right ventricular systolic pressure was calculated from the maximal transtricuspid continuous wave Doppler velocity if tricuspid valve regurgitation was measurable (198). Vena cava inferior diameter (VCI) was assessed from a subcostal view.

Statistical analysis

Continuous variables are expressed as mean±standard deviation if normally distributed, or otherwise as median (interquartile range). Normality was assessed by the Shapiro-Wilk statistic. The unpaired student's t-test and Mann-Whitney test were used when appropriate.

Effect size was calculated for each continuous variable and expressed as Cohen's d value (d). Categorical data were expressed as percentages and compared with the Pearson χ^2-test. Statistical significance (p) was always set at a 2-tailed probability level of <0.05. All statistics were performed using SAS JMP Pro (version 11.2 for Windows).

RESULTS

Study population

Twenty-four HFrEF patients (LVEF 37±10%) were included in the study. Baseline characteristics are summarized in Table 1.

Variable	Value
Age (years)	68 ± 11
Male gender (%)	92%
Ischemic heart disease (%)	83%
Body mass index (kg/m^2)	28 ± 4
Body surface area (m^2)	1.9 ± 0.2
Heart rate (bpm)	66 ± 12
Systolic blood pressure (mmHg)	124 ± 17
Diastolic blood pressure (mmHg)	54 ± 12
Echocardiography measurements	
Left ventricular ejection fraction (%)	37 ± 10
Left ventricular end-diastolic diameter (mm)	55 ± 7
Diastolic function	
Impaired relaxation	71%
Pseudonormal	13%
Restrictive filling	17%
TAPSE (mm)	17.5 ± 4.5
Right ventricular systolic pressure (mmHg)	28 ± 11
Vena cava inferior diameter (mm)	16 ± 4
Laboratory results	
Hemoglobin (g/dL)	12.0 ± 1.2
Venous Hematocrit (%)	39.0 ± 4.1
Serum creatinine (mg/dL)	1.48 ± 0.66
eGFR (mL/min/1.73m^2)	41 ± 9
Serum sodium (mmol/L)	138 ± 3
Osmolality (mmol/kg H_2O)	299 ± 8
NT-proBNP (ng/L)	1,023 (403-1,839)
Plasma aldosterone (ng/L)	199 (126-403)
Plasma renin activity (ng/mL/h)	10.8 (2.6-31.5)
Medication use	
Renin-angiotensin system inhibitor	83%
Beta-adrenergic antagonist	96%
Mineralocorticoid receptor antagonist	83%
Loop diuretic	62%

Indexed RCM (L/m2)	0.93±0.20
Measured to predicted RCM (%)	83.8±17.0
Indexed plasma volume (L/m2)	1.7±0.2
Measured to predicted plasma volume (%)	99.9±13.0

Table 1. Baseline characteristics

Plasma volume

Absolute and indexed plasma volume were 3.2±0.6 L and 1.7±0.2 L/m², respectively. On average, the plasma volume of the entire cohort was almost identical to the predicted plasma volume (99.9±13.0%). However, individual variations from 81% up to 133% were observed (Figure 1).

Subjects with measured plasma volume lower than predicted (<100%) (n=13, mean plasma volume 91,3±6,3% of predicted) were compared with subjects with plasma volume higher than predicted (≥100%) (n=11, mean plasma volume 111,2±9,2% of predicted) (Table 2). There was no significant difference in neurohumoral activity (plasma aldosterone and plasma renin activity) NT-proBNP, renal function or maintenance therapy between the two groups.

Mean measured blood volume and indexed blood volume in this cohort were respectively 5,03±1,0 L and 2,6±0,7L/m^2. Mean total blood volume was 94,0±12,4% of predicted.

	Plasma volume >100% of predicted	**Plasma volume <100% of predicted**	**p**	**d**
n	11	13		
Indexed Plasma volume (l/m2)	1,8±0.2	1.5±0.1	<0.01*	1.4
Ratio measured vs predicted plasma volume	+11.2±9.2	-9.6±6,3	<0.01*	1.6
Indexed RCM (L/m^2)	1.0±0.2	0.9±0.2	0.33	0.7
Echocardiography				
LVEF (%)	29±7	30±5	0.67	0.1
LVEDD (mm)	55±8	54±5	0.80	0.1
DDF grade			0.72	
Impaired relaxation	73%	64%		
Pseudonormal	9%	18%		

restrictive filling	18%	18%		
TAPSE (mm)	17±3	18±5	0.72	0.3
RVSP (mmHg)	26±7	29±13	0.84	0.3
VCI (mm)	16±4	15±13	0.87	0.1
SBP (mmHg)	127±18	121±17	0.50	0.9
DBP (mmHg)	52±10	55±13	0.84	0.1
Laboratory results				
Hemoglobin (g/dl)	13.1±1.2	13.5±1.3	0.35	0.3
Creatinine (mg/dl)	1.38±0.52	1.57±0.77	0.98	0.5
aldosterone (ng/L)	260 (117,;438)	189 (129; 306)	0,37	0.4
PRA (ng/L/h)	11,9 (0,42; 34,2)	9,7 (4,6; 26,65)	0,71	0.0
NT-pro BNP (ng/L)	1025 (457;2590)	1020 (374;1668)	0,54	0.1
Medication use				
ACE I			0,82	
None/≤50%/>50% (n)	2/6/3	2/6/5		
BB			0,60	
None/≤50%/>50% (n)	1/5/5	0/7/6		
MRA (n)	8	11	0,35	
Loop diuretic		5/4/4	0,47	
None/<1 mg/1 -2 mg	4/1/6			

Table 2. Subjects with measured plasma volume higher than predicted (>100%) versus measured plasma volume lower than predicted (<100%). *Loop diuretic dose is expressed in bumetanide equivalent (mg B eq) e.g. furosemide 40 mg = bumetanide 1 mg. Other HF therapy is presented as the number of patients on none, ≤50% or >50% of the target dose.*

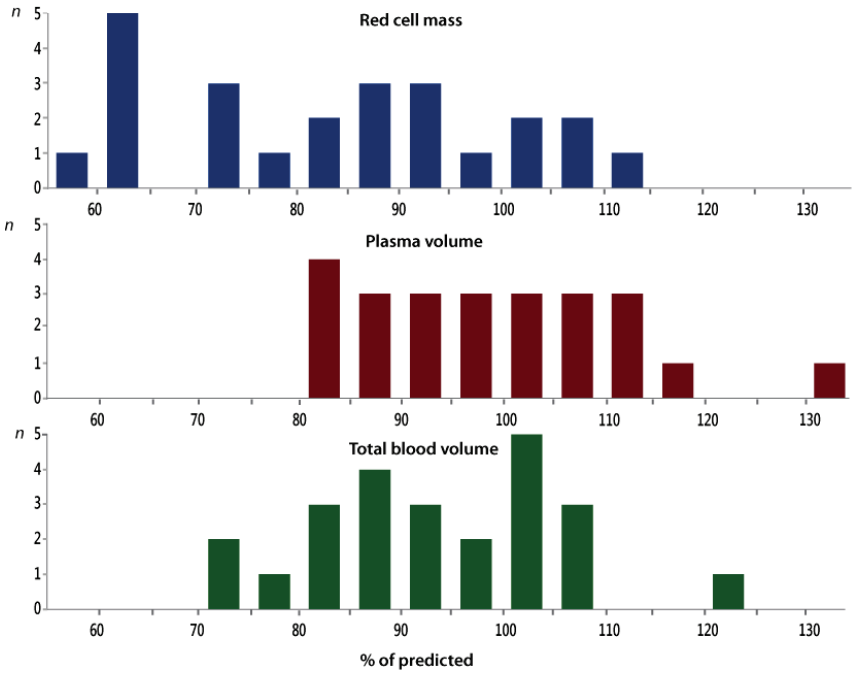

Figure 1. Measured to predicted ratio of plasma volume, red cell mass (RCM) and total blood volume in 24 chronic HFrEF patients. *On average, plasma volume is normal but heterogeneously distributed. RCM is on average lower than predicted with 75% of patients having an RCM<95%*

Red cell mass

Half the subjects (n = 12) had anemia based on a hemoglobin <13 g/dl for males and <12g/dl for females. Iron deficiency (defined as a ferritin <100 ng/ml or a transferrin saturation <20% together with a ferritin level below 300 ng/ml) is more prevalent in the anemic group, as is more pronounced kidney dysfunction (creatinine 1,65±0,64 versus 1,31 ± 0,66 mg/dl) (p values not significant between groups). Indexed plasma volume is comparable between anemic versus non anemic patients (1,7±0,2 versus 1,6±0,2 L/m^2) patients. Hemodilution (low hemoglobin and RCM ≥95%) is only present in 1 out of 12 subjects from this subgroup, while all the other subjects have a deficit in red blood cells (RCM <95%). Of the 12 patients with true anemia 5 had a plasma volume >100% of predicted. In addition, 7 out of 12 patients with normal hemoglobin levels also had a RCM ≤95%, indicating *masked* anemia (Table 3).

	Non-anemic	Anemia defined as hemoglobin <13 or 12 g/dl	Anemia defined as RCM <95%
N	12	12	18
% (original cohort)	50	50	75
Hb (g/dl)	14.2±1.0	12.3±0.4	12.9±1.1
Hct (%)	42±4	36±1	37±3
Iron deficiency (%)	25	58	50
Vit B12 (ng/L)	293±90	333±70	319±79
FZ (ng/L)	8.7±5.3	7.6±2.2	8.2±3.3
creatinine (mg/dl)	1.31±0.66	1.65±0.64	1.48±0.62
Indexed RCM	1.0±0.21	0.85±0.15	0.84±0.14
Indexed plasma volume	1.6±0,2	1.7±0,2	1,6±0.2

Table 3. Laboratory values and blood volume measurements for anemic versus non-anemic stable HFrEF patients. *Iron deficiency is determined as ferritin <100 ng/ml or the combination of transferrin saturation <20% and ferritin <300 ng/ml*

DISCUSSION

Determination of plasma volume status, defined as the % deviation of predicted, has aroused interest as a prognosticator and potential therapeutic target in heart failure patients. Indeed, limited data confirm plasma volume expansion to be present in chronic HFrEF patients with signs of fluid overload as well as in advanced (pre-transplant) chronic HFrEF patients (30-34). To the contrary, plasma volume reduction (<100%) was seen in heart failure patients on suboptimal heart failure therapy and high dosages of diuretic agents (173, 192, 199-201). However, data on accurate values of plasma volume in patients with stable chronic heart failure are rather sparse and divergent, which probably relates to different populations investigated. We report on plasma volume measurements in a cohort of clinically euvolemic, stable HFrEF patients treated with optimal doses of neurohumoral blockers. Firstly, measured plasma volume was on average normal, however with a wide distribution. Furthermore, plasma volume in stable chronic HFrEF patients seems independent of heart failure therapy, neurohumoral activation and renal function. Finally, in this cohort of stable chronic HFrEF patients, true anemia is more common than expected based on hemoglobin concentration, but seldom hemodilutional.

Plasma volume in chronic HFrEF patients is normal with a wide distribution.

The assessment of volume status relies mostly on clinical indicators, laboratory values, echocardiographic indices or invasive procedures. Tracer dilution methods for quantifying plasma volume have long existed but the lack of normal values in different patient populations and the fact that all methods are time-consuming limit their use in clinical practice. Plasma is the part of the blood containing the watery solution of electrolytes, plasma proteins, carbohydrates and lipids without formed blood elements.

We report on a stable cohort of HFrEF patients, being depicted as euvolemic, that plasma volume was heterogeneous, with individual variations from 81% up to 133% of predicted. To differentiate abnormal from normal we compared our data to healthy subjects. However, as in HFrEF patients, data on plasma volume in healthy subjects is sparse. In 1977 Feldschuh and coauthors determined *blood* volume in 160 healthy men and women based on radiolabeled albumine (202). The range of intravascular volume in this cohort was 85% to 133% of the mean blood volume in women, and 80% to 129% of the mean blood volume in men, which is similar to our findings. Hence, this further supports our findings that plasma volume in patients with stable HFREF is normal.

Although there is no universal definition, in current literature a deviation of minus 8 to 11% of predicted or plus 8 to 11% of predicted intravascular volume is generally adopted as the cutoff for intravascular volume expansion (hypervolemia) versus depletion (hypovolemia), respectively (173, 201). However, the current findings in stable clinically euvolemic HFREF patients and healthy controls demonstrate that intravascular volume is heterogeneously distributed and intravascular "euvolemia" is not within a narrow range. Therefore, the target range and cinical utility of plasma volume measurements in heart failure patients remains to be determined.

Although plasma volume was variable, all of these patients were assessed as clinically euvolemic before inclusion. Furhtermore, there were no differences in echocardiographic parameters and NT-proBNP levels between patients with < or >100% of estimated plasma volume. Therefore, it seems rather impossible to accurately predict plasma volume status in a daily clinical setting, and to assess early volume derangements. Indeed, rehospitalization rates for heart failure occurs at a rate close to 50% over 6 months (203). Therefore, detection of preclinical intravascular volume expansion may be very promising in reducing outcome rates in heart failure.

Neurohormonal activation and heart failure therapy is comparable between heart failure patients with plasma volume < or > 100% of

It is widely believed that plasma volume status is regulated through the complex interaction between afferent pressure-, volume- and osmoreceptors and efferent sodium and water retaining mechanisms (renin-angiotensin-aldosterone system (RAAS) and vasopressin) and the natriuretic system. Low blood pressure, decrease in renal perfusion pressure, reduction in tubular sodium load and activated sympathetic nervous system, will result in increased renin and aldosterone release in heart failure, promoting sodium and water retention (183). Therefore, in patients with heart failure, long-term pharmacological inhibition of the RAAS system, as well as inhibition of the natriuretic and diuretic actions of atrial natriuretic peptides, salt restriction as well as titrated diuresis should all contribute to a status of 'normal plasma volume'. Surprisingly, different degrees of neurohumoral activity (indicated by plasma aldosterone, BNP and PRA activity), hemodynamic factors (blood pressure, ejection fraction, heart rate, etc.), which strongly influence neurohumoral activity, and dosages of neurohumoral blockers, were not predictive for the hetereogenicity in plasma volume status. Therefore, we presume that the plasma volume status in chronic HFREF is complex but the neurohumoral system activation and medication use are unlikely to play a determining role.

The prevalence of anemia is higher than expected (75%), but hemodilutional anemia is very rare in stable HFrEF (4%)

Anemia in chronic heart failure can be due to an absolute deficit of red blood cells, hemodilution and/or a combination (204, 205) It is a frequent observation in heart failure which is associated with worse outcomes, presumably because of impaired peripheral oxygen delivery and nitric oxide availability that causes further neurohumoral activation (180, 206). We observed a high prevalence of hemoglobin-based definition of anemia (50%). In contrast to general belief, in this cohort of stable euvolemic chronic HFrEF patients dilutional anemia is exceptional (4%) and in 21% a combination of plasma volume expansion and deficit in red blood cells was present. Thus, in the vast majority of chronic stable HF patients with anemia a deficit in RCM is the underlying cause. Approximately half the patients had iron deficiency and on average mild renal insufficiency, which is in line with large trials (207). Moreover, if the diagnosis of anemia would be based on blood volume analyses and defined as a RCM <95%, up to 75% of subjects in this cohort would be diagnosed with anemia. In the light of recent trials demonstrating benefit of treating anemia and even iron-deficiency in non-anemic heart failure patients, revealing *masked* anemia could be an additional and interesting target for therapy (208-210).

STUDY LIMITATIONS

First, this was a small, observational, single-center study indicating that results should be considered hypothesis-generating. Second, by intention only stable HFREF patients were included in this study and the extrapolation of these results to other groups of heart failure patients should not be done. However, the fact that plasma volume status is even in this group heterogeneously distributed, is striking. Third, we normalized plasma volume and RCM for BSA and compared our data to validated reference values in a historical cohort of 784 normal men and woman (189). However, there remains a lack of validation for the normalization method used. Based on current literature, normalization for BSA is better than other parameters such as length or weight (211, 212). However, since adipose tissue has less blood volume per weight than other tissues, variability is larger in obese subjects. Therefore, accurate reference data for a broad range of BSA and age in future trials need to be determined. Finally, plasma volume assessment could be a promising strategy in heart failure but ideal target values and utility of plasma volume status driven heart failure management needs further evaluation.

CONCLUSION

This study demonstrates that plasma volume in clinically euvolemic stable and optimally treated chronic HFrEF patients is normal but heterogeneously distributed.

Anticipated factors such as differences in neurohumoral activation, renal function and loop diuretic dose could not account for this. True anemia is present in up to 75% of patients.

CHAPTER 5

Cardiovascular volume reserve in patients with heart failure and reduced ejection fraction

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Submitted

ABSTRACT

Background: Volume overload is a hallmark feature of heart failure. The pressure-based assessment of volume status has gained popularity with the use of implantable devices, yet its accuracy to detect intravascular volume expansion is not investigated.

Objectives: This study aimed to investigate the relationship between intravascular volume and intra-cardiac filling pressures in the setting of volume loading in stable HF patients with reduced ejection fraction (HFrEF).

Methods: 40 euvolemic HFrEF patients (LVEF 36±10%) (10 subjects with a pulmonary artery catheter), underwent intravascular volume expansion with 1 liter hydroxyl-ethyl-starch over 3 hours with coinciding intravascular volume measurements (technetium (^{99}Tc)-labeled red blood cell technique).

Results: Intravascular blood volume increased from 5.0±1.0 L to 5.7±1.0 L ($p<0.0001$). No change in clinical status or NT-proBNP levels (670[225;1383] ng/L vs 615[217;521] ng/L; $p=0.86$) was observed. No significant changes in echocardiographic indices of cardiac filling pressures were noticed. Invasively measured right atrial pressure and pulmonary arterial wedge pressure increased significantly immediately after start of infusion (4±2 mmHg to 8±4 mmHg; $p=0.04$ and 10±3 mmHg to 15±6 mmHg; $p=0.04$, respectively), decreased afterwards and remained stable for 3 hours (6±2 mmHg and 14±4 mmHg respectively), indicative of cardiovascular volume reserve. The accuracy of cardiac filling pressure (estimates) to predict intravascular volume expansion was very low (all AUC <0.65).

Conclusion: Euvolemic patients with HFrEF can tolerate an intravascular volume expansion of 0.7L without signs and symptoms of HF. Due to this cardiovascular volume reserve, estimates of cardiac filling pressures might be of limited value to reliable assess intravascular volume

INTRODUCTION

Volume overload secondary to unrestrained neurohumoral activation is a hallmark feature in heart failure with reduced ejection fraction (HFrEF) and an important contributor to disease progression, morbidity and mortality in HFrEF (6, 172, 173, 177). The assessment of volume status in patients with HFrEF is one of the daily "core businesses" of the practicing physician. However, physicians remain to fall short in correctly diagnosing volume status, which is reflected by the unacceptably high (re)hospitalization rates for volume overload in heart failure patients (213). Beyond body weight, objective quantification of volume status has been mainly deducted from *cardiac pressure* measurements. Many clinical signs and symptoms (exercise related dyspnea, orthopnea, bendopnea, the presence of jugular venous distention and positive hepato-jugular reflux), echocardiographic indices (inferior vena cava diameter and collapsibility, left ventricular diastolic indices), natriuretic peptide levels, or invasive assessment of cardiac filling pressures are utilized in clinical practice to diagnose volume expansion. Recently, the pressure-based assessment of volume status has gained even more popularity with the success of implantable devices. Studies with intra-cardiac pressure monitors have shown that the transition to decompensation is associated with small changes in cardiac filling pressure weeks before decompensation (52). It has been hypothesized that small amounts of intravascular volume, below the limit of detection of body weight change, are contribute significantly in these pressure increases (214). Yet, the relation between intravascular volume and intra-cardiac pressure is not straightforward in many patients. Although recently there are few studies investigating intravascular volume status at the moment of hospitalization for decompensation, no studies have examined volume handling of the cardiovascular system during the transition from euvolemia to intravascular volume overload (17, 215). Therefore, the objective of this study is to investigate the relationship between intravascular volume and intra-cardiac pressures by selectively increasing intravascular volume in euvolemic HFrEF patients.

METHODS

This prospective cohort study was carried out in a single tertiary care center (Ziekenhuis Oost-Limburg, Genk, Belgium) between September 2014 and October 2015. The study complies with the Declaration of Helsinki and the institutional review board approved the study protocol. Written informed consent was obtained from every patient before any study-specific action was performed.

Study population

Patients were eligible for study inclusion; 1) ≥18 years of age, 2) able to provide informed consent, 3) impaired left ventricular ejection fraction (LVEF) ≤40% assessed within 6 months, 4) clinical in a stable condition for at least 3 months, and 5) unchanged dosages of neurohumoral medical therapy during previous 3 months according to current guideline recommendations (13, 216). Exclusion criteria were: 1) renal replacement therapy or severe renal dysfunction with an estimated glomerular filtration rate ≤15 ml/min/1.73m^2 determined by the Chronic Kidney Disease Epidemiology Collaboration equation, 2) any clinical sign or symptom of decompensated heart failure (i.e., pulmonary rales, jugular venous distention or >1+ edema) at the time of study, or 3) elevated cardiac filling pressures defined by invasive right atrial pressure (RAP) > 10 mmHg and/or pulmonary arterial wedge pressure (PAWP) > 18 mmHg by pulmonary artery catheter (PAC) at the time of study.

Study design

All patients were admitted in the cardiology intensive care unit for research purposes. A PAC was inserted through right jugular approach with correct positioning confirmed through fluoroscopic guidance in the catheterization laboratory in the first 10 subjects the day before. At the day of the study, all patients took their maintenance medication scheduled at 8 am, and followed a standardized protocol. Subjects were placed in the semi-supine position, and an arterial and venous catheter were placed in the forearm. Hemodynamic measurements were performed in the semi-supine position at end-expiration with the balloon-tipped PAC at steady state and the zero level of the pressure transducer unit at the mid-thoracic line, halfway between the anterior sternum and the bed surface. Cardiac output was determined by calculation using the Fick equation through sampling of a mixed central venous blood gas, while assuming standard metabolic rates. Blood pressure was invasively and continuously monitored as was heart rate and PAC measurements. Baseline data was registered, and after a 60-minute equilibration period, a transthoracic echocardiography exam was performed and a venous blood sample obtained.

After baseline measurements, 1 liter of isotonic hydroxyl ethyl starch 6% was administered intravenously. The purpose was to obtain a sustained and stable expansion of the intravascular volume during a period of 3 hours. Therefore, based on pharmacokinetic properties, 500 ml was infused over 5-10 minutes (approximately 1 ml/kg/min; fast infusion), followed by a steady infusion of 0.04 mL/min/kg (slow infusion) with a maximum

of 1 liter over 3 hours. The moment of the start of the infusion was appointed as time point zero and every hour afterwards until +3 hours. Hemodynamic parameter registration, clinical assessment and a venous blood sample were repeated at each time-point. Additionally, hemodynamic parameters were also registered at the end of the fast infusion phase. Transthoracic echocardiography was repeated at + 1 hour during steady intravascular volume expansion. Urinary collections were performed by instructing patients to void empty every hour. At + 3 h, 1 mg bumetanide was intravenously administered and patients were further observed until discharge around 6 pm on the same day.

Intravascular volume measurement

Blood volume was analyzed with a technetium (^{99}Tc)-labeled red blood cell technique according to guidelines of the international committee for standardization in Hematology (184). In brief, blood volume was calculated as the zero-time distribution volume of the radio-labeled red blood cells obtained by semi-logarithmic extrapolation of values measured from 3 samples. Subsequently, plasma volume and intravascular volume expansion were calculated at every time-point based on hematocrit change from baseline. At time-point +1h intravascular plasma volume and intravascular volume expansion were measured based on ^{99}TC labeled red blood cells and calculated based on hematocrit change which showed a strong correlation (respectively R^2=0.83; p<0.001 and R^2= 0.83; p<0.001).

Laboratory measurements

Venous blood samples were each time obtained with the patient in the supine position after an adaptation period of at least 30 minutes, immediately transported in a chilled container, processed and frozen at -80°C within 30 minutes. Plasma NT-proBNP levels were measured by the Roche Diagnostics Assay (Cobas proBNP II, Roche, Rotkreuz, Switzerland). PRA was determined using the RIAZEN immunoassay (ZenTech, Liège, Belgium), with an inter- and intra-assay coefficient of variation <6%. Plasma aldosterone levels were assessed by the MaiaZen radioimmunoassay (ZenTech, Liège, Belgium), with an inter- and intra-assay coefficient of variation <7%.

Transthoracic echocardiography

Two-dimensional echocardiographic exam was performed with a commercially available system (Philips Healthcare, iE33w Androver, MA) according to published guidelines. Left ventricular ejection fraction was obtained by Simpson's biplane formula (194). Diastolic function was assessed by use of the transmitral pulsed wave doppler signal (E and A wave), tissue velocity of the lateral and septal side (lateral and septal E') of the mitral annulus and

the isovolumetric contraction and relaxation time (195, 196). Diastolic dysfunction (DDF) was categorized as normal, impaired relaxation, pseudonormal filling, restrictive filling (197). Left ventricular filling pressure was defined as elevated if E/E' >15 (mean of sepal and lateral) or E/A>2 and deceleration time<150 msec (217). Vena cava inferior diameter (IVC) and collapsibility during respiration wer assessed from a subcostal view. CVP was estimated based on the guidelines provided by the American Society of Echocardiography (198). A IVC diameter > 2.1 cm that collapses <50% with a sniff was defined as an elevated CVP corresponding with a CVP of 15 mmHg. A IVC dimeter ≤2.1 cm that collapses >50% with a sniff suggests a normal CVP pressure of 3 mm Hg. Indeterminate cases in which the IVC diameter and collapse do not fit this definition were given an intermediate value of 8 mmHg. Right ventricular systolic pressure was calculated as the sum of the maximal trans-tricuspid continuous wave Doppler velocity and estimated CVP (198).

Statistical analysis

Continuous variables were expressed as mean±standard deviation in tables and as mean and confidence intervals in figures if normally distributed, or otherwise as median [interquartile range]. Normality was assessed by the Shapiro-Wilk statistic. Values of the total HFrEF cohort and HFrEF subjects with PAC were compared with the Wilcoxon signed–rank test. For comparison of repeated measures, the paired Student's t-test or Wilcoxon signed-rank test was used as appropriate. Categorical data were expressed as percentages and compared with the Pearson χ^2-test. The predictive value to discriminate intravascular volume expansion was assessed by the use of a receiver-operating characteristic (ROC) curve analyses. To compare a normal from pathologic response, the slope of the PAWP/BSA corrected volume relationship was calculated and compared to previous data (18). Statistical significance was always set at a 2-tailed probability level of <0.05. All statistics were performed using SAS JMP Pro (version 11.2 for Windows).

RESULTS

Study population

Baseline characteristics of the study population are presented in Table 1. Study patients were on average 65±12 years old, predominantly male with a history of ischemic heart disease and a mean left ventricular ejection fraction (LVEF) of 36±10%. Baseline characteristics of the total study cohort and HFrEF patients with PAC were comparable (Table 1). By exclusion, no subjects had any clinical sign of volume overload, and all PAC patients had normal cardiac filling pressures at baseline.

Variable	Total cohort of HFrEF subjects	HFrEF subjects with PAC
n	40	10
Age (years)	65±12	68±11
Male gender	88%	80%
Ischemic heart disease	83%	80%
Body mass index (kg/m^2)	28±4	30±4
Body surface area (m^2)	2.0±0.2	2.0±0.2
Heart rate (bpm)	63±10	68±14
Systolic blood pressure (mmHg)	122±17	121±17
Diastolic blood pressure (mmHg)	56±11	55±9
Echocardiography measurements		
Left ventricular ejection fraction (%)	36±4	37±4
Left ventricular end-diastolic diameter (mm)	55±7	54±5
Diastolic function (%)	59%	50%
Impaired relaxation	26%	30%
Pseudonormal	15%	20%
Restrictive filling	12±5	12±5
E/E'	19±5	20±5
TAPSE (mm)	25±10	29±8
Right ventricular systolic pressure (mmHg)	15±5	15±2
Vena cava inferior diameter (mm)	6.8±2.2	8.0±0
Estimated CVP (mmHg)	3.7±1.7	3.3±0.8
Calculated CO (L/min)		
Laboratory results		
Hemoglobin (g/dL)	13.5±1.3	13.1±1.3
Venous Hematocrit (%)	40±4	40±4
Serum creatinine (mg/dL)	1.4±0.6	1.6±0.7
Serum Albumine (g/L)	41.4±3.4	39.8±4.0
Serum sodium (mmol/L)	138±3	138±2.2.
Serum osmolality (mmol/kg H_2O)	297±7	303±8
NT-proBNP (ng/L)	670[225;1383]	1135[521;1527]
Plasma aldosterone (ng/L)	194[127;322]	195[133;327]
Plasma renin activity (ng/mL/h)	8.6[2.6;22.1]	13.9[4.2;22.7]
PAC measurements		
PAP systolic (mmHg)		33±11
PAP diastolic (mmHg)		12±4
PAP mean (mmHg)		20±7
RAP (mmHg)		3±3
PAWP (mmHg)		8±3
CO/CI (L/min/m2)		4.2±0.8/2.1±0.4
SvO2 (%)		56±6

Intravascular volume (99Tc RBC)		
Blood volume (L)	5.0±1.0	4.7±0.7
Plasma volume (L)	3.2±0.6	3.1±0.5
Medical therapy		
Beta blocker use	95%	100%
ACE-inhibitor use	88%	80
MRA use	83%	100%

Table 1. Baseline characteristics. *ACE: angiotensin converting enzyme; CO/CI: cardiac output/cardiac index, CVP: central venous pressure; MRA: mineralocorticoid receptor antagonist; NT-proBNP, N-terminal of the prohormone of B-type natriuretic peptide , PAP: pulmonary artery pressure; PAWP: pulmonary arterial wedge pressure, RAP: Right atrial pressure, SvO2:mixed venous oxygen saturation , TAPSE, tricuspid annular plane systolic excursion*

Intravascular volume expansion

During intravascular volume expansion, blood volume increased from 5.0±1.0 L to 5.5±1.0 L at + 1 hour, to 5.7±1.0 at +2 hours and 5.6±1.0 L at +3 hours (all $p<0.0001$ vs baseline), equivalent to a relative increase of 10±5%, 13±5% and 12±5% of intravascular volume, respectively (Figure 1 and 2A). Plasma volume rose from 3.2±0.6 L to 3.6±0.6 L at +1 hour, to 3.7±0.6 L at +2 hours and to 3.7±0.6 L at +3 hours (all $p<0.0001$ vs baseline), equivalent to a relative increase of 17±7%, 25±9 % and 24±8%, respectively.

Clinical signs and symptoms in response to volume expansion

During 3 hours of intravascular volume expansion, no adverse event occurred. Clinical status of all 40 HFrEF patients remained unchanged. More specifically, subjects reported no change in dyspnea or orthopnea compared to baseline. None of the patients had pulmonary rales, increase in jugular vein distention, positive hepato-jugular reflux or occurrence of peripheral edema at hourly clinical investigation. Average urine output and weight gain during intravascular volume expansion of 3 hours were 0.49±0.25 L and +0.6±0.6kg.

Neurohumoral stimulation in response to volume expansion

PRA and serum aldosterone levels decreased from 7.6 [2.8;15.6] to 3.6 [1.7;14.0] ng/ml/h ($p=0.07$) and from 175[125;265] ng/L to 92 [71;123] ng/L ($p= 0.0001$), respectively. Baseline NT-proBNP was slightly elevated (NT-proBNP 670[225;1383] ng/L and did not change significantly during 3h of intravascular volume expansion ($p=0.86$; Table 2, Figure 1)

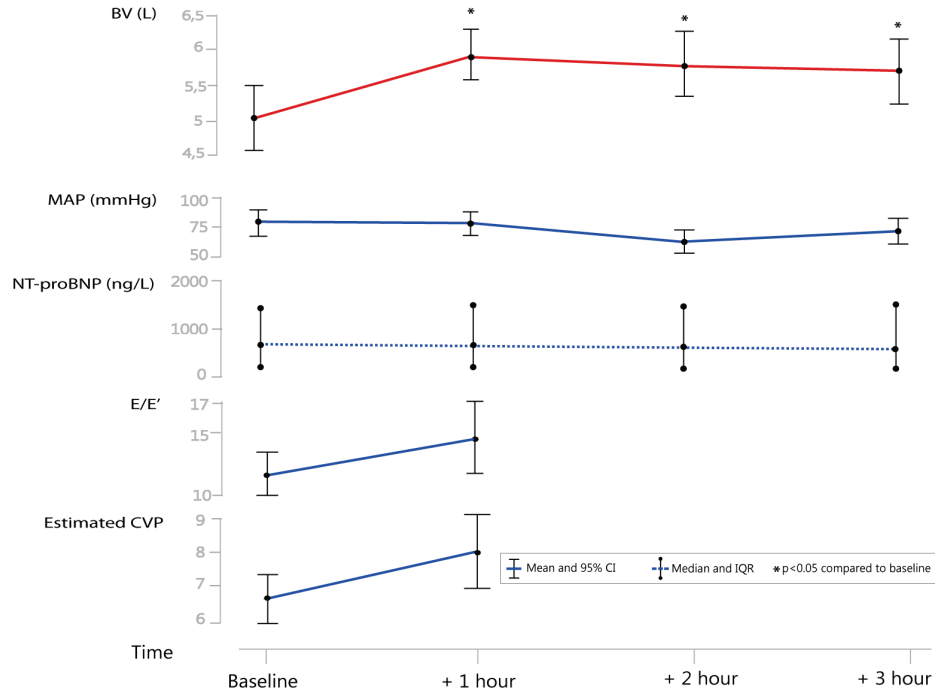

Figure 1. Clinical, laboratory and echocardiographic changes in response to plasma volume expansion. *BV: blood volume; CI: confidence interval; IQR: interquartile range; MAP: mean arterial pressure; NT-proBNP: N-terminal of the prohormone of B-type Natriuretic Peptide; estimated CVP: estimated central venous pressure;* * = p < 0.05 vs baseline.*

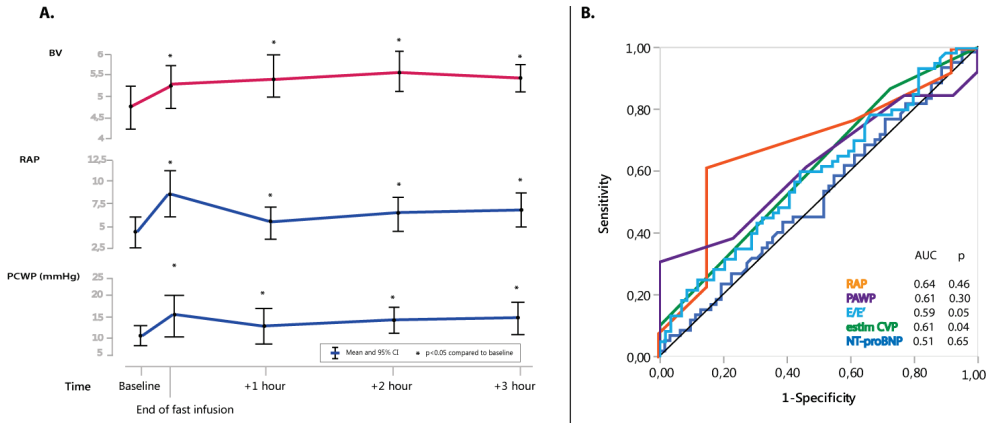

Figure 2. A: Cardiac pressure changes in response to sustained intravascular volume expansion in PAC patients. B: ROC curve for diagnostic accuracy of cardiac filling pressure (estimates) to diagnose intravascular volume expansion. *AUC: area under the curve, BV: blood volume; estim CVP: estimated central venous pressure, PAWP: pulmonary arterial wedge pressure, RAP: right atrial pressure, NT-proBNP: N-terminal pro hormone of brain natriuretic peptide; * = p < 0.05 vs baseline.*

	Baseline	Volume expansion	p
Blood volume (L)	5.0±1.0	5.6±1.0	0.0001
Plasma volume (L)	3.2±0.6	3.7±0.6	0.0001
Clinical parameters			
- Systolic Blood Pressure (mmHg)	122±17	123±17	0.5381
- Diastolic Blood Pressure (mmHg)	56±11	51±11	0.0177
- Mean arterial Pressure (mmHg)	78±10	76±11	0.1310
- Heart Rate	64±12	65±10	0.1267
- Weight (kg)	84.0±15.9	84,4±15.9	<0.0001
Laboratory values			
- Hematocrit (%)	39.9±4.0	35.6±3.8	0.0001
- NTproBNP (ng/L)	670[225;1383]	615[217;1521]	0.8615
- PRA (ng/ml/h)	8.6[2.6;22.1]	3.8[1.4;14.6]	0.0720
- Serum aldosterone (ng/L)	194[127;322]	99[79;134]	<0.0001
- Creatinine (mg/dl)	1.3±0.5	1.2±0.5	<0.0001
Echocardiographic measurements			
- Estimated CVP (mmHg)	6.8±2.2	8.0±2.9	0.1313
- E/E'	12.3±5.3	13.7±7.4	0.1267
- Cumulative urine output +3 h (L)	/	0.49±0.25	

Table 2. Evolution of intravascular volume and pressure estimates after volume expansion in total cohort of HFrEF patients. *CVP: central venous pressure; PAP: pulmonary artery pressure, PAWP: pulmonary arterial wedge pressure, PRA: plasma renin activity; RAP: right arterial pressure; Measurements during volume expansion are values at + 3 hours except for echocardiographic parameters which were obtained after 1 hour.*

Changes in cardiac pressure (-estimates) in response to volume expansion

Cardiac filling pressure estimated by echocardiography did not change significantly in response to intravascular volume expansion. There was no significant change in MAP or heart rate (Table 2 and Figure 1).

In HFrEF patients with a PAC, a significant increase in RAP and PAWP compared to baseline was observed. Average RAP and PAWP were maximal immediately after fast infusion, decreased thereafter, and remained stable during 3 hours of intravascular volume expansion (Figure 2A and Table 1 of the supplemental material). At +1 hour, a PAWP >18 mmHg was observed in 3/10 patients and a RAP >10 was observed in none. Patients with an increase in RAP at +1 hour from baseline (n=6) were compared to patients without a change or decrease in RAP (n=4). The former group had significantly higher blood volume at baseline, a significant higher increase in RAP, PAWP and PAP pressures as well as a more pronounced decrease in PRA values while intravascular expanded volume and baseline hemodynamic values were comparable between both groups (Supplemental material Table 2).

Discriminative value of cardiac filling pressure (estimates) to predict intra-vascular volume expansion.

Based on ROC curve analysis, frequently used cardiac pressure (estimates) have low accuracy to diagnose intravascular volume expansion of approximately 700 ml (all AUC<0.65) (Figure 2B).

DISCUSSION

Increased cardiac filling pressures and intravascular volume expansion, due to deficient volume and sodium homeostasis is thought to be central in the pathophysiology of decompensated HF. The objective of this study is to specifically investigate the role of intravascular volume expansion on intra-cardiac filling pressures in clinically euvolemic HFrEF patients in whom a transition from euvolemia to hypervolemia was induced. We observed that HF patients do tolerate a prolonged and significant increase of intravascular volume increase without signs and symptoms of HF. Additionally such an increase of intravascular volume only leads to a significant but limited increase in cardiac filling pressures comparable with healthy subjects. Therefore, our data suggest that stable HFrEF patients exhibit a "cardiovascular volume reserve" by which the cardiovascular system can efficiently accommodate important increases in intravascular volume. The presence of cardiovascular volume reserve in such patients contributes to the low accuracy of pressure-based assessment of intravascular volume status.

Previously, only a limited number of volume expansion experiments were conducted to study hemodynamic adaptations (218-220). These experiments used isotonic saline which

is known to almost immediately extravasate from the intravascular compartment into the interstitium. We used HES 6% and confirmed effective intravascular volume expansion with ^{99}Tc-RBC during a prolonged period of time. Moreover, the HF patients in former volume expansion studies were not on optimal medical therapy as described by current guidelines, which also can influence cardiovascular and neurohumoral adaptation to an intravascular volume increase.

During significant intravascular volume expansion of several hours in HFrEF patients, there was only a limited - albeit significant - increase in cardiac filling pressures. It has been postulated that alterations in intravascular volume can rapidly (within minutes to hours) lead to acute decompensation in HF patients (85, 221). None of the 40 HFrEF patients in this cohort developed overt signs or symptoms of congestion in response to an acute increase of almost 1 liter in intravascular volume. Cardiac filling pressures were highest directly after fast infusion but decreased and remained stable afterwards during 3 hours while intravascular volume expansion was sustained. Hence, our data are indicative of volume reserve (or capacitance reserve) of the cardiovascular compartment in stable chronic HFrEF patients. Presumably, intravascular excess volume was efficiently redistributed with only a minimal increase in intra-cardiac volume. Indeed, the compliance of the vascular space is probably log orders higher than the cardiac chambers due to its expandable nature, particularly in the pulmonary and splanchnic venous space, and spleen. Additionally, the ability of the lymphatic system to increase flow can rapidly increase (222). Moreover, almost half of the patients demonstrated even no change or a decrease in RAP following intravascular volume expansion. Interestingly, these patients had the lowest intravascular volume at baseline and highest suppression of PRA probably indicating the highest volume reserve capacity.

The relationship between a change in volume and the resultant change in pressure is dedicated by the compliance of the reservoir. Therefore, to differentiate a pathologic response from normal, the immediate (after fast infusion) increase in PAWP in response to intravascular volume expansion in our cohort of HFrEF subjects was compared with data of normal subjects in a similar experiment from Fujimoto (219) (Figure 3). In that study, healthy subjects ≥50 years of age (n=34) were given an intravascular volume expansion (0.97±0.20 L saline at 100-200 ml/min during 5-10 minutes), and pressures were observed immediately after fast infusion. The slope of increase in PAWP after the fast infusion in healthy subjects (grouped slope = 14) was comparable to the gradual slope of PAWP

increase immediate after the fast intravascular expansion in our experiment in HFrEF patients (grouped slope =13). Moreover, the slope after 1 hour (HFrEF delayed) was almost halved (grouped slope=7) further supporting the hypothesis of an efficient cardiovascular volume reserve.

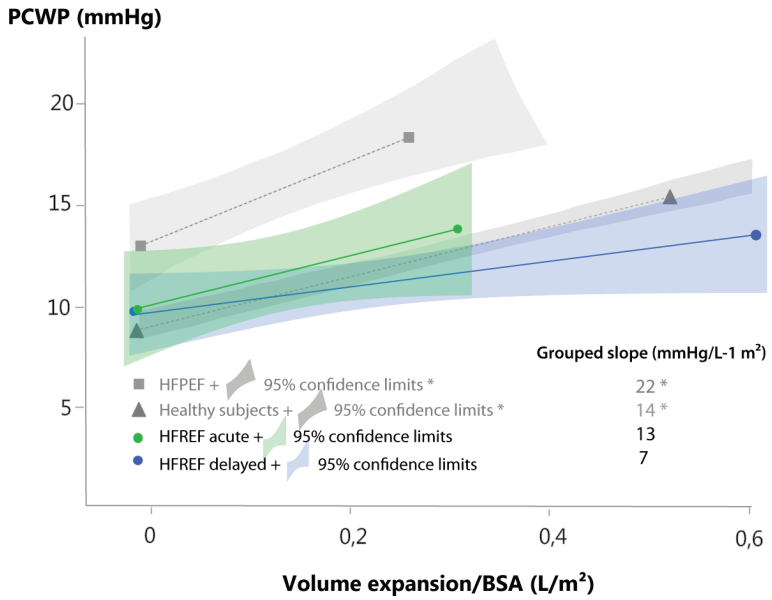

Figure 3. Pulmonary arterial wedge pressure (PAWP) relative to volume expansion in patients with heart failure with reduced ejection fraction (n=10) compared to healthy controls (n=34) and heart failure patients with preserved ejection fraction (n=11) (* Adopted from Fujimoto et al. Circulation 2013 (219)).

Obviously, the increase in intra-cardiac pressures associated with decompensation is considerably higher than observed after intra-vascular volume expansion with almost 1 liter in our cohort of HFrEF patients [25,]. Moreover, the average increase in body weight is less than 1 kg at the moment of decompensation (11). These observations indicate that a significant decline of cardiovascular volume reserve has to be present before increases in intravascular volume up to almost 1 liter can be responsible for decompensation. Animal studies have elegantly showed that marked intravascular volume expansion does not lead to increased cardiac filling pressures if sympathetic activity is low (85, 223) (224). The observed significant and rapid downregulation of RAAS hormones – which influences vascular tone as well as the natriuretic response to intravascular volume expansion – in our study is in agreement with this (9, 225, 226). Moreover, HF patients with an impaired

cardiovascular compliance such as HF patients with preserved ejection fraction (HFpEF) exhibit a much steeper PAWP/volume slope, and develop more rapidly decompensation compared to HFrEF patients. Currently, the most frequent used strategy to reduce increases in intra-cardiac pressures is the administration of diuretics to lower intravascular volume (227). However, treatment strategies which increase cardiovascular volume reserve are likely to be more successful to obviate episodes of decompensation.

Finally, Pressure-based parameters were not able to reliably detect intravascular volume expansion of 0.7 L. This finding has been reported before (228). The relationship between a change in volume and the resultant change in pressure is dedicated by the compliance of the reservoir. Ventricular end-diastolic volume and intravascular volume are often erroneously substituted when estimating intravascular volume status. Due to the cardiovascular volume reserve among other things, compliance characteristics of the vasculature bear only a limited relationship to the compliance of the left ventricle.

STUDY LIMITATIONS

The current study has several limitations. First, this was a small, observational, single-center study indicating that results should be considered hypothesis-generating. PAC measurements were only available in 10 patients. Second, the increase in intravascular volume with 0.7 L might be considered clinically not relevant in HF patients. However, multiple studies argue against this. A study investigating the pattern of weight change preceding hospitalization for HF, showed that the average increase in body weight was approximately 1 kilogram which resulted in an odds ratio of 2.77(1.13-6.80) for heart failure hospitalization (11). Additionally, the change in blood volume from admission to hospital discharge in hypervolemic patients post-diuretic therapy was only reduced with -0.7±1.1 L, while filling pressures clearly decreased (17). Moreover, it has been hypothesized that one of the mechanisms leading to acute decompensated HF is a redistribution of blood from the splanchnic capacitance veins into the effective circulating volume of up to 0.8 L (85, 229). Therefore, an increase of intravascular volume of 0.7L *is considered* sufficient to induce decompensation in HFrEF patients. Third, we studied the relationship between intravascular volume and pressure at rest to preclude changes in cardiovascular compliance as much as possible. Intra-cardiac pressures differences might have been more profound during daily activities. It has been shown that daily activity, exercise and change in body position can cause large changes in intra-cardiac pressure, however it has also been demonstrated that these do not lead to decompensation (52).

CONCLUSION

Euvolemic stable patients with HFrEF have a cardiovascular volume reserve and can efficiently handle intravascular volume expansion of 0.7L without signs or symptoms of congestion. Pressure-based assessment of intravascular volume might not be able to detect intravascular volume changes in such patients.

	baseline	Fast infusion	p	+1 hour	p	+2 hour	p	+3 hour	p
Volume									
Blood volume (L)	4.7±0.7	5.2±0.7	<0.001	5.2±0.6	<0.001	5.4±0.7	0.001	5.3±0.5	0.001
Plasma volume(L)	3.1±0.5	3.6±0.4	<0.001	3.5±0.4	<0.001	3.6±0.4	0.001	3.6±0.4	0.001
Pressure									
PAWP (mmHg)	10.4±3.3	14.6±5.5	0.008	12.6±5.8	0.129	14.0±4.2	0.011	13.6±4.4	0.039
RAP (mmHg)	4.3±2.4	8.1±3.6*	0.014	5.15±2.6	0.364	6.1±2.3	0.085	6.7±2.5	0.055
PAPm (mmHg)	20.7±6.8	26.9±8.0	0.006	23.0±7.4	0.135	24.2±6.9	0.006	25.7±5.8	0.005

Table 1: Intravascular volume and invasive hemodynamic parameters at each time point in HFrEF patients with PAC. *PAWP: pulmonary arterial wedge pressure, PAPm: Mean pulmonary arterial pressure, RAP: right atrial pressure. P between value and baseline*

	HFrEF patients with RAP increase n=6	HFrEF patients without RAP increase n=4	p-value
Volume			
Blood volume (L)	5.1±0.6	4.2±0.3	0.028
Plasma volume(L)	3.4±0.5	2.8±0.2	0.086
Volume expansion at +1 hour	+0.5±0.2	+0.5±0.2	0.624
Increase in body weight	+0.54±0.51	0.50±0.14	0.354
Urine output at +1 hour (L)	0.19±0.13	0.14±0.09	0.286
Pressure baseline			
PAWP (L)	10.5±3.4	10.3±2.6	0.830
RAP (L)	4.5±2.3	4.0±2.8	1.000
PAPm (mmHg)	21.0±6.7	20.3±7.9	0.831
Pressure increase at +1 hour			
Change PAWP (L)	+5.0±3.5	+0.3±1.7	0.054
Change RAP (L)	+2.9±1.7	-0.8±1.0	0.010
Change PAPm (mmHg)	+4.3±2.0	-0.3±2.4	0.030
Laboratory values			
PRA baseline	18.2(11.2;34.4)	3.6(1.5;12.8)	0.245
Change PRA at +1 hour (%)	+46(-119;90)	-405(-1223;81)	0.033

Table 2: intravascular volume and invasive hemodynamic parameters at baseline and +1 hour in HFrEF patients with versus without rise in RAP 1 hour after volume expansion. *PAWP: pulmonary arterial wedge pressure, PAPm: Mean pulmonary arterial pressure, PRA: plasma renin activity; RAP: right atrial pressure.*

CHAPTER 6

Renal response to intravascular volume expansion in euvolemic heart failure patients with reduced ejection fraction: mechanistic insights and clinical implications

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ABSTRACT

Background: Untreated and preclinical heart failure patients with reduced ejection fraction (HFrEF) have an impaired ability to alleviate excess intravascular volume.

Objectives: To investigate 1) the renal response to intravascular volume expansion in euvolemic and optimally treated HFrEF patients and 2) loop diuretic efficiency.

Methods: 14 healthy and 28 HFrEF patients underwent intravascular volume expansion with 1 liter hydroxyl ethyl starch 6% during 3 hours after which a loop diuretic was administered. Clinical parameters, neurohormones and urine were hourly measured.

Results: In response to intravascular volume expansion (+0.6±0.2 L; $p<0.05$) HFrEF patients demonstrated significantly lower natriuresis compared to healthy subjects (0.9±0.5 versus 1.7±0.6 g/3hours; $p<0.05$). However, natriuresis varied substantially with half of HFrEF patients exhibiting a response within the range of healthy and the other half demonstrating a significantly decreased response (1.4±0.4 vs 0.5±0.2 g/3hours; $p<0.05$). Natriuresis was associated with glomerular filtration function (eGFR), NT-proBNP and tubular fractional sodium excretion (FE_{Na}). Loop diuretic efficiency was significantly lower in HFrEF patients compared to healthy subjects (3.4±0.7 vs 2.6±1.1 g/2hours; $p<0.05$) but was only related to eGFR ($R^2= 0.47$; $p<0.05$) and independent of FE_{Na} ($R^2=0.05$; $p=0.20$). Loop diuretics increased FE_{Na} similarly in healthy subjects and HFrEF patients (9.1±2.4 vs 9.3±3.3 %; $p=0.64$).

Conclusion: The ability of the kidneys to remove excess intravascular volume is decreased in a substantial amount of euvolemic and optimally treated HFrEF patients. Renal response relates to filtration function and tubular sodium handling. In contrast, loop diuretics can surmount decreased renal tubular sodium excretion but remain dependent on eGFR.

INTRODUCTION

Signs and symptoms of volume overload are the most important reasons for hospitalization in heart failure (HF), contributing to high morbidity and mortality burden (6, 91, 230). The kidneys in HF are frequently not able to adjust sodium and water excretion to intake due to upregulated neurohumoral systems, a deficient natriuretic peptide system as well as underlying kidney dysfunction, leading gradually to a positive sodium and fluid balance (9). Furthermore, diuretic efficiency in the context of acute decompensated HF is also often impaired due to similar and other mechanisms such as reduced filtration function, renal blood flow (both influencing pharmacokinetic properties of loop diuretics), distal tubular structural remodeling, renal neurohumoral activation, etc (231).

Previous studies have demonstrated that HF is characterized by an impaired natriuretic and diuretic response to intravascular volume expansion in untreated and preclinical HF patients (232, 233). However, short term therapy with angiotensin converting enzyme (ACE)-inhibitors and subcutaneous exogenous natriuretic peptides are able to (partially) restore the renal response to volume expansion in HF patients (232, 233). Currently, there is limited knowledge regarding volume homeostasis in optimally treated euvolemic HFrEF patients. Identification of chronic HFREF patients with an impaired renal response to intravascular volume overload may allow to predict which patients are at the highest risk for decompensation. The present study was designed to evaluate the ability of the kidneys to alleviate intravascular volume expansion in optimally treated and euvolemic HFrEF patients, and to assess loop diuretic efficiency in this context. We hypothesize that the renal response to intravascular volume expansion is preserved in only a subset of patients.

METHODS

This study was carried out in a single tertiary care center (Ziekenhuis Oost-Limburg, Genk, Belgium) between September 2014 and September 2015. The study complies with the Declaration of Helsinki and the institutional review board approved the study protocol. Written informed consent was obtained from every patient before any study-specific action was performed

Study population

Patients were eligible for study inclusion if ≥18 years of age and able to give informed consent. Healthy volunteers were recruited through general announcements and had 1) no history of cardiac or renal disease except for adequately treated hypertension with guideline

recommended therapy; 2) a normal clinical examination; and 3) normal systolic function on transthoracic echocardiography.

Patients with HFrEF had 1) a clinical diagnosis of HF with evidence of impaired left ventricular ejection fraction ≤40% diagnosed within 6 months before inclusion 2) resided in NYHA functional class I-II and were stable for at least 3 months 3) were on maximal tolerated and stable doses of neurohormonal blockers and diuretic therapy according to current guideline recommendations during ≥3 months (234, 235). Exclusion criteria were: 1) renal replacement therapy or severe renal dysfunction with an estimated glomerular filtration rate (eGFR) ≤15 ml/min/1.73m^2 determined by the Chronic Kidney Disease Epidemiology Collaboration equation; (160) 2) any clinical sign or symptom of volume overload (i.e., pulmonary rales, orthopnea, jugular venous distention or ≥1+ peripheral edema).

Study design

All patients followed a standardized protocol on the day of the study. Patients were admitted and took their usual dose of medication at 8 am except for their maintenance dose of loop diuretics. Afterwards, subjects were placed in the semi-supine position. Two venous catheters were placed in the forearm and blood volume was measured with a technetium (^{99}Tc)-labeled red blood cell technique according to guidelines of the international committee for standardization in Hematology (detailed description in supplemental material) (184) .

Baseline vital parameters (blood pressure, heart rate) and a venous blood sample were obtained after a 60-minute equilibration period and all subjects were instructed to void empty. After the baseline measurements, 0,5 L isotonic hydroxyl ethyl starch (HES) 6% was infused over a 10 minutes time interval followed by an infusion of 0,5 L over a period of 3 hours. 1 liter HES 6% contains 137.0 mmol sodium or 3.15 gram sodium (conversion factor 0.02299). Start of infusion was appointed as time point zero and every hour afterwards appointed as +1 hour, +2 hours etc. At + 3 hours, 1 mg bumetanide was intravenously administered as bolus infusion in all subjects. Clinical assessment (dyspnea score, jugular vein distention, peripheral edema score, pulmonary rales), a venous blood sample and urine output were hourly collected up till +5 hours.

Renal response

The natriuretic response was defined as cumulative sodium excretion over 3 hours (gram/3 hours) during intravascular volume expansion. Loop diuretic efficiency was defined as cumulative natriuresis the first 2 hours after administration of an IV bolus of bumetanide 1

mg (gram/2 hours). It has been shown that cumulative natriuresis during 2 hours after IV administration of bumetanide ($t_{1/2}$ of bumetanide IV = 1 hour, 2 hours would be 2 half-lives) is highly accurate for loop diuretic efficiency (236). The diuretic response to intravascular volume expansion (L/3 hours) or after the IV loop diuretic (L/2 hours) was similarly determined.

Laboratory measurements and urine sampling

Venous blood samples were obtained with the patient in the supine position. Plasma N-terminal of the prohormone of B-type natriuretic peptide (NT-proBNP) levels were measured by the Roche Diagnostics Assay (Roche, Rotkreuz, Switzerland). Plasma renin activity (PRA) was determined using the Gamma-coat*radio immunoassay (DiaSorin, Sallugia, Italy). Glomerular filtration function (Estimated glomerular filtration rate (eGFR)) was assessed by the Chronic Kidney Disease Epidemiology Collaboration Formula and represents renal filtration function (161). Fractional excretion of sodium (FE_{Na}) was used as a surrogate of renal tubular handling. FE_{Na} was calculated as $FE_{Na} = U_{Na} * V / P_{Na} * eGFR$ (U_{Na} = Urinary sodium concentration (mmol/L); V = urinary output (L); P_{Na} = plasma sodium concentration (mmol/L)) (183). Urinary bumetanide was measured using high-performance liquid chromatography (supplemental material) and used to calculated the total amount of excreted bumetanide after 2 hours (mg) and natriuresis per mg of excreted bumetanide (g/mg).

Statistical analysis

Statistical analyses were performed with commercially available software (SAS JMP Pro version 11.2 for Windows). Continuous variables are expressed as mean ± standard deviation if normally distributed, or otherwise as median [interquartile range]. Normality was assessed by the Shapiro-Wilk statistic. Categorical data were expressed as percentages and compared with the Pearson χ^2-test. Values of healthy subjects and HFrEF patients or between groups of HFrEF patients were compared with the Wilcoxon-Mann-Whitney test. Effect size was calculated and expressed as Cohen's d value. For comparison of repeated measures, the Wilcoxon signed-rank test was used. Correlations between variables of renal function and natriuretic response to intravascular volume expansion or loop diuretic efficiency were calculated using Pearson's coefficient or Spearman's ρ as appropriate. Statistical significance was always set at a 2-tailed probability level of <0.05.

RESULTS

Study population

Baseline characteristics are presented in Table 1. Healthy subjects (n=14) were on average 39±22 years old with normal systolic function (mean LVEF 59±7%), and eGFR (97.4±27.6 ml/min/1.73m^2). Baseline values of NT-proBNP and PRA were within the normal range.

HFrEF patients (mean LVEF 33±6; n=28) were on average 66±12 years old, predominantly male with a history of ischemic heart disease. NTproBNP levels were elevated (1174 [315;1690] ng/L) and eGFR (65.8±26.3 ml/min/1.73m^2) on average mildly impaired. Baseline PRA levels (7.6 [2.8;15.6] ng/ml/h) were elevated.

	Healthy subjects	**HFrEF subjects**	**p**
n	14	28	
Baseline characteristics			
Age (years)	39±22	66±12	<0.05*
Male gender (%)	36%	86%	<0.05*
Ischemic heart disease (%)	0	86%	<0.05*
BSA (m2)	1.9±0.2	1.9±0,2	0.83
Heart rate (bpm)	69±11	64±10	0.99
Mean arterial blood pressure (mmHg)	87±10	78.10	<0.05*
Left ventricular ejection fraction (%)	59±7	33±6	<0.05*
Laboratory values			
Hemoglobin (g/dL)	13.5±1.1	13.3±1.4	0.44
eGFR (mL/min/1.73m^2)	97.4±27.6	65.8±26.3	<0.05*
NT-proBNP (ng/L)	69[33;93]	1174[315;1690]	<0.05*
PRA (ng/mL/h)	0.8[0.6;2.0]	7.6[2.8;15.6]	<0.05*
Medication use			
Renin-angiotensin system inhibitor use (%)	29%	90%	<0.05*
Beta-adrenergic antagonist use (%)	14%	93%	<0.05*
MRA use(%)	7%	76%	<0.05*
Loop diuretic (%)	0%	45%	<0.05*
Blood volume measurement			
Blood volume/BSA (L/m^2)	2.6±0.4	2.5±0.3	0.96

Table 1. Baseline characteristics of Healthy subjects and Heart Failure patients with reduced ejection fraction. *BSA: body surface area; eGFR, estimated glomerular filtration rate according to the Chronic Kidney Disease Epidemiology Collaboration; FE_{Ne} :Fractional excretion of sodium, MRA: mineralocorticoid receptor antagonist; NT-proBNP, N-terminal of the prohormone of B-type natriuretic peptide, PRA: plasma renin activity*

Intravascular volume expansion

Blood volume measurement was performed in 4 of the 14 healthy subjects and in 18 of 28 HFrEF patients. A similar and sustained increase in intravascular volume was demonstrated in all subjects up till +3 hours (+0.5±0.2 L at +1h, +0.6±0.2 L at +2h and +0.6±0.2 L at +3h; all p compared to baseline <0.0001:).

Response to intravascular volume expansion in healthy subjects versus HFrEF patients

During 3 hours of volume expansion, there was no significant change in clinical status in healthy subjects or HFrEF patients.

Natriuretic and diuretic response to intravascular volume expansion were significantly lower in HFrEF patients versus healthy subjects, respectively 0.9±0.5 vs 1.7±0.6 g/3 hours ($p<0.05$) and 0.49±0.27 vs 0.94±0.31 L/3 hours ($p<0.05$) (Figure 1 and Table 2). Although baseline levels were significantly different, the absolute and relative change over 3 hours in PRA level and NT-proBNP levels in both groups were similar. eGFR increased in both groups during intravascular volume expansion (Table 1 Supplemental Material). FE_{Na} during intravascular volume expansion was non-significantly higher in healthy subjects compared to HFrEF patients (2.8±1.0 vs 2.4±1.4 % ; p=0.09) (Table 2).

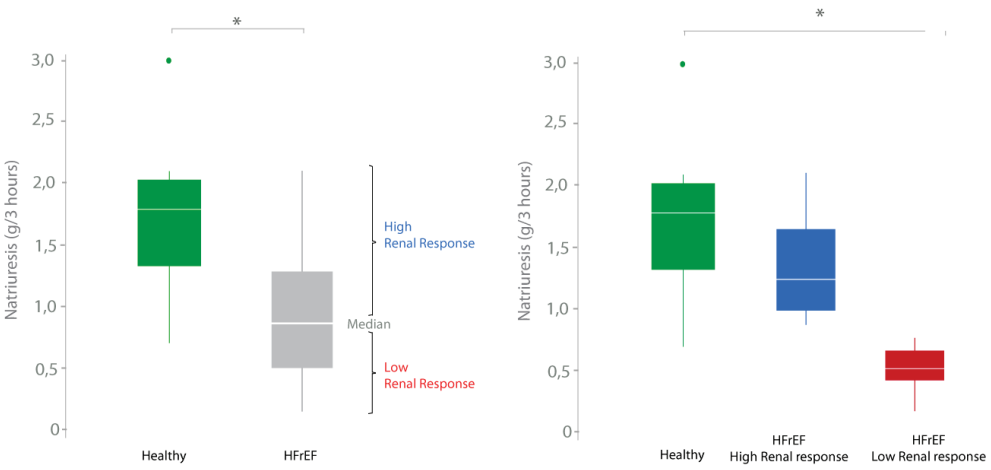

Figure 1. Natriuretic response to intravascular volume expansion in healthy subjects and HFREF patients (* = p<0.05)

	Healthy subjects	HFrEF patients	p
Intravascular volume expansion			
Natriuretic response (g/3 hours)	1.7±0.6	0.9±0.5	<0.05*
Cumulative Diuresis (L/3 hours)	0.94±0.31	0.49±0.27	<0.05*
FENa (%)	2.6±0.7	2.2±1.1	0.09
IV loop diuretics			
Loop diuretic efficiency (g/2 hours)	3.4±0.7	2.6±1.1	<0.05*
Cumulative diuresis (L/2 hours)	1,1±0.4	0.9±0.3	0.12
FENa (%)	9.1±2.4	9.3±3.3	0.64
Exreted loop diuretic (mg) (2 hours)	0.34±0.11	0.28±0.16	0.61
Natriuresis/excreted loop diuretic (g/mg)	9.9±2.8	11.0±6.5	0.89

Table 2. Renal response to intravascular volume expansion in Healthy subjects and HFrEF patients. *FE_{Ne} :Fractional excretion of sodium*

HFREF patients with high versus low natriuretic response to intravascular volume expansion

Notably, there was a large spread in natriuresis in HFrEF patients as well as in healthy subjects. The median natriuretic response in the group of HFREF patients was 0.75 g over 3 hours. HFrEF patients with a natriuretic response to intravascular volume above the median ("High natriuretic response") demonstrated a response within the range of healthy subjects (1.4±0.4 vs 1.7±0.6 g/3 hours; p=0.09). In contrast, HFrEF patients with a response below the median ("low natriuretic response") demonstrated a significantly decreased renal response to volume expansion (0.5±0.2 vs 1.7±0.6 g/3 hours ;p<0.05) (Figure 1).

Factors associated with natriuretic response to intravascular volume expansion in HFREF patients

HFREF patients with low natriuretic response had a significantly lower eGFR (53.6±28.3 vs 74.6±25.7 ml/min/1.73 m^2; p<0.05) and FE_{Na} during intravascular volume expansion (1.6±0.8 vs 3.2±1.5 %; p<0.05) compared to HFrEF patients with high natriuretic response (Figure 2). Whereas the product of eGFR and tubular sodium excretion was strongly correlated with natriuretic response (R^2=0.98; p<0.05), eGFR and FE_{Na} separately were both moderately correlated (R^2 respectively 0.33 and 0.41; both p<0.05) (Table 3).

There was no significant difference among both groups regarding, baseline PRA values, change in PRA values, change in NT-proBNP levels, maintenance therapy of neurohumoral blockers or loop diuretics. However, baseline NT-proBNP values were significantly higher in patients with a low compared to high natriuretic response to intravascular volume expansion (1690 [1029;3452] vs 500 [167;1174] ng/L ; $p<0.05$) (Figure 2, Table 3 and Table 2 of the supplemental material). Since plasma levels of NT-proBNP are influenced by eGFR, HFrEF patients were divided in 4 subgroups based on eGFR < or > 60 ml/min/1.73 m^2 and low or high natriuretic response. Still, HFrEF patients with a low natriuretic response had significantly higher baseline values of NT-proBNP compared to the other group in a similar eGFR-class, which corresponded to patients with a significantly lower tubular sodium excretion during intravascular volume expansion (Figure 3).

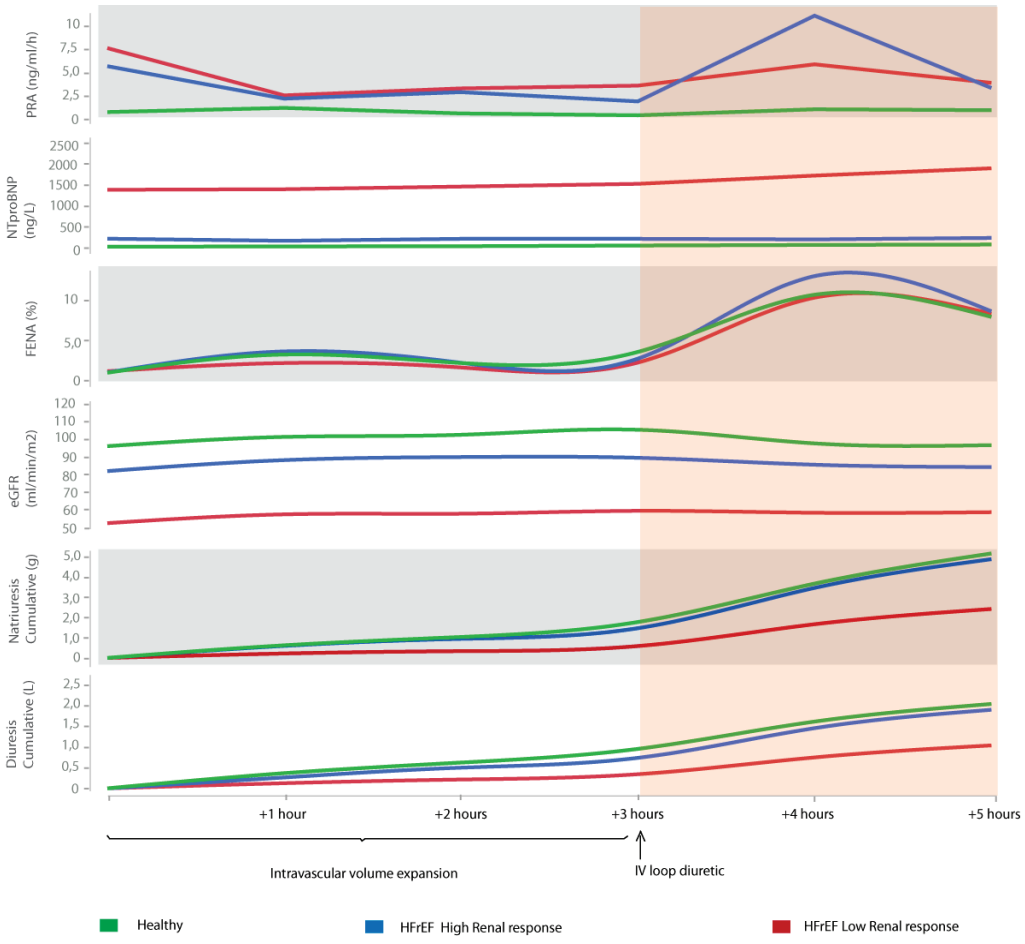

Figure 2. Evolution of clinical and biochemical markers during intravascular volume expansion and after IV loop diuretics in healthy subjects, HFrEF patients with high and low natriuretic response.

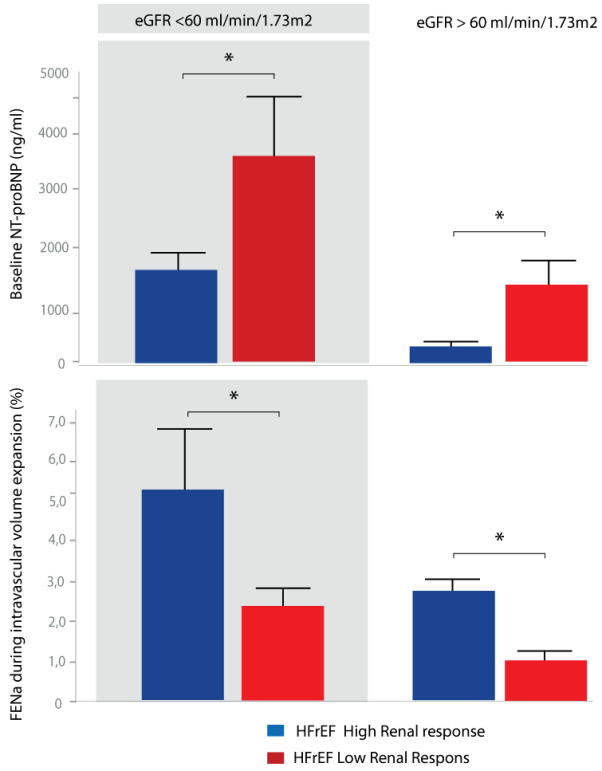

Figure 3. NT-proBNP values and FE_{Na} in patients with high versus low renal response stratified by eGFR.

	Natriuretic response to intravascular volume expansion		**Loop diuretic efficiency**	
	R^2	p	R^2	p
eGFR x FE_{Na}	0.98	<0.05*	0.97	<0.05*
eGFR	0.33	<0.05*	0.47	<0.05*
FE_{Na}	0.41	<0.05*	0.05	0.20

Table 3. Correlation between natriuretic response to intravascular volume expansion or loop diuretic efficiency and parameters of renal function in (glomular filtration function (eGFR) and tubular sodium excretion (FE_{Na})). *Baseline eGFR, estimated glomerular filtration rate according to the Chronic Kidney Disease Epidemiology Collaboration; FE_{Na}: Fractional excretion of sodium*

Loop diuretic efficiency

Loop diuretic efficiency was significantly higher in healthy subjects versus HFREF patients (3.4±0.7 vs 2.6±1.1 g/2 hours; p<0.05) and in HFREF patients with high versus low natriuretic response to intravascular volume expansion (3.1±1.3 vs 2.0±0.8 g/2 hours; p<0.05). Loop diuretic efficiency was moderately correlated with eGFR (R^2= 0.51; p<0.001) but not with FE_{Na} (R^2= 0.03; p=0.45). FE_{Na} after IV loop diuretics was comparable between healthy subjects, HFREF patients with high and low natriuretic response (respectively 9.1±2.4, 9.3±2.6 vs 9.0±3.9%; p=0.82). There was no significant relation between PRA levels, use of neurohumoral blockers or maintenance therapy with oral loop diuretics and loop diuretic efficiency in HFrEF patients (all p>0.05). The excreted amount of loop diuretic in urine after 2 hours and natriuresis per excreted mg of loop diuretic was not significantly different between healthy subjects and HFrEF patient or between HFrEF patients with high versus low renal response (Table 2 and Table 4).

	HFrEF natriuresis >median N=14	**HFrEF natriuresis <median N=14**	**p**	**d**
Baseline				
n	14	14		
Heart rate (bpm)	67±14	61±5	0.45	
Mean arterial blood pressure (mmHg)	77±9	80±11	0.71	
Left ventricular Ejection fraction(%)	37±10	34±12	0.58	
Laboratory values				
eGFR (mL/min/1.73m2)	74.6±25.7	53.6±28.3	<0.05*	0.70
Plasma renin activity (ng/ml/h)	7.0[1.4;18.5]	7.7[3.3;15.5]	0.61	
NT-proBNP (ng/L)	500[167;1174]	1690[1029;3452]	<0.05*	0.79
Maintenance therapy				
Renin angiotensin blocker use	100%	80%	0.19	
Beta blocker use	92%	94%	0.83	
MRA use	69%	86%	0.18	
Loop diuretic use	31%	50%	0.10	
Blood volume measurement				
Blood volume/BSA (L/m2)	2.4±0.3	2.5±0.2	0.20	
Intravascular volume expansion				
Cumulative Natriuresis (g/3 hours)	1.4±0.4	0.5±0.2	<0.05*	1.50
Cumulative Diuresis (L/3 hours)	0.71±0.23	0.33±0.16	<0.05*	1.12
FENa (%)	3.2±1.5	1.6±0.8	<0.05*	1.63

IV loop diuretics

Loop diuretic Efficiency (g/2 hours)	3.1±1.3	2.0±0.8	<0.05*	1.10
Cumulative Diuresis (L/2 hours)	1.17±0.23	0.71±0.30	<0.05*	1.31
FENa (%)	9.0±3.9	9.3±2.6	0.92	
Exreted loop diuretic (mg)	0.31±0.17	0.27±0.17	0.51	
Natriuresis/excreted loop diuretic (g/mg)	11.2±8.3	11.1±4.7	0.78	

Table 4. Baseline characteristics and renal response to intravascular volume expansion and loop diuretic administration in HFrEF patients with low versus high natriuretic response to intravascular volume expansion. *BSA: body surface area; eGFR, estimated glomerular filtration rate according to the Chronic Kidney Disease Epidemiology Collaboration; FE_{Na} :Fractional excretion of sodium, MRA: mineralocorticoid receptor antagonist; NT-proBNP, N-terminal of the prohormone of B-type natriuretic peptide, PRA: plasma renin activity*

DISCUSSION

Sodium and fluid avidity, leading to volume overload is thought to be central in the pathophysiology of HF. Therefore, the ability of the kidneys to adequately respond to increased fluid and sodium intake is of critical importance in euvolemic HF patients to prevent volume overload and disease progression. We conducted a mechanistic study to compare volume homeostasis between healthy subjects and clinically euvolemic and optimally treated HFrEF patients by assessing the renal response to intravascular volume expansion before and after loop diuretic administration.

Our main findings are that the natriuretic response to intravascular volume expansion is heterogeneous but significantly impaired in a substantial amount of HFrEF patients even though they appear euvolemic and optimally treated. The natriuretic response is associated with baseline eGFR as well as the capacity to increase tubular sodium excretion during intravascular volume expansion. Importantly, the administration of a loop diuretic is able to surmount the reduced renal tubular sodium excretion in euvolemic treated HFrEF patients but efficiency remains dependent on eGFR.

Normal volume homeostasis

An average Western diet contains up to 6 gram of sodium per day (99), which is almost entirely absorbed by the gastrointestinal tract (183). The body strictly controls plasma osmolality within narrow limits, therefore extracellular volume increases after a salty meal (79). The subsequent activation of volume receptors leads to a decrease in renin release within minutes to hours, and the release of natriuretic peptides from the myocardium,

stimulating natriuresis and diuresis (9, 183, 225). Thus, the body regulates extracellular volume by adjusting sodium output to intake. In HF, the kidneys are frequently not able to adjust sodium output to intake, causing a positive sodium and fluid balance and expansion of extracellular volume

This study is based on these physiologic concepts and intended to mimic a *prolonged expansion of the intravascular volume* with an iso-osmotic solution containing a physiologic concentration of sodium (137 mmol/L). The prolonged expansion of the intravascular volume (+0.6±0.2 L) was confirmed via radionucleotide technique. Importantly, this is in contrast with previous studies which used saline 0.9% infusions, which only provides a brief intravascular expansion due to rapid extravasation into the interstitial compartment (218, 233). Additionally, natriuresis was chosen as the principal determinant of the renal response as sodium is the principal determinant of volume homeostasis and loop diuretic function (237).

Treated HFrEF patients have a heterogeneous renal response to intravascular volume expansion

Similar to previous studies performed in patients with preclinical HF or HF patients without neurohumoral blockers, we observed an overall impaired natriuresis in response to intravascular volume expansion in HFrEF patients compared to healthy subjects (218, 233). However, the natriuretic response was heterogeneous: while some HFrEF patients had a severely decreased renal response, up to half of the HFrEF patients had a response within the range of healthy subjects.

Determinants of impaired renal response to volume expansion

Natriuresis depends on filtration of sodium (expressed by eGFR) and on tubular handling of the filtered sodium (expressed by FE_{Na}). As sodium is freely filtered by the glomerulus, eGFR is an important determinant of natriuresis (9). Whereas a person with an eGFR of 125 ml/min/1.73m^2 roughly filters 0.5 kg sodium per 24 hours, a person with an eGFR of 30 ml/min/1.73m^2 will still filter 150 mg sodium per day which fairly exceeds sodium intake. However, as demonstrated by the strength of correlation between natriuretic response and eGFR, tubular handling of filtered sodium is at least as important as filtration for eventual natriuresis. Therefore, patients with a normal eGFR can have a significantly impaired natriuretic response to intravascular volume expansion and vice versa.

Tubular sodium handling is multifactorial. In normal circumstances 99% of filtered sodium is reabsorbed in the renal tubuli. The largest part of sodium is reabsorbed in the proximal tubules driven by Starling forces. In contrast, more distal tubular sodium reabsorption depends substantially on the activity of the RAAS and natriuretic peptide system (9). Notably, after intravascular volume expansion PRA levels were significantly decreased in all groups indicating that the increase in intravascular volume was sensed and resulted in downregulation of RAAS in healthy subjects as well as in HFrEF patients regardless of low or high natriuretic response. But, no difference in RAAS activity could be demonstrated in HFrEF patients with different renal responses. Secondly, although no difference in relative change between groups was apparent, NT-proBNP levels were significantly higher in patients with a low natriuretic response. Elevated levels of natriuretic peptides may cause down-regulation of natriuretic peptide receptors in the kidney as well as upregulation of neutral endopeptidase which degrades the natriuretic peptides or phosphodiesterase resulting in deficient distal tubular sodium handling (238-240). Interestingly, in a group of preclinical HF patients, the natriuretic response to intravascular volume expansion could be partially restored by administering an ACE-inhibitor or by administration of exogenous BNP (232, 233). Nevertheless, our data demonstrate that even in stable euvolemic and well treated HFrEF patients (90% of HFrEF patients were on blockers of the RAAS , 93% on beta blockers and 76% on MRA), half of HFrEF patients remain to have an impaired natriuretic response to volume expansion.

Loop diuretic resistance

To further clarify contributing factors to the impaired tubular sodium handling, 1 mg bumetanide was intravenously administered after 3 hours of intravascular volume expansion. In the setting of volume overload in acute decompensated HF patients, loop diuretic efficiency has emerged as a strong indicator of prognosis and represents the interaction between cardiac function, renal function and volume status (231). Impaired diuretic efficiency in acute decompensated HF can be due to reduced filtration function, renal blood flow, distal tubular structural remodeling, or renal neurohumoral activation. As expected, the diuretic efficiency (=gram sodium excreted /mg bumetanide) remained impaired in HFrEF patients. Importantly, this seems mainly related to a decreased filtration of sodium in patients with reduced eGFR. Indeed, drug delivery in HFrEF patients - regardless of natriuretic response to volume expansion - was excellent as a similar amount of excreted urinary bumetanide compared to healthy subjects was found in the urine. Glomerular filtration of diuretics bound to albumin is only a minor pathway in which loop

diuretics are delivered to their tubular sight of action (231). Rather, active secretion by proximal tubular cells, a process which is dependent on renal blood flow is primarily responsible for tubular diuretic delivery. Furthermore, the potential for the loop diuretic to increase the FE_{Na} among normal versus HFrEF patients was similar as was the ratio of natriuresis over excreted bumetanide. Therefore, in contrast to congested and volume overloaded HF patients, optimal treated HF patients with an acute increase in intravascular volume can exhibit a *similar tubular response to loop diuretics* compared to normal subjects. Finally, there was also no significant relation between PRA levels or maintenance therapy and loop diuretic efficiency in our cohort of HFrEF patients.

Implications for clinical practice and future research

During persistent intravascular volume overload (as during decompensation), assumed that the observed natriuretic response can be maintained, HFrEF patients with a high natriuretic response can generate a cumulative sodium output of almost 12 gram sodium over 24 hours, while HFrEF patients with low natriuretic response can only generate 4 gram of sodium output over 24 hours. Therefore, while a positive sodium balance is unlikely in the former group, sodium (and fluid) accumulation is very probable in the latter group if consuming a normal western diet.

Our data suggest that stable euvolemic chronic HF patients are heterogenous regarding their ability to alleviate intravascular volume excess. Phenotyping these patients based on their normal or impaired renal response to intravascular volume overload may be helpful to the treating cardiologist to further guide therapy and give recommendations on salt and fluid restrictions. Surely, performing an intravascular volume expansion test to measure natriuretic response and FE_{Na} is cumbersome. Intuitively cardiologists take NT-proBNP and eGFR into account in the management of HFrEF patients. HFrEF patients - even though they are clincally euvolemic and under maximal neurohumoral blockade – with moderately elevated NT-proBNP levels depicted a group susceptible to sodium retetention.

Additionally, based on these mechanistic data, we speculate that HFrEF patients with an impaired natriuretic response to volume loading could have the largest benefit of new and emerging chronic heart failure therapies like sodium/glucose cotransporter 2 (SGLT-2) inhibitors and angiotensin-receptor/neprilysin inhibitors (ARNI). Both drugs target natriuresis by respectively inhibiting the glucose-sodium cotransporter in the proximal tubule or increasing levels of BNP, and have shown to decrease rehospitalization rates and mortality in HFrEF patients (241, 242). However, further insights on the effects of these

therapies on volume homeostasis and renal sodium handling in HF patients are still awaited for.

STUDY LIMITATIONS

The current study has several limitations. First, this was a small, observational, single-center study indicating that results should be considered hypothesis generating. Second, intravascular volume expansion was only sustained for three hours so if the renal response to sustained intravascular expansion is identical is unclear. Third, infusion of 1 liter HES 6% intravascular leads to dilution of most plasma components (creatinine, albumine etc.). Therefore, the change in plasma components should be interpreted accordingly. Since all patients underwent the same studyprotocol, the difference in evolution of laboratory parameters between groups was studied. Finally, the exact mechanism responsible for impaired fractional sodium excretion at the level of the tubuli could not be retrieved.

CONCLUSION

The ability of the kidneys to remove excess intravascular volume is heterogeneous and decreased in a substantial number of euvolemic and optimal treated HFrEF patients. The renal response relates to both renal filtration function and tubular sodium handling. Loop diuretic efficiency remains dependent on eGFR, but loop diuretics can surmount renal tubular impairment. Phenotyping chronic HFrEF patients based on their renal response could help guide therapy.

SUPPLEMENTAL MATERIAL

Urinary bumetanide measurement

Urinary bumetanide was measured using the Hitachi LaChrom Elite HPLC with a diode array detector. Chromotographic separation was achieved on the Purospher STAR column RP-18 endcapped 150x3mm, 3µm particles at the flow rate of 0.5 mL/min. The mobile phase consisted of acetonitrile and water, containing 0.145% TEA and 0.65% H3PO4, pH 3.3. The retention time was 13.6 minutes and the total run time was 19 minutes. The detector was set at 274nm. A stock solution of bumetanide was prepared in methanol at a concentration of 1mg/ml. A calibration curve was constructed for each assay by adding known amounts of bumetanide to drug-free urine. A concentration range from 50 to 5000 ng/ml was used. All the standards were run in duplicate.

Blood volume measurement

Total blood volume was measured with a technetium (^{99}Tc)-labeled red blood cell technique, according to guidelines of the international committee for standardization in Hematology (184). Methods are described in detail in a previous publication (243). In brief, blood was taken from the patient and labeled with ^{99}Tc in the nuclear lab. The resulting labeled blood was reinjected into the patient. Afterwards, 5 mL of blood was collected at 10-min intervals for 30 min. Radioactivity was measured in an automated counter (Veenstra/COMECER, Joure – The Netherlands). Baseline blood volume was calculated as the zero-time volume of distribution of the radio-labeled red blood cells. Blood volume at each timepoint was calculated based on baseline blood volume and hematocrit change.

	Healthy subjects	**HFrEF subjects**	**p**
Change in vital parameters			
Change MAP (mmHg)*	+1[-5;3]	-3[-12;2]	0.27
Change HR (bpm)*	+4[1;9]	+3[0;8]	0.50
Change in laboratory parameters			
Change eGFR (ml/min/1.73m2)*	+7±5	±7±5	0.69
Change PRA (ng/ml/h)*	-0.3[-1.0;0.0]	-1.3[-5.8;0.2]	0.27
Change PRA (%)*	-32[-60;-7]	-48[-55;-1]	0.85
Change NTproBNP (ng/L)*	+8[0;12]	+21[-11;95]	0.28
Change NTproBNP (%)*	+8[0;21]	+3[-2;11]	0.40

Table 1 supplemental material: Change in clinical and laboratory values during intravascular volume expansion in heathy subjects and HFREF patients. *eGFR, estimated glomerular filtration rate according to the Chronic Kidney Disease Epidemiology Collaboration; HR: heart rate; MAP: mean arterial pressure; NT-proBNP, N-terminal of the prohormone of B-type natriuretic peptide, PRA: plasma renin activity , * the average/median change from baseline over 3 hours.*

	HFrEF natriuresis > median N=14	HFrEF natriuresis < median N=14	p
Intravascular volume expansion			
Change in vital parameters			
Change MAP (mmHg) *	0 [-9;3]	-7[-14;-2]	0.06
Change HR (bpm) *	+5[+2;8]	+2[-2;7]	0.20
Change in laboratory parameters			
Change eGFR (ml/min/1.73m2)*	+7.3±5.3	+6.4±4.1	0.38
Change PRA (ng/ml/h)*	-0.1[-5.3;0.7]	-1.3[-5.8;0.9]	0.55
Change PRA (%)*	-44[-52;6]	-43[-68;-3]	0.58
Change NTproBNP (ng/L)*	+14[-5;68]	+32[-68;109]	0.96
Change NTproBNP (%)*	+3[-1;10]	+5[-6;16]	0.83

Table 2 Supplemental Material: Change in clinical and laboratory values during intravascular volume expansion in HFrEF patients with high vs low natriuretic response to intravascular volume expansion *. eGFR, estimated glomerular filtration rate according to the Chronic Kidney Disease Epidemiology Collaboration; HR: heart rate; MAP: mean arterial pressure; NT-proBNP, N-terminal of the prohormone of B-type natriuretic peptide, PRA: plasma renin activity , * the average/median change from baseline over 3 hours.*

CHAPTER 7

Intrarenal venous flow alterations during transition from euvolemia to intravascular volume expansion in heart failure patients with reduced ejection fraction

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ABSTRACT

Background: Intrarenal blood flow alterations may help to better understand impaired volume handling in heart failure (HF).

Objectives: To study 1) intrarenal flow patterns in chronic HF patients with reduced ejection fraction (HFrEF) during the transition from euvolemia to intravascular volume overload, and 2) the relationship between intrarenal flow patterns and diuretic efficiency.

Methods: Arterial resistive index (RI), venous impedance index (VII) and venous flow pattern (continuous vs discontinuous) were assessed in 40 euvolemic HFrEF patients by intrarenal Doppler ultrasonography at baseline, during 3 hours of intravascular volume expansion with 1-liter hydroxyl ethyl starch 6%, and 1 hour after the administration of a loop diuretic. Clinical parameters, echocardiography, and laboratory values were assessed. Cumulative urine output was collected after 3 and 24 hours.

Results: In response to intravascular volume expansion VII increased significantly (0.2±0.3 to 0.7±0.2; $p<0.001$). This was reversed after IV diuretic administration. In contrast, RI changed non-significantly after expansion (0.6±0.1 to 0.7±0.1; $p=0.131$). There was a non-significant change in echocardiographic estimates of central venous pressure. Compared to higher VII, patients with lower VII following volume expansion generated a greater spontaneous and loop diuretic-induced amount of diuresis (0.6±0.2 vs 0.4±0.3 L/3 hours; $p=0.025$ and 2.5±0.4 vs 1.8±0.5 L/24 hours; $p=0.001$).

Conclusion: In HFrEF patients, intravascular volume expansion results in significant blunting of venous flow, before a significant change in cardiac filling pressures could be demonstrated. The observed impaired renal venous flow is correlated with less diuretic efficiency. Intrarenal venous flow patterns may be of interest to evaluate renal congestion.

INTRODUCTION

Congestive heart failure is characterized by signs and symptoms of volume overload, contributing to a high morbidity and mortality burden. There is increasing recognition that the capacity of the kidneys to compensate for fluid overload relates not only to the underlying intrinsic renal function but also to renal blood flow in part influenced by increased venous pressure (178, 244). Currently, we have very limited insight at the bedside to distinguish these major factors influencing diuretic efficiency.

Renal vascular ultrasound has been used to assess the degree of severity of renal artery stenosis or to assess vascular and endothelial dysfunction. The intrarenal resistance index (RI) is calculated as the maximum flow velocity minus diastolic flow velocity, divided by maximum flow velocity of the Pulsed Doppler waveform of the interlobar renal artery (245) (figure 1). RI reflects renal arterial flow, and changes in response to alterations in arterial resistance and capacitance. Whereas a normal RI is approximately 0.6, an RI >0.7 is seen in renal artery stenosis, hypotension, renal vein thrombosis, arterial stiffness, etc (246). In contrast, renal venous flow is normally continuous, and the venous impedance index (VII) low. VII is calculate as the peak maximum flow velocity minus maximum flow velocity at nadir, divided by peak maximum flow velocity of the Pulsed Doppler waveform of the interlobar renal veins (245) (Figure 1). The VII changes in response to alterations in venous compliance, which are determined by central venous pressure and/or renal interstitial pressure (247, 248). Interestingly, recent reports have demonstrated that renal flow indices strongly correlate with clinical outcomes in HF independent of eGFR, and other conventional prognostic factors of HF (249, 250). Whether such alterations are directly associated with volume expansion or removal has not been demonstrated.

Therefore, the objectives of this study are to 1) study intrarenal flow patterns in HFrEF patients during the transition from euvolemia to intravascular volume overload and 2) investigate the relationship between intrarenal flow patterns and the ability to alleviate intravascular volume excess.

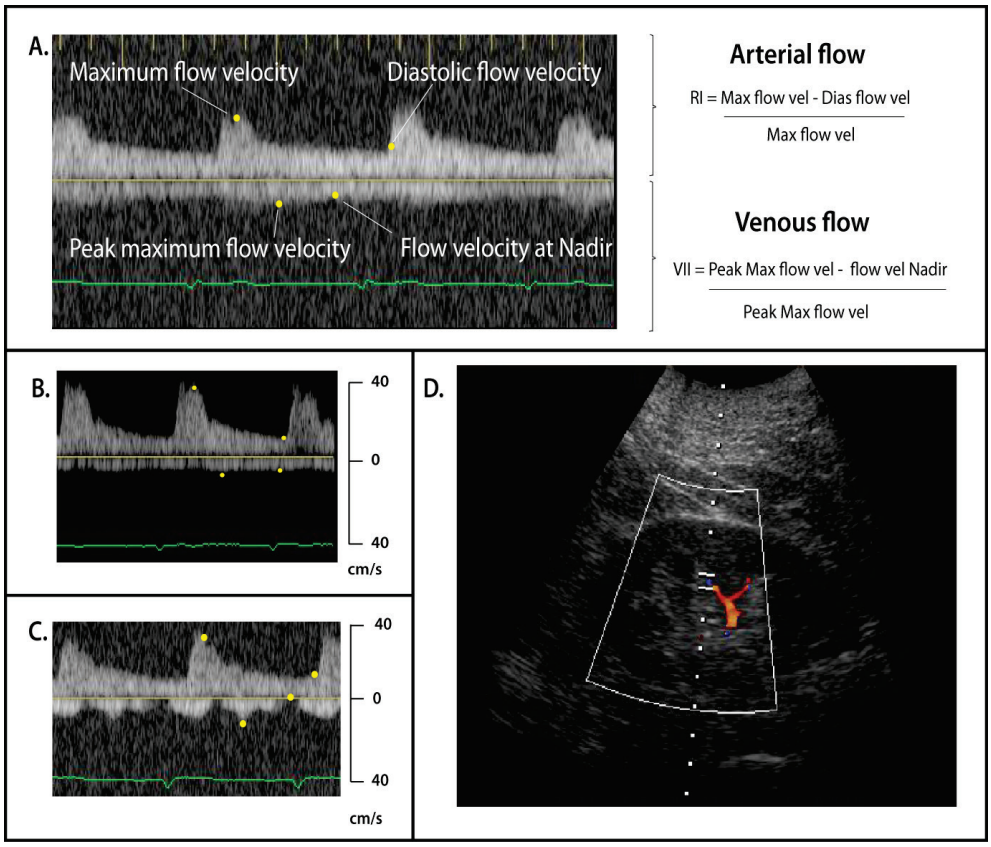

Figure 1. Intrarenal doppler ultrasonography *A. Pulsed wave Doppler sample in the interlobar renal vessel. Intrarenal arterial flow (upward . Doppler signal) and venous flow (downward Doppler signal); B. Continuous venous flow; RI=0.7 and VII=0.1; C. Discontinuous venous flow , RI=0.6 and VII=1.0; D. Collor Doppler flow image of the right kidney.*

METHODS

This study was carried out in a single tertiary care center (Ziekenhuis Oost-Limburg, Genk, Belgium) between September 2014 and October 2015. The study complies with the Declaration of Helsinki and the institutional review board approved the study protocol. Written informed consent was obtained from every patient before any study-specific action was performed

Study population

Patients were eligible for study inclusion if ≥18 years of age and able to give informed consent. Patients with chronic HFrEF had 1) a clinical diagnosis of heart failure with evidence

of impaired left ventricular ejection fraction ≤40% diagnosed at least 6 months before inclusion 2) stable NYHA class ≤3 for at least 3 months 3) stable doses of medical therapy according to current guideline recommendations during ≥3 months. Exclusion criteria were: 1) renal replacement therapy or severe renal dysfunction with an estimated glomerular filtration rate ≤15 ml/min/1.73m^2 determined by the Chronic Kidney Disease Epidemiology Collaboration equation; any 2) clinical sign or symptom of volume overload (i.e., pulmonary rales, orthopnea, jugular venous distention or ≥1+ peripheral edema).

Study design

All patients followed a standardized protocol on the day of the study. Each patient took their usual dose of medication at 8 am except for their maintenance dose of loop diuretics. Subjects were placed in the semi-supine position and a venous catheter was placed in the forearm. After a 60-minute equilibration period, all subjects were instructed to empty their bladder and baseline vital parameters (blood pressure, heart rate, weight), transthoracic echocardiography, intrarenal Doppler ultrasonography and a venous blood sample were obtained. After baseline measurements, 0,5 liter of isotonic hydroxyl ethyl starch (HES) 6% was infused over 10 minutes followed by an infusion of 0,5 liters over a period of 3 hours in order to maintain a stable intravascular volume expansion of 3 hours. 1 liter HES 6% contains 137.0 mmol sodium or 3,15 gram sodium (conversion factor 0.02299). Start of infusion was appointed as time point zero and every hour afterwards appointed as +1 hour, +2 hours etc. At + 3 hours, 1 mg bumetanide was intravenously administered as bolus infusion in all subjects. Clinical assessment (dyspnea score, jugular vein distention, peripheral edema score, pulmonary rales), vital parameters, a venous blood sample and urine output were hourly collected up till +3 hours. Subsequently, urine output was collected up till +24 hours. Transthoracic echocardiography and intrarenal Doppler ultrasonography were obtained at baseline, +1 hour (during intravascular volume expansion) and +4 hours (1 hour after IV loop diuretic administration). Subjects were discharged from the hospital around +5 hours. In-hospital intake of oral fluid was 100 ml in all patients. After hospital discharge patients were instructed to maintain their usual low-salt diet and maximum intake of 1.5 liter over 24 hours.

Laboratory measurements and urine sampling

Plasma N-terminal of the prohormone of B-type natriuretic peptide (NT-proBNP) levels were measured by the Roche Diagnostics Assay (Roche, Rotkreuz, Switzerland). Estimated

glomerular filtration rate (eGFR), a measure of glomerular filtration function, was calculated using the Chronic Kidney Disease Epidemiology Collaboration Formula.

Transthoracic echocardiography

Two-dimensional echocardiographic exam was performed with the use of a commercially available system (Philips Healthcare, iE33w Androver, Massachusetts) at baseline and at +1 hour. Images were acquired in the left lateral decubitus position. All reported echocardiography measurements were averaged from 3 consecutive cycles and assessed as recommended by the American Society of Echocardiography. The modified Simpson's biplane method was used to calculate ejection fraction. Tricuspid annular plane systolic excursion (TAPSE) was measured in an apical four-chamber view by placing the 2D cursor at the tricuspid lateral annulus and measuring the distance of systolic annular right ventricular excursion along a longitudinal line. Ratio of E/E' was assessed in a standardized manner with the use of the transmitral pulsed-wave Doppler signal and tissue velocity of the lateral and septal side of the mitral annulus. Maximal vena cava inferior diameter (IVC) and collapsibility during respiration was assessed from a subcostal view. CVP was estimated based on the guidelines provided by the American Society of Echocardiography (198). A IVC diameter > 2.1 cm that collapses <50% with a sniff was defined as an elevated CVP corresponding with a CVP of 15 mmHg. A IVC dimeter ≤2.1 cm that collapses >50% with a sniff suggests a normal CVP pressure of 3 mm Hg. Indeterminate cases in which the IVC diameter and collapse do not fit this definition were given an intermediate value of 8 mmHg.

Intrarenal Doppler ultrasonograpy

Intrarenal Doppler ultrasonography was performed with the use of a commercially available system (Philips Healthcare, iE33w Androver, Massachusetts) with a sector transducer frequency range of 2.5 to 5 MHz at baseline, +1 hour and +4 hours. Doppler echography was recorded of the right kidney with the patient in the left semi-lateral decubitus position, or in case of unsatisfactory image quality the left kidney. The ECG signal was simultaneously recorded by the ultrasound system. Color Doppler images were used to determine interlobar vessels. Pulsed Doppler waveforms of the interlobar arteries and veins were recorded simultaneously. The renal resistive index (RI) at a lobar artery was calculated as the maximum flow velocity minus diastolic flow velocity, divided by maximum flow velocity (245) (Figure 1). The venous impedance index (VII) was calculated as the peak maximum flow velocity minus maximum flow velocity at nadir, divided by peak maximum flow velocity (251). In addition, Doppler waveforms were divided into 2 flow patterns: continuous and

discontinuous. Discontinuous flow was defined as a pattern in which velocity at the nadir was zero. If the nadir was zero, VII was set as 1.0, therefore VII ranged from 0 to 1. All measurements were averaged over 3 cardiac cycles during sinus rhythm.

Reproducibility

Two observes (P.N. and P.M.) independently assessed RI, VII and intrarenal flow patterns in 15 patients. To test intra-observer variability, a single observer analyzed the data twice on occasions separated by a 1-month interval. Reproducibility was assessed as the mean percentage of error (absolute difference divided by the mean of the 2 observations).

Statistical analysis

Statistical analyses were performed with commercially available software (SAS JMP Pro version 11.2 for Windows). Continuous variables are expressed as mean ± standard deviation in tables and as mean and 95% confidence intervals in figures if normally distributed, or otherwise as median [interquartile range]. Normality was assessed by the Shapiro-Wilk statistic. Categorical data were expressed as percentages and compared with the Pearson χ^2-test. Values between groups of HFrEF patients were compared with the Wilcoxon test. Effect size between HFrEF patients with VII above versus below the median was calculated and expressed as Cohen's d value. For comparison of repeated measures, the Wilcoxon signed-rank test was used. Correlations were calculated using Pearson's coefficient or Spearman's ρ as appropriate. Independence between renal function and VII was confirmed by multiple regression modelling. Statistical significance was always set at a 2-tailed probability level of <0.05.

RESULTS

Study population

Subjects with HFrEF (n=40; LVEF 36±10%,) were on average 65±12 years old and eGFR was on average mildly impaired (64±26 ml/min/1.73m^2). Baseline estimated CVP was normal (6±2 mmHg). Renal arterial RI was within de range of normal (0.6±0.1). VII was on average 0.2±0.3 with 68% of HFrEF patients demonstrating a continuous flow pattern. There was no significant correlation between VII and baseline glomerular filtration function (R^2= 0.1, p=0.089). VII and the presence of continuous vs discontinuous flow was not related to any other baseline characteristic including renal and cardiac function. Other baseline characteristics are presented in Table 1.

	HFrEF n=40
Age (years)	65±12
BSA (m2)	2.0±0.2
Male gender	88%
Ischemic CMP	83%
Mean arterial pressure (mmHg)	78±10
Heart rate (bpm)	63±10
Hypertension	50%
Diabetes	28%
Laboratory values	
Hemoglobin (g/dl)	13.5±1.3
Hematocrit (%)	40±4
eGFR (ml/min/1.73m2)	64±26
NTproBNP (ng/L)	670[225;1383]
Echocardiographic values	
Left ventricular ejection fraction (%)	36±10
TAPSE (mm)	19±5
E/E'	12±6
IVC maximum diameter (mm)	15±5
Estimated CVP	6±2
Intrarenal Doppler ultrasonography	
RI	0.6±0.1
VII	0.2±0.3
Continuous venous flow pattern	68%
Maintenance therapy	
Angiotensin converting enzyme-inhibitor/angiotensin receptor blocker-use	88%
Beta adrenergic antagonist use	95%
Mineralocorticoid receptor use	83%
Loop diuretic use	66%

Table 1. Baseline characteristics. *BSA: body surface area; CVP: central venous pressure; eGFR, estimated glomerular filtration rate according to the Chronic Kidney Disease Epidemiology Collaboration;, IVC: inferior vena cava; MAP: mean arterial pressure;; NT-proBNP, N-terminal of the prohormone of B-type natriuretic peptide, RI: resistive index; TAPSE: Tricuspid annular plane systolic excursion; VVI:venous impedance index*

Response to intravascular volume expansion in HFrEF patients

At +1 hour, the average infused amount of HES 6% was 0.6±0.1L. There was no significant change in clinical status in HFrEF patients in response to intravascular volume expansion. Based on echocardiography, no significant change in left ventricular ejection fraction, E/E', tricuspid annular plane systolic movement (TAPSE), estimated CVP or maximal IVC width from baseline was noticed (all p>0.05) (Table 1 supplemental material). RI changed non-significantly. VII increased significantly in HFrEF patients from 0.2±0.3 at baseline to 0.7±0.2

during intravascular volume expansion (p<0.001) (Figure 2). Spontaneous diuresis and natriuresis after 3 hours of volume expansion were respectively 0.5±0.3 L and 1.0±0.5 g (Table 2).

	HFrEF n=40	**P –value of change from baseline**
Intravascular volume expansion		
Intrarenal Doppler ultrasonography +1 hour		
RI	0.7±0.1	0.131
VII	0.7±0.2	<**0.001**
Continuous flow pattern	17%	
urinecollection		
Cumulative diuresis (L/3 hours)	0.5±0.3	
Cumulative natriuresis (g/3 hours)	1.0±0.5	
IV loop diuretic		
Intrarenal Doppler ultrasonography +4 hours		
RI	0.6±0.1	0.094
VII	0.4±0.2	0.750
Continuous flow pattern	70%	
Urinecollection at +24 h		
Cumulative diuresis (L/24 hours)	2.1±0.7	
Cumulative natriuris (g/24 hours)	4.9±1.9	

Table 2. Parameters during intravascular volume expansion and after IV diuretic in HFrEF patients

Loop Diuretic efficiency

At 3 hours, 1 mg of bumetanide was intravenously administered. At that point, the average infused amount of HES 6% was 0.9±0.1 liter. The average urine output from +3 tot +4 hours was 0.6±0.2 L. RI changed non-significantly. VII decreased significantly in HFrEF patients (from 0.7±0.2 to 0.4±0.2; p<0.001) but VII 1 hour after loop diuretic administration was not significantly different from baseline (p=0.750). After 24 hours, cumulative diuresis and natriuresis were respectively 2.1±0.7 L and 4.9±1.9 g. There was no significant change from baseline in echocardiographic parameters obtained 1 hour after loop diuretic administration (+4 hours) (supplemental material Table 2).

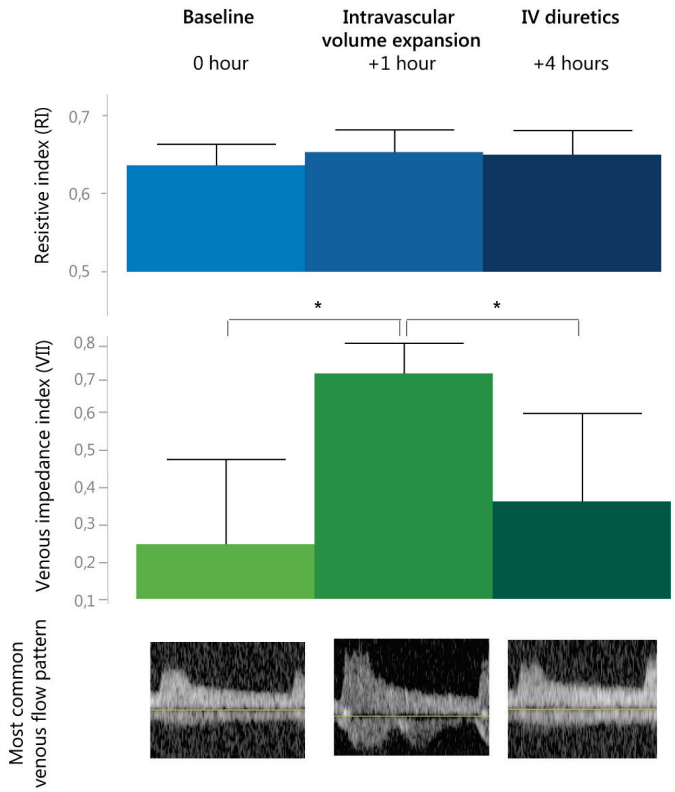

Figure 2. Change in arterial resistive index and venous impedance index at baseline, during intravascular volume expansion, and after IV diuretic in HFrEF patients.

HFrEF patients with high versus low VII during intravascular volume expansion

The median VII during intravascular volume expansion in HFrEF patients was 0.74. HFrEF patients were divided in 2 groups based on VII above (VII ≥0.74, "High VII") or below the median (VII<0.74 "low VII"). In HFrEF patients with high VII the prevalence of hypertension was significantly higher. Furthermore, these patients were characterized by baseline higher levels of NT-proBNP. There was no significant difference in renal function between groups (70±27 vs 59±26 ml/min/1.73m2; p=0.198) (Table 3 and Table 2 of the supplemental material). More than half of HFrEF patients with high VII during intravascular volume expansion had a discontinuous flow pattern at baseline compared to 1 out of 4 HFrEF patients with low VII (Table 3).

HFrEF patients with a low VII during intravascular volume expansion had a significantly better cumulative spontaneous natriuresis and diuresis after 3 hours compared to HFrEF

patients with high VII (1.2±0.4 vs 0.7±0.5 g/3 hours; p=0.002 and 0.6±0.2 vs 0.4±0.3; p=0.025). Futhermore, cumulative diuresis and natriuresis after 24 hours was significantly higher in patients with a VII below the median during intravascular volume expansion vs above the median (2.5±0.4 vs 1.8±0.5 L/24 hours, p=0.001 and 5.9±1.2 vs 4.1±1.4 g/24 hours; p=0.005) (Figure 3). VII remained significantly associated with cumulative diuresis at +3 and +24 hours after correction for eGFR (p=0.025 and p= 0.009, respectively).

	HFrEF with VII>median	**HFrEF with VII<median**	**p-value between groups**	**Cohen's d-value**
Baseline				
Intrarenal Doppler ultrasonography				
Baseline RI	0.7±0.1	0.6±0.1	0.065	
Baseline VII	0.5±0.3	0.3±0.2	**0.003**	0.7
Continuous venous flow pattern	48%	83%		
Intravascular volume expansion				
Intrarenal Doppler Ultrasonography				
RI	0.7±0.1	0.7±0.1	0.468	
VII	0.9±0.1	0.5±0.2	**<0.001**	2.0
Continuous venous flow pattern	0%	40%		
Urine output during first 3 hours				
Natriuresis (g/3 hours)	0.7±0.5	1.2±0.4	**0.002**	1.0
Diuresis (L/3 hours)	0.4±0.3	0.6±0.2	**0.025**	1.1
IV loop diuretics				
Intrarenal Doppler Ultrasonography				
RI	0.7±0.1	0.6±0.1	0.098	
VII	0.4±0.3	0.3±0.2	0.109	
Continuous venous flow pattern	60%	80%		
24 urine collection				
Cumulative diuresis (L/24 hours)	1.8±0.5	2.5±0.4	**0.001**	1.2
Cumulative natriuresis (g/24 hours)	4.1±1.4	5.9±1.2	**0.005**	1.1

Table 3. Characteristics of HFREF patients with VII < or > the median. *BSA: body surface area; CVP: central venous pressure; eGFR, estimated glomerular filtration rate according to the Chronic Kidney Disease Epidemiology Collaboration;, FE_{Na} :Fractional excretion of sodium; IVC: inferior vena cava; MAP: mean arterial pressure;; NT-proBNP, N-terminal of the prohormone of B-type natriuretic peptide, RI: resistive index; TAPSE: Tricuspid annular plane systolic excursion; VVI:venous impedance index*

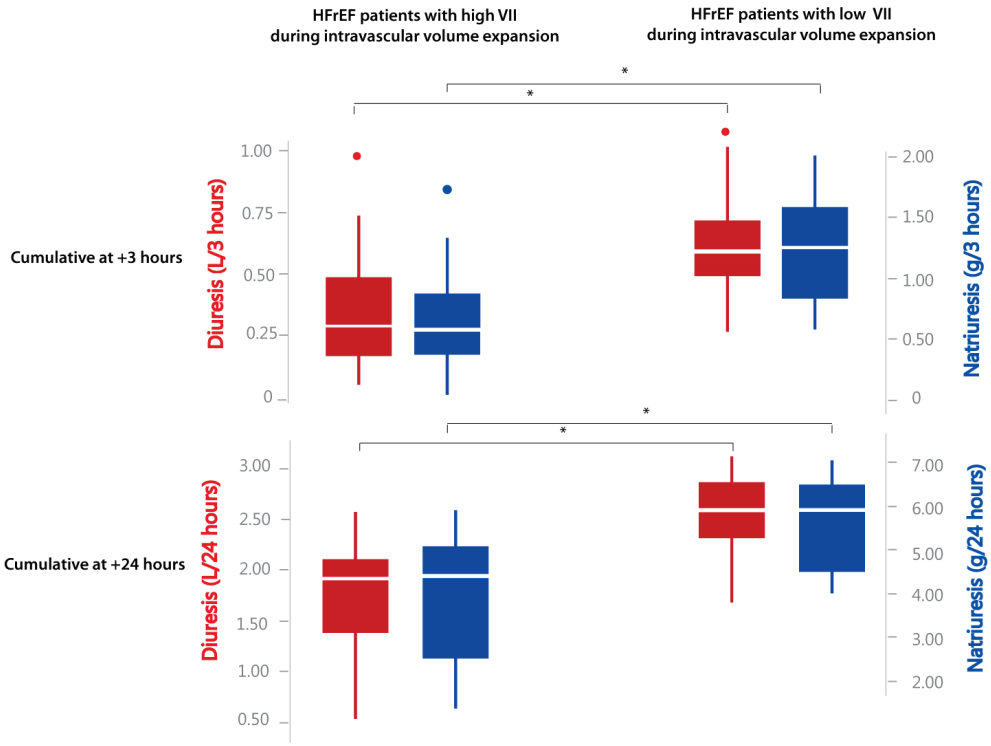

Figure 3. Diuresis (red) and natriuresis (blue) after +3 hours (upper panel) and after 24 hours (lower panel) in HFrEF patients with high versus low venous impedance index during intravascular volume expansion.

Reproducibility

Intra- and inter-observer variability of RI and VII measurements were as follows: RI: 0±9 and 3±12 %, respectively and VII 4±13 and 5±12%. Classifications of intrarenal flow patterns were consistent between intra- and interobserver assessments.

DISCUSSION

We conducted a study on renal arterial and venous flow index alterations in optimal treated HFrEF patients undergoing the transition from euvolemia to intravascular volume overload. Our main findings are that intravascular volume expansion with 0.6 L leads to a significant blunting of venous – not arterial - flow in chronic HFrEF patients, which can be reversed after the administration of IV diuretics. Additionally, blunting of renal venous flow in HFrEF patients during intravascular volume expansion was related to a lower diuretic response, independent of the underlying renal function.

Physiology of intrarenal flow and effects of increased central venous pressure

Under normal conditions, blood-flow in the renal arterial circulation is antegrade and maintained during diastole. When renal arterial vascular resistance increases or compliance reduces, a decrease in renal diastolic blood flow occurs, which is more pronounced than the decrease in the systolic component (252). This results in an increased RI. Importantly, the Doppler waveform is altered not by vascular resistance alone but by the interaction of vascular resistance and compliance (e.g. large arterial distensibility, pulse pressure, etc.). Therefore, aging, atherosclerosis, stiffening of the large arteries, obstructive uropathy, etc. will result in an increased RI (253). However, since resistance of veins is less variable, venous flow alterations are merely the consequence of changes in interstitial pressure or venous pressure leading to a decrease of vessel compliance (254, 255). Previously, an increased intrarenal VII has been associated with increased CVP in HF and tricuspid regurgitation (250).

Effective handling of salt and water by the nephrons requires an intact and intricate balance between both the inflow and outflow of the renal vasculature. Importantly, right atrial pressure (i..e. central venous pressure) is transmitted directly to the renal veins. Consequently, elevated CVP leads to renal capillary pressure increase, and decreased venous vessel compliance. Since the kidneys are encapsulated organs, renal congestion will be accompanied by increased renal interstitial pressure, and decreased interstitial compliance which can eventually reduce glomerular filtration pressure (255). This is extensively studied in the context of acute decompensated heart failure where elevated CVP is identified as one of the main drivers of worsening renal function (178, 244, 256).

Intravascular volume expansion leads to a change in intrarenal flow

Our mechanistic study allowed to directly assess intrarenal hemodynamic flow changes during intravascular volume expansion in euvolemic HFrEF patients. A significant increase in VII in HFrEF patients was observed after intravascular volume expansion with 0.6 L. Importantly, no significant change in echocardiographic estimates of central venous pressure could be demonstrated implicating that the changes in VII were already present before overt increase in venous pressure. The increased pulsatility or discontinuation of the venous flow signal were typically observed a few milliseconds after the p-wave and t-wave of the simultaneous recorded ECG. Therefore, they might reflect the impairment in renal venous flow when right atrial pressure is highest (257)(Figure 4). We hypothesize that small increases in the intra-atrial a- and v-wave (representing atrial and ventricular contraction)

together with a decreased venous compliance secondary to intravascular volume expansion will induce larger pressure waves in the inferior vena cava and intermittently blunt (decrease or interrupt) forward flow in the interlobar veins. Our data demonstrate that an increase of 0.6 Liter of intravascular volume already leads to renal venous congestion, with decreased compliance of renal veins and discontinuity of flow before a significant increase in intra-cardiac filling pressure estimates could be observed by echocardiography.

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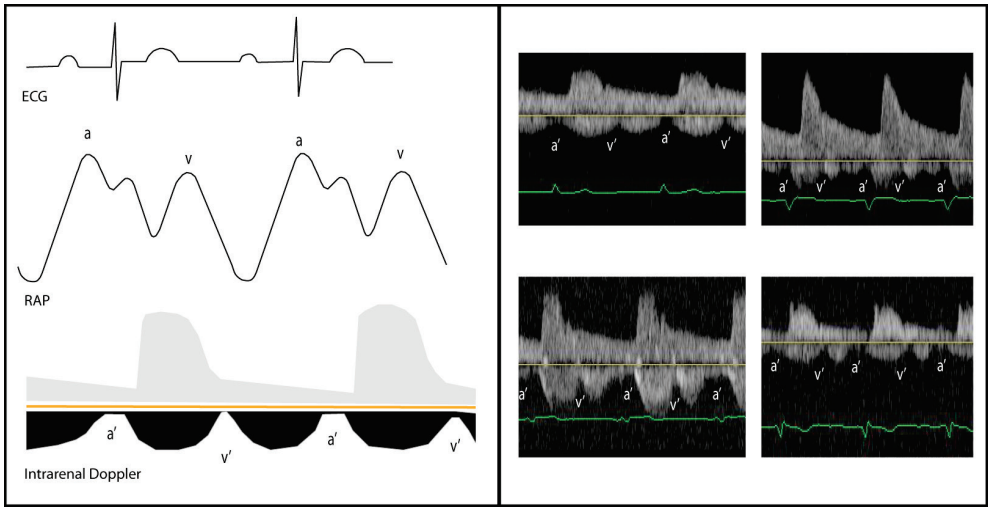

Figure 4. Increased pulsatility and discontinuation of venous flow in the intrarenal doppler signal reflects the right atrial pressure curve. *Left panel shows an ECG, right atrial pressure curve and intra renal Doppler signal. Right panel shows the intrarenal doppler signal of 4 different HFrEF patients with VII > median during intravascular volume expansion. a: atrial contraction; ECG: electrocardiogram,; RAP: right atrial pressure; v: ventricular contraction*

RI in response to intravascular volume expansion did not significantly change, which probably relates to higher intravascular hydrostatic pressure and different wall properties of the arterial vascular bed. Importantly, 1 hour after administration of an IV diuretic the increase in VII was reversed which is probably due to a combination of efficient intravascular volume redistribution and volume reduction secondary to diuresis. Therefore, VII – even more than RI - seems be a parameter which can rapidly reflect changes in intrarenal venous vessel compliance and/or renal venous congestion.

Higher VII relates to worse natriuresis and diuresis in response to intravascular volume expansion and loop diuretic administration

The group of HFrEF patients with the highest VII during intravascular volume expansion demonstrated a significantly lower diuretic and natriuretic response after 3 hours. Similarly, after IV diuretics, urine output and natriuresis from baseline up till +24 hours were significantly lower in this group. Importantly, this impaired natriuretic response was observed independent of the underlying renal function.

Venous congestion can influence natriuresis and diuresis in many ways. First, water and sodium filtration depends on differences in oncotic and hydrostatic pressure between the glomerulus and Bowman's space. Increased interstitial renal pressure as a result of venous congestion can reduce the hydrostatic driving force for filtration. Secondly, intratubular water and sodium are reabsorbed along the tubulus dependent on - again- Starling forces and neurohumoral activity. In the presence of renal venous congestion interstitial pressure rises and lymph flow massively increases washing out interstitial proteins, and decreasing interstitial colloid osmotic pressure (258, 259). These changes facilitate sodium and water reabsorption in the peritubular capillaries and diminish back flux into the lumen of the tubules, especially in the proximal tubules (9). Furthermore, there is longstanding evidence that renal venous congestion influences the sympathetic nervous system and renin-angiotensin-aldosterone system (260-262). Increased levels of intrarenal neurohormones stimulate reabsorption of sodium and water in the distal tubules (9). A negative correlation between sodium and water excretion and renal interstitial pressure was previously demonstrated (255). Intriguingly, we observed that even mild impairment of renal venous flow, was already correlated with significant impairment of diuretic and natriuretic capacity in our cohort of HFrEF patients. Though HFrEF patients with higher VII during intravascular volume expansion had higher baseline levels of NT-proBNP and higher values of E/E' during intravascular volume expansion (indicative of more ventricular wall stress), baseline renal function was non significantly different among groups. Indeed, VII remained significantly associated with cumulative diuresis at +3 and +24 hours after correction for eGFR. Therefore, it seems that intrarenal congestion may not only influence spontaneous diuretic capacity but also loop diuretic efficiency.

As intrarenal flow indices in HF reflect intrarenal compliance and not renal function per se, our data might explain why renal flow indices strongly correlate with clinical outcomes in HF, independent of eGFR and other conventional prognostic factors of HF(249, 250, 263).

Interestingly, a recent study showed that discontinuous intrarenal venous flow patterns – more than arterial RI - correlated strongly with clinical outcomes in HF patients and this correlation was even present in the lower CVP stratum. This further supports our findings that assessment of renal venous flow alterations is clinically relevant even before increases in CVP are detectable (85, 250).

STUDY LIMITATIONS

The current study has several limitations. First, this was a small, mechanistic, single-center study indicating that results should be considered hypothesis-generating. Our study was not able to determine how concomitant factors such as neurohormonal activity, eGFR or hypertension contributed to the intrarenal flow patterns. Despite these limitations, our data may warrant future studies to further clarify the role of intrarenal flow patterns in the pathophysiology of sodium avidity and the clinical value in HF.

CONCLUSION

In HFrEF patients, intravascular volume expansion leads to significant changes in renal venous - but not arterial - flow before a significant increase in cardiac filling pressures is present. Patients with higher VII have significantly lower natriuretic and diuretic response to intravascular volume expansion. Intrarenal flow patterns, which are readily clinically obtainable, may be of additional interest to the classic evaluation of HFrEF patients regarding interpretation of renal congestion.

SUPPLEMENTAL MATERIAL

	HFrEF n=40	P –value of change from baseline
Intravascular volume expansion		
Echocardiographic values +1 hour		
Left ventricular ejection fraction (%)	34±9	0.066
TAPSE (mm)	18±6	0.787
E/E'	13±7	0.067
IVC maximal diameter (mm)	16±5	0.261
Estimated CVP (mmHg)	7±2	0.216
IV loop diuretic		
Echocardiographic values +4 hours		
Left ventricular ejection fraction (%)	33±9	0.066
TAPSE (mm)	18±6	0.787
E/E'	13±6	0.110
IVC maximal diameter (mm)	16±5	0.357
Estimated CVP (mmHg)	6±2	0.788

Table 1: Echocardiographic values during intravascular volume expansion and after IV diuretics in HFrEF patients *.CVP: central venous pressure; elevated CVP: central venous pressure; IVC:inferior vena cava TAPSE: Tricuspid annular plane systolic excursion; VVI:venous impedance index*

	HFrEF with VVI>median	HFrEF with VVI<median	p	Cohen's d-value
Baseline				
Age (years)	68±12	62±12	0.146	
BSA (m2)	1.9±0.2	2.0±0.2	0.322	
Ischemic CMP	76%	89%	0.330	
MAP (mmHg)	74±10	82±12	0.067	
Heart rate (bpm)	66±13	63±9	0.563	
Hypertension	75%	15%	**0.020**	
Diabetes	40%	30%	0.751	
Laboratory values				
Hb (g/dl)	13.4±1.4	13.6±1.1	0.221	
Hct (%)	40±4	40±4	0.792	
eGFR (ml/min/1.73m2)	59±26	70±27	0.198	
NTproBNP (ng/L)	1290(606;2231)	450(122;1195)	**0.021**	0.7
Echocardiographic values				
LVEF (%)	35±11	38±10	0.418	
TAPSE	18±5	20±6	0.165	

E/E'	14±6	10±4	**0**.480	
Estimated CVP	6±2	6±2	0.264	
IVC max	16±4	13±5	0.099	
Maintenance therapy				
Angiotensin converting enzyme-inhibitor/angiotensin receptor blocker-use	94%	78%	0.167	
Beta adrenergic antagonist use	94%	94%	0.967	
Mineralocorticoid receptor use	94%	72%	0.086	
Loop diuretic use	66%	66%	0.903	
Intravascular volume expansion				
Echocardiographic values				
Left ventricular ejection fraction	33±8	35±8	0.605	
TAPSE	17±5	19±7	0.250	
E/E'	17±10	11±3	**0.035**	0.9
Estimated CVP	8±2	7±3	0.061	
IVC max	18±5	14±5	0.177	
IV loop diuretics				
Echocardiographic values				
Left ventricular ejection fraction	35±9	32±8	0.282	
TAPSE	18±5	17±5	0.678	
E/E'	15±8	10±3	0.171	
Estimated CVP	6±2	6±2	0.264	
IVC max	18±6	14±4	0.160	

Table 2: Characteristics of HFREF patients with VII < or > the median. *BSA: body surface area; CVP: central venous pressure; eGFR, estimated glomerular filtration rate according to the Chronic Kidney Disease Epidemiology Collaboration;, FE_{Na} :Fractional excretion of sodium; IVC: inferior vena cava; MAP: mean arterial pressure;; NT-proBNP, N-terminal of the prohormone of B-type natriuretic peptide, RI: resistive index; TAPSE: Tricuspid annular plane systolic excursion; VVI:venous impedance*

CHAPTER 8

Cardiac output and renal dysfunction, definitely more than impaired flow

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2016 May 17;67(19):2209-12

Nowadays, patients with heart failure (HF) rarely present in cardiogenic shock. Instead, HF has become a chronic systemic disease whereby symptoms and disease progression relates to unrestrained neurohumoral stimulation leading to water and sodium retention (6). Since the kidneys are responsible for fluid homeostasis, it is not surprising that renal function is one of the strongest predictors for outcome in HF, outperforming other prognosticators directly reflecting cardiac function (264). The interdependence between heart and kidney has been a topic of extensive research for decades. Intuitively, progressive renal dysfunction is often attributed to hypoperfusion of the kidney due to progressive impairment of cardiac output. However, a drop in systemic blood pressure, venous congestion and intra-abdominal pressure are hemodynamic parameters stronger associated with worsening renal function in heterogenous populations of HF (178, 244, 265, 266). In this issue of the *Journal,* Hanberg and coauthors provide further data to support the disconnect between cardiac output and renal function in a HF population with careful cardiac and hemodynamic profiling. They concluded a post-hoc sub-analysis from the randomized and registry portions of the Evaluation Study of congestive Heart Failure and Pulmonary Artery Catheter Effectiveness (ESCAPE) Trial to specifically evaluate the cardiac output - renal function association (256). The study population consisted of advanced HFREF patients (mean ejection fraction 23±12%) with average cardiac index (CI) of 2.3±2.1/min and right atrial pressure of 14±9 mmHg. The authors investigated the correlation between CI and renal function in the combined study cohort (>500 patients), as well as across different well-characterized patient subgroups in which reduced cardiac output was expected to play an important role (i.e. high right atrial pressure, more impaired renal function or low systolic blood pressure). Overall no positive association between CI and renal function was found. Therefore, this analysis of ESCAPE is consistent with previous data, and together with the finding of lack in in success of treatment strategies focused at improvement of renal function through enhanced cardiac output, it's evident to conclude that reduced CI is not a hemodynamic driver for renal dysfunction in patients hospitalized for HF who are not in shock. As always, to better appreciate these findings, a brief review of cardio-renal physiology might be insightful.

GLOMERULAR FILTRATION IS NOT THE SUM OF HEMODYNAMIC FACTORS

The kidneys preserve the body's fluid- and electrolyte balance to protect tissue perfusion as well as intra- and extracellular volume homeostasis. To do this, the kidneys must filter a sufficient – rather fixed - amount of blood per time from the renal glomerular capillaries into

the Bowman's capsule (filtration function) and precisely regulate tubular water and solute reabsorption (tubular function). Renal blood flow (RBF) is normally around 20% of the cardiac output, and determined by the difference between renal arterial and venous pressure, the intra-abdominal pressure and the tonus of the renal vasculature (178, 244, 265, 267). The glomerular filtration rate (GFR) depends on RBF, but more importantly on Starling forces between the glomerular capillaries and Bowman's space. Importantly, due to the essential role of the kidney, the human body possesses extensive mechanisms to preserve GFR. First, an important decrease in cardiac output will lead to a redistribution of blood volume within the body to preserve kidney perfusion. Secondly, within the kidney intrinsic autoregulation mechanisms will keep GFR within narrow limits (Figure 1). Therefore, GFR is a complex interplay of hemodynamic factors and autoregulation mechanisms.

FILTRATION FRACTION AND TUBULAR SODIUM AVIDITY: THE BIGGER PICTURE

Although it seems appealing now to finally end the tale on cardiac output and renal function, the bigger picture should not be missed. GFR has proven to be a very important prognostic *marker*, but it is probably not a good *target* to improve outcome. Moreover, during effective decongestion and uptitration of renin-angiotensin blockers, a small GFR decrease has even been associated with improvement in survival and less readmissions in HF (179, 268).

As the body aims to preserve GFR, the filtration fraction (FF) - which is the ratio of GFR/RBF - will be altered. As a result, two comparable levels of GFR can reflect a different situation (Figure 1). When FF is increased, a state of high water and sodium avidity, characteristic for HF is induced. This results in high proximal tubular sodium reabsorption enhancing neurohumoral activation, and resistance to the action of natriuretic peptides (Figure 1) (9). Therefore, any strategy that intends to lower FF or targets sodium reabsorption in the proximal tubules might have large benefits in decompensated HF. For example, renin-angiotensin blockers, which mediate *efferent* arteriolar vasodilatation and therefore an increase in RBF and decrease in FF, have proven to lead to increased diuretic and natriuretic capacity in chronic and acute HF, even in the face of a potential drop in GFR (269). Serelaxin, a recombinant human relaxin-2, which significantly reduces rehospitalization and short term mortality in acute HF (secondary endpoints) and preserves renal function, increases RBF and reduces FF but does not significantly affect GFR (270). Interestingly, the increase in RBF (up to 50%) probably relates to a reduction in venous congestion and vasodilation of the afferent and efferent arteriole unloading the glomerulus (271). Furthermore, Acetazolamide,

an old and largely forgotten diuretic, and sodium-glucose transporter-2 (SGLT-2) inhibitors - which recently demonstrated striking effects on cardiovascular endpoints in type II DM patients – both inhibit proximal tubular sodium transport (272, 273). These drugs should enhance distal tubular flow in the nephron counteracting salt retention, facilitating decongestive treatment, and boosting loop diuretic responsiveness (241). Although results of large randomized clinical trials should be awaited for, these strategies look very promising.

Finally, an important obstacle in optimizing decongestive strategies in HF and the major shortcoming of the study of Hanberg, is that only metrics of renal filtration function are analyzed. Surely, GFR remains an estimate of the renal "reserve" available to relieve congestion and to respond to the insult posed by HF. However, GFR, based on serum creatinine, is an inaccurate reflection of only the filtration function of the kidney while other components, such as renal tubular avidity for fluid and sodium are not revealed. Currently, loop diuretic response, defined as sodium output over loop diuretic dose, may be one of the best markers of the cardio-renal interaction since this better reflects the renal reaction to volume status on the filtration and tubular level (237). However, practical difficulties with urine analysis, and failure to identify the specific etiology and best treatment strategy in case of a poor response lack a clinical use. Recently, a urinary spot analysis after administration of diuretic agent has been investigated which excellently reflects natriuretic response and might make an individualized approach in HF patients feasible (236)

In conclusion, the results of Hanberg convincingly show that renal filtration function during acute HF is not driven by changes in cardiac output. Future research should focus on other metrics of renal function in order to diagnose a state of increased water and salt avidity. Meanwhile, the principal target in the treatment of acute HF patients must remain efficient decongestion and restoring a neutral salt and water balance without specifically wanting to improve cardiac output.

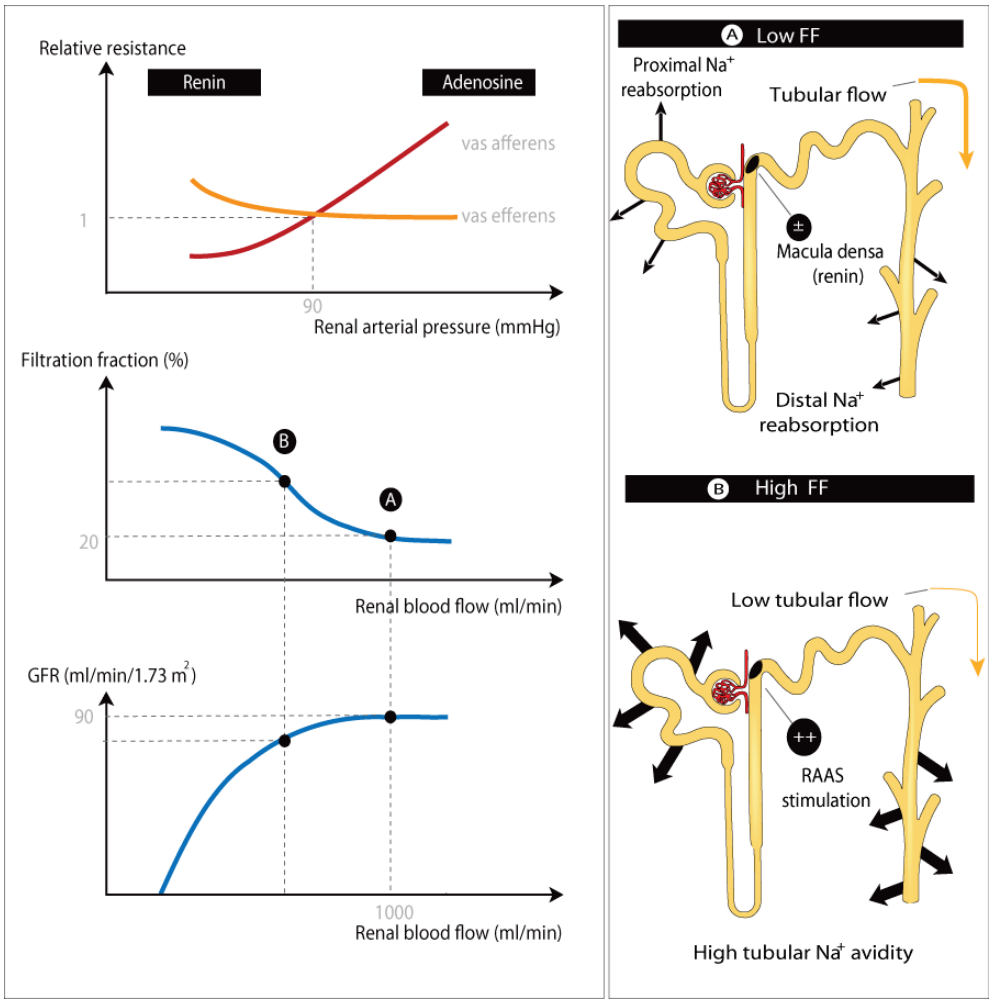

Figure 1. Filtration fraction (FF) is more important than glomerular filtration rate (GFR) in Heart failure. *GFR is kept constant over a wide range of renal arterial perfusion pressures by adapting the resistance of the afferent (adenosine) and efferent arteriole (renin). Therefore, comparable levels of GFR can reflect different situations. When renal perfusion is low, GFR is preserved resulting in an increase of FF. This leads to higher oncotic and lower hydrostatic pressure in the peritubular capillaries strongly facilitating sodium and water reabsorption. The increased reabsorption of sodium in the proximal tubule reduces its availability to the macula densa, further stimulating renin release, which will increase the FF even more. Also, distal tubular flow will be lower, which enhances the response to aldosterone and impairs the action of natriuretic peptides (9). (adapted from Medical Physiology, Boron and Boulpaep (183))FF: filtration fraction, GFR: glomerular filtration rate; RAAS: renin-angiotensin aldosterone system.*

CHAPTER 9

The acute cardiorenal syndrome

Burden and mechanisms of disease

Petra Nijst, Wilfried Mullens

Current Heart Failure reports.

2014 Dec;11(4):453-62

ABSTRACT

Worsening renal function during the treatment of acute decompensated heart failure, so called acute cardio-renal syndrome, is very common, and complicates the treatment course. The underlying pathophysiology of WRF involves variable contributions of renal hemodynamics, neurohormonal activity and oxidative stress. Historically, WRF has been associated with adverse outcomes. However, emerging data support therapeutic strategies that permit WRF while effectively treating congestion as they are associated with improved outcomes.

INTRODUCTION

Renal dysfunction is a common finding in heart failure (HF) and is one of the most potent prognostic indicators in these patients (274). The 'cardiorenal syndrome (CRS)' is a state in which therapy to relieve heart failure (HF) symptoms is limited by a decline in renal function, which often coincides with the development of resistance to loop diuretics (275). 'Worsening renal function (WRF)' is a more concrete and quantitative description of the cardiorenal syndrome, characterized as an 0.3-0.5 mg/dL rise in serum creatinine or a decrease in glomerular filtration rate (GFR) of 9-15 ml/min during HF admission (276, 277). WRF rather than CRS has been utilized in most research studies because this definition is more universally accepted for outcomes data (278). The CRS has been classified into five subtypes based on the organ primarily involved (heart or kidney) and on whether the failure is acute, chronic or secondary (Table 1) (279). Multiple different mechanisms - such as hemodynamic derangement, neurohormonal upregulation and oxidative stress - may contribute to this complex syndrome. Nonetheless, congestion – defined as raised cardiac filling pressures - seems to be a principal factor in disease mechanism and outcome. Increasing evidence indicates that the adverse prognosis associated with WRF is related to the mechanism underlying renal dysfunction, rather then simply a further reduction in glomerular filtration rate itself.

Type	Denomination	Description	Example
1	**Acute cardiorenal**	Acute heart failure leading to acute kidney disease	Acute coronary syndrome leading to acute heart and kidney failure
2	**Chronic cardiorenal**	Chronic Heart Failure leading to kidney failure	Chronic Heart failure
3	**Acute renocardiac**	Acute kidney disease leading to acute heart failure	Uremic cardiomyopahty
4	**Chronic renocardiac**	Chronic kidney disease leading to heart and kidney failure	Left ventricualr hypertrophy due to kidney failure
5	**Secondary**	Systemic disease leading to heart and kidney failure	Sepsis, vasculits, diabetes mellitus, etc.

Table 1. Classification of the cardiorenal syndromes

In this review we will focus on the acute CRS (type I) and describe the burden, contemporary pathophysiologic insights of WRF during ADHF and therapeutic strategies in this domain.

KIDNEY FUNCTION: GFR AND OTHER BIOMARKERS

The kidneys serve three essential functions: filtration mainly coordinated by the glomeruli, homeostasis of the body's fluid- and electrolyte balance carried out by the renal tubuli, and secretion and/or activation of hormones playing an important role in ia. erythropoiesis (erythropoietine), calcium metabolism (vitamin D) and regulating blood pressure (renin). The GFR is the net flow rate of plasma ultrafiltrate across the capillary walls in the glomerulus. Glomerular ultrafiltration depends on the number of functional nephrons, the area of the glomerular filtration barrier, and hydrostatic and colloid osmotic pressure differences between the glomerular capillaries and Bowman's space (Starling forces). In the acute setting, alterations in Starling forces, based on hemodynamic driven variations in renal blood flow (RBF), are primarily responsible for GFR alterations since the other GFR-determining factors are more stable. RBF - or renal perfusion - depends on the arterio-venous pressure difference in the glomerulus and the resistance of the renal vasculature. An important feature of the renal circulation is the presence of renal autoregulation mechanisms, which try to keep RBF and GFR within narrow limits by adjusting resistance of the *afferent* arterioles in response to renal arterial pressure fluctuations, and thus compensating filtration fraction (FF; FF is the ratio of GFR over RBF). In contrast, *efferent* arteriolar resistance, capillary resistance and venous resistance all change very little over a wide range of renal arterial pressures (183). Importantly, serum creatinine depends on age, diet, gender and muscle mass and small rises in serum creatinine can reflect large reductions in GFR. Furthermore, GFR mainly represents the filtration function of the kidneys, yet is frequently used as a surrogate for *overall* renal function.

However, renal tubular function is also of great interest, especially in decompensated HF patients where an imbalance in fluid and salt homeostasis is the main problem (280). The ability to sustain the filtration *and* tubular functions of the kidney is vital to alleviate congestion by therapeutic interventions in HF. A large number of markers for renal tubular damage exist (kidney injury molecule (KIM) – 1, neutrophil gelatinase-associated lipocalin (NGAL), N-acetyl-beta-glucosaminidase (NAG), etc.). It was recently demonstrated that in the acute setting of ADHF the degree of AKI is low, based on NGAL concentrations (281). Hence, tubular injury is a minor cause of WRF (183). Unfortunately, none of these tubular injury biomarkers seem suitable for *follow up* of renal tubular function in HF, due to influence from volume status, renal inflammation and neurohormonal activity (280).

Other interesting biomarkers that could give insights in renal function - especially in neurohormonal activity - are the blood urea nitrogen (BUN)-to-creatinine ratio and serum sodium concentration. An increased ratio of BUN-to-creatinine level is frequently interpreted as pre-renal azotemia. Based on the same physiological concepts, renal urea handling closely parallels neurohormonal activation. The BUN-to-creatinine ratio can serve as a useful tool to differentiate renal dysfunction with a strong neurohormonal component as opposed to renal dysfunction from other causes (282). Hyponatremia - defined as serum sodium < 135 mmol per liter - is an important indicator for impaired water excretion by the kidneys rather than sodium depletion. Hyponatremia is the result of deregulated arginine vasopressine release (AVP) and insufficient tubular flow, contributing to excessive water retention in HF. Both elevated BUN-to-creatinine ratio and hyponatremia are associated with an increased risk for adverse outcomes in HF patients (283, 284).

BURDEN OF RENAL DYSFUNCTION AND WRF IN HF

Renal dysfunction (defined as an estimated GFR less than 60 mL/min/ 1.73 m^2) is present in up to 60% of stable outpatients and hospitalized acute decompensated HF (ADHF) patients and thus a strikingly prevalent comorbidity in HF (285-288). The Acute Decompensated Heart Failure National Registry (ADHERE) comprising 118,465 patients revealed that moderate renal dysfunction (GFR 30-59 ml/min/1.73 m2), severe renal dysfunction (GFR 15-29 ml/min/1.73 m2) and kidney failure (GFR < 15 ml/min/1.73 m2) occurs in respectively 43.5 %, 13.1 % and 7.0 % on admission of ADHF patients. In-hospital mortality increased from 1.9 % for patients with normal renal function to 7.6 % for patients with severe dysfunction (286). Importantly, baseline glomerular filtration rate (GFR) appears to be a stronger predictor of mortality and morbidity in HF patients than left ventricular ejection fraction or NYHA functional class (264).

It is obvious that patients with pre-existing renal dysfunction are vulnerable to developing WRF (264, 289). Other risk factors for renal decline relate to comorbidities and therapy. *WRF* has an incidence range from 20% to 30% in ADHF patients (286, 290, 291). Whereas baseline renal function is invariably linked to worse outcomes and WRF has been associated with worse long-term outcomes in most observational studies (276, 289, 292), recent studies about the prognostic value of WRF in the setting of ADHF are mixed. Depending on contextual factors - and thus underlying mechanism of WRF - decreases in GFR can be associated with neutral or even better clinical outcome in HF. Metra and coauthors observed

in a large observational study that WRF only has a negative clinical association when occurring in patients with persistent fluid overload. Therefore, WRF might merely reflect the result of effective decongestion - thus hemoconcentation – as well as intensified therapy with angiotensin converting enzyme–inhibitors (ACE-I) or angiotensin receptor blockers (ARB) (16) in patients who are adequately decongested, while in those who are not WRF might be the result of persistent volume overload and/or neurohormonal derangements (179, 289). Furthermore - and surprisingly -, the prognosis of *improvement* in renal function (IRF) during hospitalization of ADHF is similar to that of WRF (293). Indeed, it has previously been shown that IRF patients often experienced WRF before or after the index hospitalization, implying that the ability to improve renal function may be predominantly present in those patients who tend to develop renal impairment (266).

MECHANISMS OF WRF

WRF arises typically within days of hospitalization, suggesting a direct causative effect of the hemodynamic derangement associated with HF decompensation and/or iatrogenic treatment adjustments (292). It is generally accepted that hemodynamic derangements (elevated venous pressure, elevated intra-abdominal pressure, low cardiac output, blood pressure drops) alter RBF and therefore are the main drivers for GFR decline during acute episodes of HF. Although, less understood, also complex neurohormonal (RAAS, SNS) and inflammatory (oxidative injury) derangements play a role in the complex pathophysiology of WRF (Figure 1).

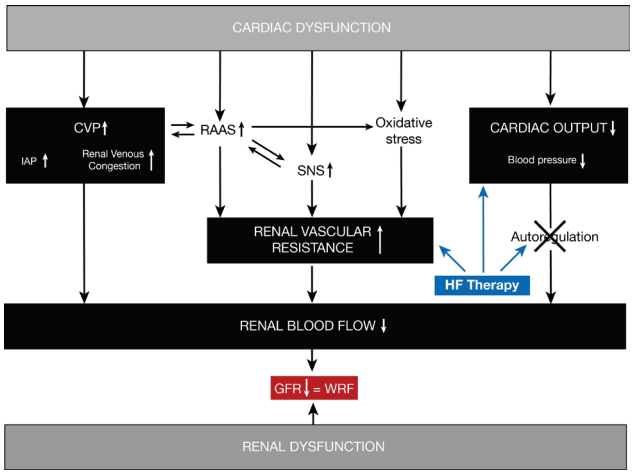

Figure 1. Pathophysiology of the cardiorenal syndrome

Cardiac output

Traditionally, worsening renal function in HF had been attributed to low-output failure or hypotension resulting in renal hypoperfusion (289, 294). Inadequate RBF prompts renin release by the juxtaglomerular cells of the afferent arterioles through low-flow states in the ascending limb of the loop of Henle and pressure-sensing baroreceptors. Activation of the renin-angiotensin-aldosterone cascade will lead to afferent and efferent arteriolar vasoconstriction and thereby decreasing GFR (274). Furthermore, RAAS activation leads to enhanced sodium and water reabsorption in order to try to preserve renal perfusion and renal filtration fraction. In extreme cases, persistant hypoperfusion may lead to renal ischemia. However, recent data from large registries argue against impaired cardiac output being the main culprit for the pathogenesis of WRF. The proportion of patients presenting with WRF due to low "renal preload" is small since renal autoregulation mechanisms immediately adapt afferent arteriolar resistance and thus lead to a compensatory adjustment in filtration fraction, which preserves GFR. Importantly, WRF is also present in a population of HF with preserved ejection fraction (HFPEF) where it is expected cardiac output is preserved. A prospective study in patients admitted for ADHF with underlying HFPEF observed a prevalence of 12% of WRF during admission for ADHF. Impaired baseline renal function and especially patients with the combination of impaired baseline renal function and WRF had increased risk of overall and cardiovascular mortality (295). Furthermore, a large trial of pulmonary artery catheter–guided management of 433 individuals admitted with acute decompensated congestive heart failure (Evaluation Study of Congestive Heart Failure and Pulmonary Artery Catheterization Effectiveness [ESCAPE]) found no correlation between baseline renal function and cardiac index (290), and improvement in cardiac index did not result in improved renal function, prevention of death, or prevention of rehospitalization (178, 265, 290, 291). These findings were confirmed in the Dopamine in Acute Decompensated Heart Failure (DAD-HF) I and II trials, (296, 297) where low dose dopamine showed no beneficial effect on renal function. Nonetheless, it is important to recognize the exceptional severe situations of heart failure with very low cardiac output or extremely high renal vascular resistance (caused by the activation of the neurohormonal axis) and thus markedly diminished RBF. In these situations renal impairment can be reversible with advanced cardiac or renal support.

Congestion and central venous pressure

In contrast to the small proportion of patients presenting with a low cardiac output (298), congestion is present in almost every patient with acute decompensated HF (12, 299), and a pivotal factor in the pathophysiology and outcome of WRF. In the ESCAPE trial renal function did not worsen when treatment was directed at lowering invasively measured cardiac filling pressures (central venous pressure (CVP) and pulmonary capillary wedge pressure (PCWP)), whereas it did worsen in the treatment arm guided by clinical assessment alone (290). The presence of venous congestion as measured by elevated CVP, both on admission and during follow up, seems to be one of the strongest hemodynamic determinants of development of WRF (178, 244, 300-302). As already adduced, Metra recently emphasized in a large observational study that the negative impact of WRF on outcome was only present in association with persistent congestion (16).

A rise in CVP can diminish RBF through backward transmission of venous pressure. The raised CVP might distend the venules surrounding the distal ends of the tubules so that the lumen of the tubule can be obliterate (255, 303). Also, since the kidneys are encapsulated organs, increased central venous pressure could cause an increase in renal interstitial pressure further attenuating RBF (255, 304). Moreover, angiotensin II (AT II) concentrations increase with increasing renal venous pressure (301, 305). This will lead to a further fall in GFR and will increase sympathetic system activity. The combination of hemodynamic and neurohormonal alterations induced by venous congestion cause progressive renal impairment (306).

Different treatment strategies can be applied to treat congestion and lower CVP (307). Large trials evaluated the effect of different diuretic strategies (high dose versus low dose diuretics, intravenous bolus versus continuous infusion strategies; Diuretic Optimization Strategies Evaluation (DOSE)-trial (308)) and diuretic versus ultrafiltration (UF) regimens (Ultrafiltration versus intravenous diuretics for patients hospitalized for Acute Decompensated congestive heart failure (UNLOAD) trial (309) and Cardiorenal Rescue Study in Acute Decompensated Heart Failure (CARRESS) trial) in volume overloaded patients. WRF was seen more in patients treated with high doses of diuretics in DOSE and undergoing ultrafiltration in CARRESS, yet there was no difference on the prevalence of WRF between treatment groups in UNLOAD although outcome was better in UF patients in the latter study. Therefore, none of the treatment strategies in these studies is convincingly superior. Notably, no sustained difference in fluid loss between the different treatment

strategies groups was seen, and clinical decongestion was only reached in a minority of patients, i.e. 9% with pharmacologic therapy and 10% with ultrafiltration after 96 hours in CARRESS.

Blood pressure

Recently, our group showed that changes in systemic blood pressure during ADHF treatment, due to aggressive vasoactive and diuretic therapy, is another strong hemodynamic determinant of dynamic renal function. This observation was consistent with previous analysis in the ESCAPE trial (293) and in the pre-RELAX-AHF trial (310). The underlying mechanism is still not well understood. Theoretically, intra-glomerular pressure should remain independent from systemic blood pressure to a certain extent by autoregulation mechanisms. However, whether such autoregulatory mechanisms still hold true in a pathophysiological situation such as ADHF on a background of treatment with ACE inhibitors/ARBs and in the setting of aggressive diuretic and vasoactive therapy is unclear (266). Although, blood pressure and CVP are both accessible parameters to focus on during treatment of ADHF, their correlation with changes in renal function remains relatively weak. This seems logical since RBF – and subsequently GFR - depends on renal perfusion *pressure* but also on renal *vascular resistance.* It would therefore be of interest to determine if dynamic renal changes correlate with directly measured renal perfusion changes. Nonetheless, significant drops in blood pressure should be avoided during treatment of ADHF.

Intra-abdominal pressure

The prevalence of raised intra-abdominal pressure (IAP; defined as ≥ 8 mmHg) in patients with advanced heart failure admitted with hemodynamic derangements may be as high as 60% despite the lack of abdominal complaints or overt ascites (265). Elevated IAP results in an indirect increase in central venous pressures as well as directly "compressing" the kidneys (and thus increase renal pressure), both leading to reduction in RBF (311-313). Indeed, elevated IAP is associated with more impaired renal function at baseline. Also, reduction of IAP following intensive medical therapy seems to be associated with improvement in renal function. In contrast, persistently elevated IAP after therapy was associated with worsening of renal function regardless of central hemodynamic measures (265). In the latter group, prompt reduction in IAP has been observed with the use of mechanical fluid removal, either by paracentesis (in the presence of ascites) or ultrafiltration

(314). Importantly, as IAP will be trans-diaphargmatically transmitted, and give rise to elevation of intra-thoracic pressures, it is important to recognize that measured intravascular pressures are not reflective of intravascular volumes, and inappropriate diuretic admission might increase the risk of intravascular underfilling.

Renin-angiotensin-aldosterone system

Renin-angiotensin-aldosterone system (RAAS) upregulation in HF is a (mal)adaptive response to altered hemodynamics and sympathetic signalling. Moreover, neurohormonal factors are more stimulated in the presence of reduced renal function (315, 316) and in the presence of congestion (261). Renin, AT II and aldosterone cause both systemic and renal vasoconstriction (i.e., hemodynamic effects), and therefore reduce RBF and enhance tubular sodium reabsorption. In the early stages of HF, GFR is well maintained by compensatory increases in filtration fraction. However, in advanced stages or when blood pressure decrease beyond the limits of the renal autoregulation mechanisms, GFR becomes more dependent on afferent arteriolar flow and hence neurohormonal activity. Furthermore, high levels of AT II directly contributes to intrinsic kidney damage since AT II upregulates cytokines transforming growth factor-β, tumor necrosis factor-α nuclear factor-κB and interleukin-6, and stimulates fibroblasts, resulting in cell growth, inflammation, and fibrotic damage in the renal parenchyma (317, 318). RAAS inhibitors have well-established long-term major clinical benefits in HF, and seem to have beneficial effects on renal function. Indeed, a recent meta-analysis established the greater benefit of RAAS inhibition on survival in HF patients who develop WRF versus those who do not. This stands to reason, as it has been observed there is a greater stimulation of RAAS in the presence of reduced renal function and this can confer greater potential for improvement when the RAAS is adequately blocked (319). However, since RAAS inhibitors reduce glomerular filtration rate (GFR) they are frequently unfairly omitted by clinicians.

Sympathetic nervous system

Overactivation of the sympathetic nervous system (SNS) is thought to contribute to progression of HF and WRF. Renal sympathetic activity is predominantly exerted by sympathetic innervation of the renal vasculature, the proximal tubular segment of the nephron and juxtaglomerular renin-containing granular cells. Growing evidence supports the presence of renal sympathetic overdrive in renal dysfunction through RAAS upregulation, mechano- and chemoreflex activation

and endothelial dysfunction with decreased bioavailability of nitric oxide (NO) (320). Moreover, SNS overactivity leads to direct renal afferent and efferent arteriolar vasoconstriction and renin release, both leading to decreasing RBF and ultimately GFR. Beta blockers may counteract the negative effects of chronic renal SNS activation (321). The CIBIS-II trial (322) and MERIT-HF (323) trial clearly demonstrated the advantage of beta blockers in patients with HF and renal dysfunction. Recently, a lot of interest has emerged in the application of renal denervation therapy as a pilot study of catheter-based renal sympathetic denervation found significant improvements in GFR in 24% of patients with resistant hypertension (324, 325). Therefore, several studies investigating the effect of renal denervation in HF patients with renal dysfunction are ongoing and seem promising.

Oxidative stress

Growing evidence supports oxidative injury as a common link between progressive cardiac and renal dysfunction. Neurohormones are strong precipitants and mediators of an oxidative injury cascade that lead to widespread endothelial dysfunction, inflammation and cell death in the heart and kidneys. AT II seems to be particularly important in this process, exerting many deleterious effects through the activation of NADPH oxidase and NADH oxidase. AT II activates these 2 enzymes within vascular smooth muscle cells, cardiac myocytes, and renal tubular epithelial cells, generating superoxide, a reactive oxygen species (326-328). Interestingly, ACE inhibitors and angiotensin receptor blockers can increase the availability of nitric oxide, an important factor in endothelial function. (329). Furthermore, anemia and low levels of erythropoietin (EPO), common findings in renal dysfunction and HF, may contribute to the renal oxidative imbalance. The effect of EPO on nitric oxide synthesis is unclear, but it does appear to decrease oxidative stress as well as increase hemoglobin, which acts as an antioxidant (274, 330). Additionally, *bardoxolone methyl,* an oral antioxidant inflammation modulator, which reduces inflammation and oxidative stress, can induce a sustained increase in GFR in chronic kidney disease associated with type 2 diabetes (331). However, in the BEACON trial, this promising agent was associated with an increase in cardiovascular events, especially heart failure events, and therefore the trial was terminated earlier (332). A possible explanation for this observation is drug-drug interactions, which reduce the bioavailability of angiotensin-receptor blockers and angiotensin converting enzyme-inhibitors. Therefore, other antioxidative agents, which do

not subvert essential HF therapies, could still have large potential in the treatment of the cardiorenal syndrome.

CLINICAL TRIALS ON DECONGESTION

Since renal dysfunction and WRF portends such a poor prognosis, therapeutic strategies that improve renal function in HF are very alluring and widely studied. Multiple therapies for ADHF were investigated in large randomized trials, of which some included only patients with WRF after initial therapy for ADHF (Table 2). In essence, all the agents tried to improve RBF - hence GFR - by increasing cardiac output (inotropes, vasodilators), decreasing congestion (diuretics, ultrafiltration, vaptans), and lower renal vascular resistance (rolofylline, serelaxin, nesiritide, renal denervation, etc.). However, none of the current studied therapeutic strategies have proven beneficial effects on outcome. Confounding effects of persistent congestion, use of other background therapies, baseline renal function, etc. probably account for that. However, provisional results on serelaxin and renal denervation therapy are promising, but need to be confirmed in on-going large trials. Additionally, for the future, it seems worthwhile to investigate the potential of antioxidative agents in the treatment of the cardiorenal syndrome.

THERAPEUTIC APPROACH FOR WRF

Current guidelines state that treatment of signs and symptoms of ADHF should be achieved *without WRF*. When WRF arises during treatment of ADHF these guidelines recommend varied strategies as e.g. other diuretic regimens, inotropic support, ultrafiltration, dialysis, vasodilator association, etc. (333) However, as indicated above, none of these treatment strategies have proven benefit on outcome. Interestingly, therapeutic strategies where the focus lies on effectively decongesting the patient*, with permissive WRF,* have a beneficial impact on mortality compared to persistent fluid overload. Therefore, the goal of treatment of ADHF should be to effectively reach decongestion, while avoiding significant blood pressure drops (266) and preserve RBF. This most often can be achieved by careful titration of diuretics. While loop diuretic agents will continue to play a key role through their strong diuretic effect on the loop of Henle, combination strategies with more proximally acting agents like acetazolamide or distal acting agents like thiazides hold great promise. Furthermore, vasodilation therapy such as high dose nitrates or nitroporusside will have beneficial effects on central hemodynamics (lowering of filling pressures including CVP, increase in CO), with increased RBF often leading to enhanced diuresis (307). Ultrafiltration

therapy could still be an interesting approach in those patients with persistent volume overload despite of diuretic fine-tuning. However, uncertainty of the rate of fluid removal with risk of hypotension due to intravascular underfilling, and the invasiveness of the procedure (central line, anticoagulants, mechanical removal, etc.) still hamper widespread use of the technique. Finally, ACE-I and beta blockers have well-established effects in chronic ambulatory HF patients, also in those *with* renal dysfunction. They should be continued if possible during ADHF.

CONCLUSION

Renal dysfunction is present in a large group of HF patients, and WRF occurs in 20-30% of patients admitted for ADHF. The underlying pathophysiology of WRF is related to an imbalance in interactions of the failing heart, the neurohormonal and inflammatory system, as well as HF therapies influencing glomerular filtration. Although, GFR reflects only a part of renal function, and reduced glomerular filtration rate is associated with worse outcomes, prognosis of WRF relates more to the underlying cause than WRF itself. (334). Unfortunately, limited progress has been made in the last years with respect to differentiation of potential subtypes of WRF and individualizing therapy. To date, no specific agent has proven effective as therapy for WRF, so strategies aiming at effectively decongestion the patient through careful titration of diuretic- and vasoacting agents are still the preferred strategy. Notably, provisional data on serelaxin and renal denervation seem promising.

Firt Author, year (reference)	Acronym, Study design	Therapy, target	Outcome (mortality and/or hospitalisation)	Effect on renal function
Felker; 2011; (308)	**DOSE-AHF** double blind, 2-by-2 design, 308 pts	Diuretics; **decongestion**	no benetit	more (transient) WRF in high-dose group
Konstam; 2007, (335)	**EVEREST** double blind, placebo controlled; 4133 pts	Vasopressine V2 receptor blocker (tolvaptan); **decongestion**	no benefit	more WRF in treatment group
Giamouzis; 2010, (297)	**DAD-HF I** 2 treatment groups, 60 pts	Dopamine, **cardiac output and renal vasodilatation**	No benefit	WRF after 24 hours (early WRF) less frequent.
Triposkiadis; 2013, (296)	**DAD-HF II** 3 treatment groups, 161 pts	Dopamine; **cardiac output and renal vasodilatation**	No benefit	No effect
O'Connor, 2011, (336)	**ASCEND-HF** placebo-controlled, 7141 pts	Natriuretic peptide (Nesiritide); **renal vasodilatation, RAAS-inhibiton, decongestion**	no benefit	No effect
Chen; 2013; (337)	**ROSE-HF** double blind, placebo controlled; 3 groups, 360 pts	Natriuretic peptide (nesiritide); **renal vasodilatation, RAAS inhibition, decongestion** Dopamine; **renal vasodilation**	no benefit	No effect
Massie; 2010; (338)	**PROTECT;** , placebo controlled; 2033 pts	Adenosine antagonist (rolofylline**), renal vasodilatation**	no benefit	No effect
Costanzo, 2007; (309)	**UNLOAD:** 2 groups, 200 pts	Ultrafiltration vs IV diuretics; **decongestion**	less hospitalizations for HF in ultratiltration group	No effect
Bart, 2012, (339)	**CARRESS** 2 groups, 188 pts	Ultrafiltration versus stepped diuretic therapy; **decongestion**	No benefit (more complications in ultrafiltartion arm)	More WRF in ultrafiltration group
Teerlink, 2013, (340, 341)	**RELAX-AHF** double-blind, placebo controlled; 1161 pts	Recombinant human relaxin-2 (seralaxine), **renal vasodilation**	Under investigation	Lower incidence of WRF on day 2
2014-2015	**DIASTOLE, RDT-PEP, Symplicity-HF, PRESERVE, REACH, RSD4CHF, Re-ADAPT-HF, etc.**	Renal denervation; **SNS inhibiton**	Under investigation	Under investigation

Table 2. Important RCT's evaluating the effect of therapy on WRF in HF patients

PART III

Neurohumoral activation in heart failure patients on optimal medical therapy and patients with heart failure and recovered ejection fraction

OBJECTIVE | To study neurohumoral activation in patients with Heart Failure. Subsequently, to explore the contribution of different (medical and device-related) therapies to myocardial recovery

CHAPTER 10

The importance of plasma renin activity in patients with heart failure and reduced ejection fraction on optimal medical therapy

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Submitted

ABSTRACT

Background: Renin-angiotensin-aldosterone system (RAAS) activation in heart failure with reduced ejection fraction (HFREF) is detrimental through promotion of adverse ventricular remodeling and retention of salt and water.

Aims: The objective of this study is to describe RAAS activity in distinct HFREF populations and to assess its prognostic impact.

Methods: Venous blood samples were prospectively obtained in 76 healthy volunteers, 72 patients hospitalized for acute decompensated HFREF, and 78 ambulatory chronic HFREF patients without clinical signs of congestion. Sequential measurements were performed in patients with acute decompensated HFREF.

Results: Plasma renin activity (PRA) was significantly higher in ambulatory chronic HFREF (7.6 ng/ml/h [2.2;18.1]) compared to patients with acute decompensated HFREF (1.5 ng/ml/h [0.8;5.7]) or healthy volunteers (1.4 ng/ml/h [0.6;2.3]) (all $p < 0.05$). PRA was significantly associated with arterial blood pressure and renin-angiotensin system blocker dose. A progressive rise in PRA (+4 ng/ml/h [0.4;10.9]; $p<0.001$) was observed in acute decompensated HFREF patients after 3 consecutive days of decongestive treatment. Only in acute HFREF, before initiation of decongestive treatment, PRA levels in the highest tertile are associated with increased cardiovascular mortality or heart failure readmissions ($p=0.035$).

Conclusion: PRA is significantly elevated in ambulatory chronic HFREF patients but is not associated with worse outcome. In contrast, in acute HFREF patients, PRA is associated with cardiovascular mortality or heart failure readmissions.

INTRODUCTION

Renin-angiotensin-aldosterone system (RAAS) activation in heart failure with reduced ejection fraction (HFREF) has detrimental long-term effects such as water and salt retention as well as promoting adverse ventricular remodeling. Outcomes in HFREF patients have drastically improved during the past two decades through strategies that have targeted RAAS activation (342-345). Plasma renin activity (PRA) and plasma aldosterone levels are biomarkers that quantitatively reflect RAAS activation and might be used for risk stratification in HFREF. Indeed, previous studies have linked higher levels of RAAS activation to more advanced disease stages and worse outcomes in both acute and chronic HFREF (8, 346-349). Most of these studies have focused on PRA as renin is the rate-limiting step of the RAAS, and a more reliable reflection of RAAS activation compared to serum aldosterone (350, 351). However, these studies largely predate the current era of HFREF treatment in which angiotensin-converting enzyme inhibitors (ACE-i), angiotensin receptor blockers (ARB), beta-blockers and mineralocorticoid antagonists (MRA) are guideline-recommended therapies. Moreover, whether RAAS activation during decongestive therapy has prognostic significance remains unclear (182). Therefore, the objective of this study is to describe the extent of RAAS activation, and its prognostic impact in well-characterized HFREF populations on optimal medical therapy.

METHODS

Study design

This prospective cohort study was carried out in a single tertiary care center (Ziekenhuis Oost-Limburg, Genk, Belgium) between September, 2011, and October, 2015. The study complies with the Declaration of Helsinki and the institutional review board approved the study protocol. All subjects provided written informed consent before any study-specific intervention was performed.

Study population

Patients were eligible for study inclusion if ≥18 years of age and able to give informed consent. *Healthy volunteers* were recruited through general announcements and had 1) no history of cardiac or renal disease; 2) a normal clinical examination; and 3) normal cardiac function on transthoracic echocardiography.

Patients with acute decompensated HFREF had 1) the presence of ≥3 signs or symptoms of volume overload (edema, jugular venous distention, orthopnea, rales or pulmonary

vascular congestion on chest X-ray); 2) plasma N-terminal of the prohormone of B-type natriuretic peptide (NT-proBNP) levels >1,000 ng/L; 3) a left ventricular ejection fraction ≤45%; and 4) a clinical diagnosis of heart failure with evidence of impaired left ventricular ejection fraction ≤40% within 6 months before inclusion 5) on optimal medical therapy according to current guideline recommendations (234, 235) 6) were hospitalized with an anticipated treatment strategy of intravenous loop diuretics. Exclusion criteria were 1) administration of intravenous diuretics before study inclusion; 2) mechanical ventilation; 3) inotropic or vasopressor support; 4) concurrent diagnosis of an acute coronary syndrome; 5) renal replacement therapy; or 6) ventricular assist devices, including the use of an intra-aortic balloon pump, at any time during the index hospitalization.

Ambulatory patients with chronic HFREF had 1) a clinical diagnosis of heart failure with evidence of impaired left ventricular ejection fraction ≤40% within 6 months before inclusion; 2) no hospital admission for worsening heart failure signs or symptoms within 6 months before inclusion; 3) stable New York Heart Association (NYHA) functional class I-III for ≥3 months; 4) unchanged pharmacological therapy with ACE-i, ARB, beta-blockers, MRA and diuretics during the last 3 months prior to inclusion; 5) optimal medical therapy according to current guideline recommendations (234, 235).

Study endpoint

Cardiovascular mortality and heart failure readmissions (defined as hospitalizations because of signs or symptoms of congestion or low cardiac output that warranted treatment with parenteral drugs) were prospectively registered in all study patients from inclusion up till 3 years after which they were censored.

Laboratory measurements

Venous blood samples were obtained at the moment of study inclusion with the patient in the supine position after an adaptation period of 30 minutes. Plasma NT-proBNP levels were measured by the Roche Diagnostics Assay (Roche, Rotkreuz, Switzerland). PRA was determined using the Gamma-coat*radio immunoassay (DiaSorin, Sallugia, Italy). Plasma aldosterone levels were assessed by the Aldosterone Maia radioimmunoassay (Adaltis, Rome, Italy).

Within the subpopulation of acute decompensated HFREF sequential venous blood samples were obtained before the start of intravenous therapy (baseline), after 3 days of decongestive therapy, and during ambulatory follow-up approximately 6 weeks after

discharge. Treating physicians were blinded to test results and treatment during hospitalization was at their own discretion.

Statistical analysis

Continuous variables are expressed as mean±standard deviation, if normally distributed, or otherwise by median [interquartile range]. Normality was assessed by the Shapiro-Wilk statistic. Categorical data are expressed as percentages and compared with the Pearson χ^2-test. One-way analysis of variance (ANOVA) testing or the Kruskal-Wallis *H* test were used as indicated. Repeated measures within the acute decompensated HFREF group were compared using the paired Student's *t*-test or the Wilcoxon signed-rank test as appropriate. Univariate and multivariate regression analysis was used to search for associations between PRA and anticipated predictors. Cumulative survival rates were calculated according to the Kaplan-Meier method with the log-rank test used for comparison among tertiles of PRA. Statistical significance was always set at a 2-tailed probability level of <0.05. All statistics were performed using SAS JMP Pro (version 11.2 for Windows).

RESULTS

Study population

Seventy-six healthy volunteers, 72 patients with acute decompensated HFREF and 78 ambulatory chronic HFREF patients were included. Table 1 summarizes their baseline characteristics. Compared to healthy controls, acute and chronic HFREF patients were older and had a severely impaired left ventricular ejection fraction (LVEF; 25±10 vs 33±7, respectively). Neurohormonal blocker use was high in both cohorts of HFrEF patients. However, compared to chronic ambulatory HFrEF patients, less patients with acute decompensated HFrEF were on maintenance therapy with ACE-inhibitor or ARB (50% vs 87%). Instead, 26% of acute decompensated patients were taking oral vasodilators (hydralazine and/or nitrates). Loop diuretic use was highest in the cohort of acute HFREF patients.

RAAS activation in distinct populations of heart failure with reduced ejection fraction

PRA was significantly higher in ambulatory chronic HFREF patients (7.6 ng/ml/h [2.2;18.1]) compared to acute decompensated HFREF patients (1.5 ng/ml/h [0.8;5.7]) or healthy volunteers (1.4 ng/ml/h [0.6;2.3]) (boh p <0.0001). There was no significant difference in PRA levels between acute decompensated HFREF patients and healthy volunteers (p=0.13)

(Figure 1). Plasma concentrations of aldosterone were the lowest in acute decompensated HFREF but not significantly different from healthy subjects (p=0.08) or ambulatory chronic HFrEF patients (p=0.19) and comparable among the 2 other groups (p=0.76). Overall, PRA was significantly associated with blood pressure and ACE-i/ARB dose after multivariate regression analysis. There was no significant association between PRA and either loop diuretic dose or NT-proBNP level (Table 2).

	Healthy volunteers	**Acute decompensated HFREF**	**Ambulatory chronic HFREF**
	n=76	**n=72**	**n=78**
Age (years)	42±16	67±11	66±12
Male gender	51%	76%	77%
Heart rate (bpm)	68±11	81±19	66±10
Systolic blood pressure (mmHg)	130±17	128±23	124±17
Diastolic blood pressure (mmHg)	76±10	71±15	63±12
Ischemic cardiomyopathy	N/A	58%	62%
Left ventricular ejection fraction (%)	65±6	25±10	33±7
Medical therapy			
ACE-i/ARB use (%)	0	50%	87%
≤50% of target dose		35%	47%
>50% of target dose		15%	40%
Beta-blocker use (%)	0	72%	97%
≤50% of target dose		57%	45%
>50% of target dose		15%	52%
MRA use (%)	0	49%	81%
Loop diuretic use (%)	0	64%	49%
Hydralazine/nitrate use (%)	0	26%	8%
Laboratory results			
Creatinine (mg/dl)	0.93±0.21	1.43±0.68	1.29±0.51
NT-proBNP (ng/l)	47 [30;73]	4011 [2018;10608]	608 [271;1,407]
PRA (ng/ml/h)	1.4 [0.6;2.3]	1.5 [0.8;5.7]	7.6 [2.2;18.1]
Plasma aldosterone (ng/l)	247 [165;346]	179 [134;292]	213 [144;374]

Table 1. Baseline characteristics of the study population. *ACE-i, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; HFREF, heart failure with reduced ejection fraction; MRA, mineralocorticoid receptor antagonist; NT-proBNP, N-terminal of the prohormone of B-type natriuretic peptide; NYHA, New York Heart Association; PRA, plasma renin activity*

	Univariate			Multivariate		
	Beta	**S.E.**	**p**	**Beta**	**S.E.**	**p**
Age (years)	0.58	0.94	0.54			
Male gender	0.02	0.02	0.17			
Left ventricular ejection fraction (%)	-2.55	0.71	0.0004	-1.27	0.84	0.133
Heart rate (bpm)	-1.38	0.10	0.155			
Systolic blood pressure (mmHg)	-3.85	1.06	0.0003			
Diastolic blood pressure (mmHg)	-4.20	0.94	<0.0001	-3.17	0.98	0.0015
ACE-i/ARB dose (% of guideline recommended dose)	4.54	0.96	<0.0001	3.15	1.18	0.0085
Beta-blocker dose (% of guideline recommended dose)	3.20	0.96	0.0010	-1.24	1.38	0.370
MRA dose (% of guideline recommended dose)	3.46	0.94	0,0003	1.11	1.22	0.363
Loop diuretic dose (mg bumetanide equivalents)	0.63	0.94	0.50			
Creatinine (mg/dl)	1.05	0.93	0.26			
NT-proBNP (ng/l)	-0.92	0.93	0.33			

Table 2. Univariate and multivariate regression analysis for significant determinants of plasma renin activity in the total study population. *Beta and standard error (S.E.) for continuous ariables reported per standard deviation change. MRA, mineralocorticoid receptor antagonist; NT-proBNP; N-terminal of the prohormone of B-type natriuretic peptide*

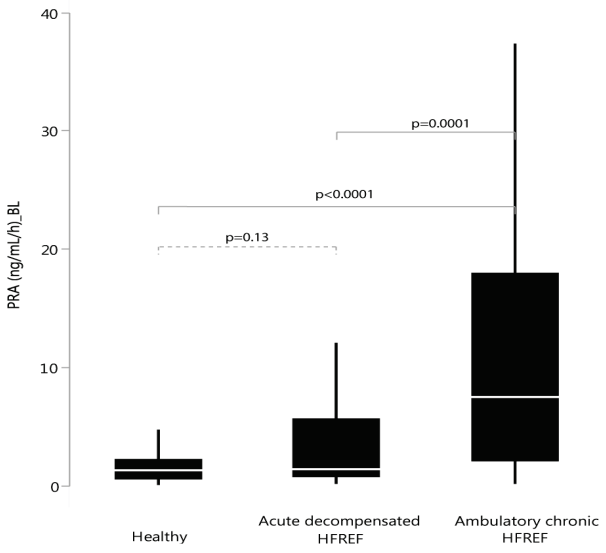

Figure 1. Plasma renin activity (PRA) in healthy volunteers, patients with acute decompensated HFREF and ambulatory chronic HFREF

PRA during decongestive therapy and uptitration of neurohormonal blockers in patients with acute decompensated heart failure

During decongestive treatment (from baseline to day 3) in patients with acute decompensated HFREF, the absolute and relative increase in PRA was +2.4 ng/ml/h [-0.2;6.9] and +107% [-12%;553%], respectively (both p <,0,001). Seventy-one percent of patients showed an increase in PRA during decongestion and uptitration of neurohormonal blockers (Figure 2, Table 3). Additionally, from day 3 until 6 weeks after discharge, neurohormonal blockers were further uptitrated, and 58% of patients showed an additional increase in PRA, which was however non-significant (+0,5 ng/ml/h [-2.3;7.8] (p=0.35)) (Figure 2). Taken together, from admission until 6 weeks follow up 79% of patients showed an increase in PRA (+4 [0.4;10.9] ng/ml/h or +238% [30;654] compared to baseline; p<0,001)]. Similar trends were observed with regards to serum aldosterone concentration.

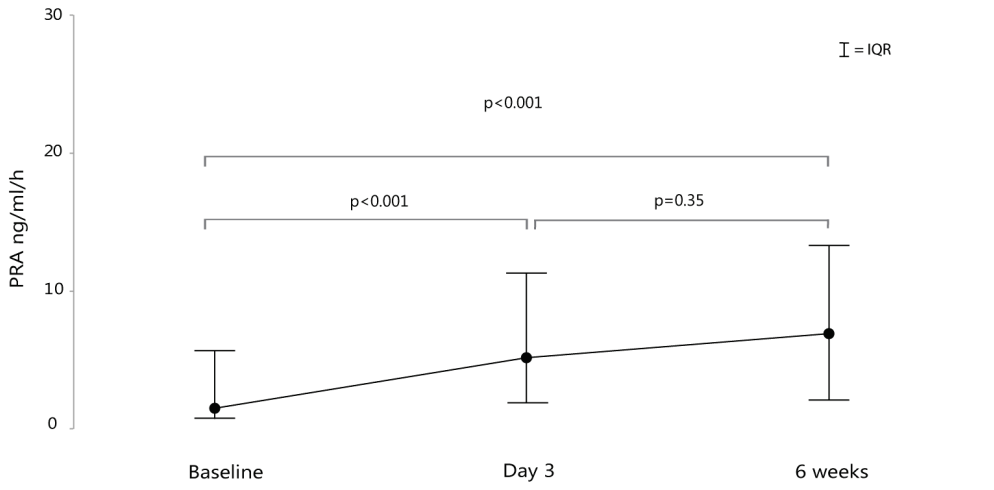

Figure 2. Plasma renin activity from admission until 6 weeks after discharge in patients with acute heart failure and reduced ejection fraction. *PRA levels rise during decongetive therapy and neurohumoral uptitration*

	Baseline (BL)	Day 3 (D3)	6 weeks (6W)	p BL-D3	p BL-6W	p D3-6W
ACE-I or ARB use	50%	68%	68%	<0.0001	<0.0001	<0.0001
○ ≤50% of target dose	35%	55%	38%			
○ >50% of target dose	15%	13%	30%			
Beta-blocker use	72%	88%	97%	0.0038	0.0038	<0.0001
○ ≤50% of target dose	57%	66%	68%			
○ >50% of target dose	15%	22%	29%			
MRA use	49%	90%	86%	<0.001	0.006	<0.0001
Loop diuretic use	64%	100%	70%		0.0005	
○ Loop diuretic dose (mg bumetanide equivalent)	1 [0;2]	N/A	0.5 [0;1]			
○ Cumulative loop diuretic dose used during D1-D3 (mg	N/A	4 [2;5]	N/A			
○ bumetanide equivalent)						

Table 3. Use of neurohormonal blockers and loop diuretics in patients presenting with acute decompensated heart failure and reduced ejection fraction at baseline (BL), after 3 days (D3) of decongestive treatment, and at 6 weeks (6W) after discharge

PRA and clinical outcome

During the entire follow-up period, 36 events occurred in patients with acute decompensated HFrEF (22 patients died from cardiovascular causes and 14 patients were readmitted for worsening heart failure). PRA levels at admission in the highest tertile were associated with a significantly increased event rate (log rank=0.035) (Figure 3). There was no difference in outcome between patients with a PRA rise versus decline during recompensation (from baseline until day 3) (log rank=0.96) (Figure 4, Table 3 Supplementary Appendix). In the subgroup with ambulatory chronic HFREF patients, 1 death and 6 heart failure readmissions occurred. PRA levels were not associated with clinical outcome (Log rank =0.99) (Figure 3). Baseline characteristics of acute decompensated and chronic stable HFrEF patients per tertile are presented in the supplementary appendices.

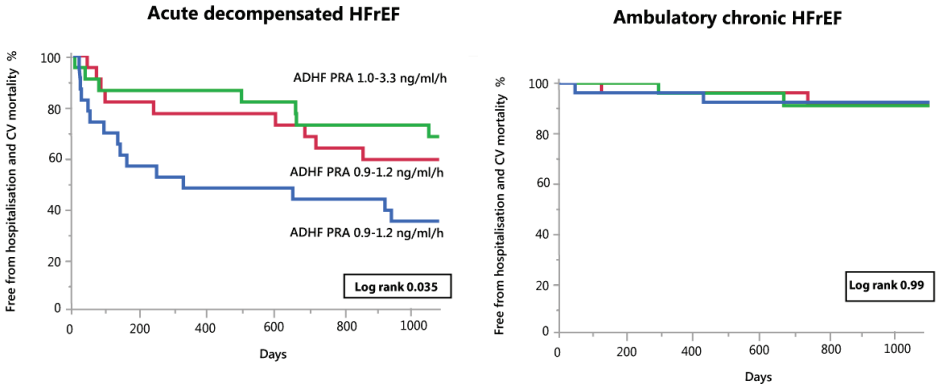

Figure 3. Kaplan-Meier curves for the combined endpoint of heart failure associated hospitalization and cardiovascular mortality in patients with acute decompensated HFREF (left panel) and ambulatory chronic HFREF (right panel) according to tertiles of PRA.

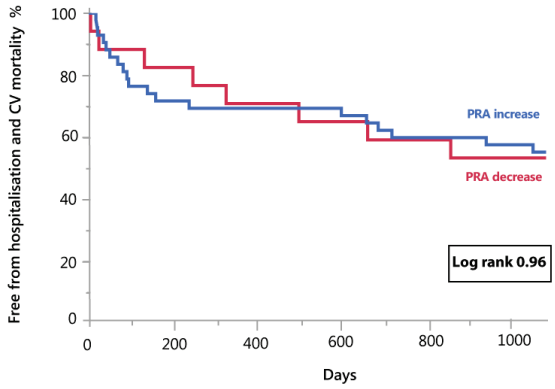

Figure 4. Kaplan-Meier curve for the combined endpoint of heart failure associated hospitalization and cardiovascular mortality in patients hospitalized for acute heart failure with PRA increase versus PRA decrease during decongestive therapy.

DISCUSSION

Longitudinal data from distinct well-characterized HFREF populations in the current era of treatment with neurohormonal blockers provide a unique opportunity to examine RAAS activation. The primary findings of this study are 1) significant PRA is present in ambulatory chronic HFREF patients without signs and symptoms of congestion, while PRA seems depressed during episodes of acute decompensated HFREF with clear signs of volume overload; 2) PRA levels only correlate significantly to worse outcomes in patients with acute decompensated HFREF before initiation of decongestive treatment; and 3) treatment with neurohormonal blockers significantly influence neurohormonal levels.

The renin-angiotensin-aldosterone system

To better appreciate the study results, a brief review of the RAAS is useful. Renin, an enzyme released by juxtaglomerular cells of the renal afferent arteriole, starts a cascade in which angiotensinogen is cleaved first into angiotensin I, which is further metabolized to angiotensin II by angiotensin-converting enzyme. Angiotensin II causes systemic and renal arteriolar vasoconstriction, promotes renal tubular sodium and water reabsorption, and is a potent stimulator of aldosterone release from the adrenal glands. Upon an acute drop of the cardiac output, RAAS activation helps to preserve organ perfusion in general and the glomerular filtration rate in particular (352-354). Renin is released from the afferent arteriole in response to 3 main stimuli: 1) decreased arterial blood pressure sensed by baroreceptor cells in the afferent arteriolar vessel wall; 2) decreased chloride concentrations in macula

densa cells lining the renal tubules at the end of Henle's loop; and 3) sympathetic nerve system activation (9, 225). As a result, renin release is physiologically inhibited by normal or elevated systemic blood pressure and a diet high in salt (355-357). Persistent and excessive RAAS activation causes adverse ventricular remodeling and contributes to fluid retention with signs and symptoms of congestion (358-364).

High plasma renin activity in ambulatory chronic heart failure with reduced ejection fraction

More than two decades ago, before the standard use of neurohormonal blockers in HFREF, Francis et al compared neurohormonal activation - including PRA - in healthy volunteers versus asymptomatic HFREF patients versus HFREF patients with signs and symptoms of congestion. The authors concluded that neurohormonal activation already occurred in patients with left ventricular dysfunction before the onset of symptoms, which was further exaggerated as overt heart failure ensued and diuretics were added to therapy (8). Remarkably, important increases in neurohormonal activation were mainly seen in the patients with symptomatic HFREF, while the PRA increases in asymptomatic patients were modest. This is in contrast to our findings in a contemporary cohort of HFREF patients, where the most pronounced PRA rise was observed in ambulatory chronic HFREF patients without signs and symptoms of congestion but well treated with ACE-I, ARB, beta-blockers and MRA.

Indeed, ACE-i and ARB tend to lower aldosterone concentrations, but increase PRA, while beta-blockers might lower both, and MRA increase both (365-371). Similarly, hypertensive patients treated with neurohormonal blockers demonstrate increased PRA and plasma aldosterone levels (372-374). Yet, the individual response to medication varies greatly due to genetic polymorphisms (347, 375-379). Our data corroborate this as we observed a wide spread in both PRA and plasma aldosterone levels among patients with ambulatory chronic HFREF. Therefore, most probably the PRA and serum aldosterone levels do not reflect disease-related RAAS activation and are not a reliable surrogate for downstream receptor activation (380, 381).

Plasma renin activity in acute heart failure with reduced ejection fraction

As neurohormonal activation is often perceived as the key driver in HFREF disease progression, it may seem odd that PRA and serum aldosterone levels are significantly lower in patients with signs and symptoms of congestion. Furthermore, this contradicts former observations in medication naive HFREF patients (8). Yet, most chronic HFREF patients in

the current study were on maximal tolerated dosages of neurohormonal blockers and also have rather low blood pressure. Both are powerful predictors of PRA levels in our overall population (Table 2). In contrast, most patients with acute decompensated HFREF present with elevated rather than low arterial blood pressure, a finding also present in the current study (6). Intriguingly, this might indicate that the RAAS in advanced HFREF treated with neurohormonal blockers remains appropriately responsive to hemodynamic changes including blood pressure and volume overload. Also, the most important increase in RAAS activation during the treatment of acute HFREF is seen in the first days of hospitalization, which seem to be linked to decongestive therapy (reduction in plasma volume as well as intensified diuretic therapy) and introduction and/or uptitration of neurohormonal blockers. Therefore, one might speculate that PRA could be a potential surrogate for effective circulatory volume assessment.

Prognostic value of PRA

PRA levels only correlate significantly to worse outcomes in patients with acute decompensated HFREF before decongestive treatment. High levels of neurohormones in stable HFREF patients are not predictive for rehospitalization due to water and salt retention or death. Also, the relation of high PRA levels and negative outcome in acute decompensated might not be driven by higher neurohormonal activation but rather reflect more advanced disease in this subgroup, reflecting volume overload and low pressure, both known to be related to worse outcome (50-51). In conclusion, it seems that the association between a RAAS biomarker and adverse outcomes applies only to the setting and not to its absolute value.

STUDY LIMITATIONS

We recruited and compared 2 groups of HFREF patients. Although we were able to characterize these groups in detail, it is uncertain to what extent observed differences in RAAS activation were due to heterogeneity between groups. The fact that all patients were recruited from a single institution and the limited sample size makes findings hypothesis-generating and ask for separate confirmation.

CONCLUSION

PRA is decreased in a state of acute decompensation compared to ambulatory chronic HFREF. An increase in PRA activity is observed in the majority of patients during

decongestive treatment and neurohormonal blocker uptitration. However, increased PRA is only associated with adverse outcomes in the setting of acute decompensated HFREF before initiation of decongestive treatment

SUPPLEMENTARY MATERIAL

	Lowest PRA tertile 0.2-0.9 ng/ml/h n=24	Median PRA tertile 1.0-3.3 ng/ml/h n=24	Highest PRA tertile >3.3 ng/ml/h n=24	p
Baseline characteristics				
- Age	68±14	65±13	69±12	0.70
- Male (%)	73	68	83	0.52
- Ischemic etiology (%)	64	45	57	0.81
- LVEF (%)	24±10	23±10	27±9	0.38
- Maximal aerobic capacity (ml/kg/min)	13±4	13±4	12±3	0.57
Baseline parameters				
- HR (bpm)	78±20	88±19	79±16	0.18
- Systolic Blood pressure (mmHg)	132±20	136±19	114±23	**0.001**
- Diastolic blood pressure (mmHg)	73±11	77±19	66±13	**0.04**
Baseline Medical therapy				
- ACE/ARB use (%)	50	45	59	0;24
○ ≤50% of target dose	20	31	50	
○ >50% of target dose	30	14	9	
- BB use (%)	73	50	87	0;23
○ ≤50% of target dose	55	36	61	
○ >50% of target dose	18	14	26	
- MRA use (%)	41	32	70	**0.03**
- Loop diuretic use (%)	64	55	74	0.40

Laboratory values				
- Creatinine (mg/dl)	1.4±0.9	1.3±0.5	1.6±0.6	0.43
- NT-proBNP (ng/l)	4317 [2649;7559]	2979 [1699;11498]	3189 [1428;13428]	0.89
- PRA (ng/ml/h)	0.6 [0.3;0.8]	1.5 [1.2;2.5]	9.5 [5.7;12;1]	**<0.001**
- Aldosterone (ng/ml)	154 [103;185,5]	173 [130;261]	398 [180;765]	**<0.001**

Table 1: Characteristics of acute decompensated HFREF patients according to PRA tertiles.*ACE-i, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; HR, heart rate; LVEF, left ventricular ejection fraction; MRA, mineralocorticoid receptor antagonist; NT-proBNP, N-terminal of the prohormone of B-type natriuretic peptide; PRA, plasma renin activity*

	Lowest PRA tertile 0.2-3.3 ng/ml/h n=26	**Median PRA tertile 3.5-15 ng/ml/h n=26**	**Highest PRA tertile >15.0 ng/ml/h n=26**	**p-value**
Baseline characteristics				
- Age	70±12	65±12	63±11	0.11
- Male (%)	70	64	85	0.18
- Ischemic HF etiology (%)	73	52	58	0.28
- LVEF (%)	33±8	33±8	31±5	0.65
- Maximal aerobic capacity (ml/kg/min)	14±3	15±4	16±6	0.43
Baseline parameters				
- HR (bpm)	65±9	67±11	67±9	0.70
- Systolic Blood pressure (mmhg)	132±18	120±16	119±15	**0.01**
- Diastolic blood pressure (mmhg)	67±11	62±11	60±13	0.08
Medical therapy				
- ACE/ARB use (%)	86	100	96	0.41
○ ≤50% of target dose	54	64	44	

○ >50% of target dose	32	36	52	
- BB use (%)	96	96	100	0.38
○ ≤50% of target dose	32	44	64	
○ >50% of target dose	64	52	36	
- MRA use (%)	83	87	95	0.99
- Loop diuretic use (%)	46	40	62	0.28
Laboratory values				
- Creatinine (mg/dl)	1.3±0.6	1.2±0.5	1.3±0.4	0.80
- Pro BNP (ng/l)	942[376;2595]	531[122;1104]	608[204;1504]	0.13
- PRA (ng/ml/h)	0.9[0.7;2.3]	7.6[5.1;11.4]	24.9[17.6;37.1]	**<0.001**
- Aldosterone (ng/ml)	189[126;259]	231[153;341]	348[157;507]	**0.03**

Table 2 Appendix: Characteristics of chronic ambulatory HFREF patients according to PRA tertiles *ACE-i, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; HR, heart rate; LVEF, lef ventricular ejection fraction; MRA, mineralocorticoid receptor antagonist; NT-proBNP, N-terminal of the prohormone of B-type natriuretic peptide; PRA, plasma renin activity*

	PRA increase during decongestive therapy n=42	**PRA decrease during decongestive therapy n=17**	**p**
Baseline characteristics			
- Age	68±13	65±13	0.38
- Male (%)	67	88	0.09
- Ischemic etiology (%)	62	41	0.31
- LVEF (%)	24±4	21±7	0.65
- Maximal aerobic capacity (ml/kg/min)	12±4	14±4	0.24

Baseline parameters			
- HR (bpm)	82±19	87±21	0.17
- Systolic Blood pressure (mmHg)	129±23	121±21	**0.04**
- Diastolic blood pressure (mmHg)	70±15	80±15	0.35
Baseline Medical therapy			
- ACE/ARB use (%)	43	53	0.48
○ ≤50% of target dose	29	47	
○ >50% of target dose	14	6	
- BB use (%)	71	65	0.59
○ ≤50% of target dose	62	41	
○ >50% of target dose	9	24	
- MRA use (%)	45	47	0.79
- Loop diuretic use baseline (%)	60	65	0.56
- Cumulative loop diuretic dose during 3 days of decongestive therapy (mg bumetanide equivalent)	4.0±2.8	4.2±3.1	0.97
Laboratory values			
- Creatinine (mg/dl)	1.4±0.6	1.3±0.6	0.87
- NT-proBNP (ng/l)	4997[2433;10659]	2979[1570;10729]	0.53
- PRA (ng/ml/h)	1.3[0.7;4.5]	3[1.2;8.7]	**0.02**
- Aldosterone (ng/ml)	172[132;293]	236[169;566]	0.26

Table 3 Appendix: Characteristics of acute HFREF patients according to PRA increase or decrease on day 3 of decongestive therapy compared to baseline PRA level *ACE-i, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; HR, heart rate; LVEF, lef ventricular ejection fraction; MRA, mineralocorticoid receptor antagonist; NT-proBNP, N-terminal of the prohormone of B-type natriuretic peptide; PRA, plasma renin activi*

CHAPTER 11

Neurohormonal profile in patients with heart failure and myocardial recovery after cardiac resynchronization therapy

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Submitted

ABSTRACT

Background: Heart failure (HF) patients with myocardial recovery (HFrecEF; LVEF ≥ 50%) have a distinct HF phenotype. Ten percent of HF patients with reduced ejection fraction (HFrEF) demonstrate myocardial recovery after cardiac resynchronization therapy (CRT).

Aims: This study aims to describe the neurohormonal profile of HFrecEF patient versus persistent HFrEF patients after CRT and healthy subjects.

Methods: Venous blood samples were collected in 75 healthy volunteers, 75 CRT patients with persistent reduced ejection fraction and 75 HFrecEF patients at least 6 months after CRT implant. The combined endpoint of all-cause mortality and HF hospitalizations was prospectively registered up to 2 years after inclusion.

Results: HFrecEF patients after CRT (LVEF 55±3) had significantly lower levels of PRA (3.3 (1.0±12.5) vs 7.7 (2.4;17.2) ng/ml/h, p=0.44), NTproBNP (213 (129;431) vs 973 (346;1903) ng/L, p<0.001) and hsTroponine (9(6;14) vs 14(9;21) ng/L;p=0001) compared to patients with persistent reduced LVEF (LVEF 31±8). However, these levels were still higher than in normal subjects (all p<0.001). The event rate in HFrecEF patients was significantly lower than in HFrEF patients (log-rank 0.04; RR 0.15 CI (0.01; 0.84); p=0.028).

Conclusion: Patients with myocardial recovery after CRT have excellent 2 year prognosis with significantly lower serum levels of neurohormonal activation and myocardial injury compared to patients with persistent reduced LVEF after resynchronization. However, these levels remain above the upper limit of normal.

INTRODUCTION

In the strictest sense, *myocardial recovery* is a return to both normal structure and normal function of the heart (382). Although the definition can differ in the literature, HF patients with recovered ejection fraction (HFrecEF) are commonly specified as *patients with a previous reduced left ventricular ejection fraction (LVEF) but with a recuperation to ≥50%.* Pharmacologic therapy, coronary revascularization and cardiac devices can all contribute to recovery. The prevalence of myocardial recovery in a general HFrEF population is estimated around 10% (383). These patients represent a distinct HF phenotype with biochemical properties and a natural history that differs from the traditional HF population such as HF with reduced and preserved ejection fraction (HFrEF and HFpEF) (383). However, these patients remain to have a higher event rate of heart failure admissions, suggesting that there is no true cardiac recovery (383).

In patients with dyssynchrony and a persistent LVEF<35% despite optimal neurohormonal blockade, cardiac resynchronization therapy (CRT) can lead to myocardial recovery in 10% of patients (384). Patients with HFrecEF after CRT appear to be a special subgroup of HF (and even among HFrecEF) patients. Whereas treatment with neurohormonal blockers did not improve LV function and geometry sufficiently, reduction of dyssynchrony by CRT did lead to myocardial recovery. This might indicate that dyssynchrony was the principal etiological factor of the cardiomyopathy in CRT-HFrecEF patients. Therefore, insights into neurohormonal activation and myocardial injury markers in CRT-HFrEF patients might provide further insight regarding pathophysiology and treatment strategies.

METHODS

Study design

This observational cohort study was carried out in a single tertiary care center (Ziekenhuis Oost-Limburg, Genk, Belgium). The study complies with the Declaration of Helsinki and the institutional review board approved the study protocol.

Healthy subjects were eligible for study inclusion if ≥18 years of age and recruited by general announcement. After confirmation of a normal clinical investigation and history as well as a normal cardiac function on echocardiography, subjects were included. CRT patients were recruited in the ambulatory CRT clinic by 2 dedicated device specialist after confirmation of

clinic stability and an echocardiographic assessment of LVEF by Biplane Simpson-method. All subjects provided written informed consent before any study-specific intervention was performed. After inclusion, a venous blood sample was obtained. CRT patients with myocardial recovery (CRT-HFrecEF) were compared with CRT patients with persistent reduced LVEF (CRT-HFrEF) and healthy subjects. Subjects were prospectively followed from the day of inclusion up till 2 years after inclusion.

Study population

Healthy volunteers had 1) no history of cardiac or renal disease; 2) a normal clinical examination; and 3) normal cardiac function on transthoracic echocardiography.

CRT-HFrEF patients had received CRT for a class I indication at least 6 months before inclusion and fulfilled all of the following inclusion criteria (385); 1) left ventricular ejection fraction <40% on transthoracic echocardiography; 2) no hospital admission for worsening heart failure signs or symptoms within the past 6 months; 3) optimal medical therapy according to current guideline recommendations including the maximally tolerated dose for neurohormonal blockers; 4) unchanged pharmacological therapy with ACE-i, ARB, beta-blockers, MRA and diuretics during the last 3 months (234, 235).

CRT-HFrecEF: Inclusion criteria were similar to CRT-HFrEF patients with the exception that LVEF was ≥ 50% on transthoracic echocardiograpy.

Laboratory measurements

Venous blood samples were obtained at the moment of study inclusion at least 6 months after CRT implantation, with the patient in the supine position after an adaptation period of 30 minutes. Plasma NT-proBNP levels were measured by the Roche Diagnostics Assay (Cobas proBNP II, Roche, Rotkreuz, Switzerland). PRA was determined using the RIAZEN immunoassay (ZenTech, Liège, Belgium), with an inter- and intra-assay coefficient of variation <6%. Plasma aldosterone levels were assessed by the MaiaZen radioimmunoassay (ZenTech, Liège, Belgium), with an inter- and intra-assay coefficient of variation <7%. Venous blood samples for high sensitivity troponin (hsTroponin) assessment were collected in heparin-coated vacutainer tubes. hsTroponin was measured using the Elecss Cobas 5th Generation hsTroponin assay (Roche Diagnostics, F Hoffmann-La Roche Ltd, Basel, Switzerland). The 99th percentile upper limit of normal is 14 ng/L for this assay, with a coefficient of variation <10% at 9ng/L.

Study endpoint

All-cause mortality and heart failure readmissions (defined as hospitalizations because of signs or symptoms of congestion or low cardiac output that warranted treatment with parenteral drugs) were prospectively registered in all study patients from inclusion up till 2 years (730 days) after inclusion.

Statistical analysis

Continuous variables are expressed as mean±standard deviation, if normally distributed, or otherwise by median [interquartile range]. Normality was assessed by the Shapiro-Wilk statistic. Categorical data are expressed as percentages and compared with the Pearson χ^2-test. One-way analysis of variance (ANOVA) testing or the Kruskal-Wallis *H* test were used as indicated. Univariate regression analysis was used to search for associations between PRA or serum aldosterone and anticipated predictors. Cumulative survival rates were calculated according to the Kaplan-Meier method with the log-rank test used for comparison among groups. The proportional hazards model was used to calculate the reduction is risk ratio for the combined endpoint in HFrecEF patients with corresponding 95% confidence intervals (CI). Statistical significance was always set at a 2-tailed probability level of <0.05. All statistics were performed using SAS JMP Pro (version 11.2 for Windows).

RESULTS

Seventy-five healthy subjects, 75 CRT-HFrEF patients and 75 CRT-HFrecEF patients were included. Table 1 summarizes their baseline characteristics.

	Healthy volunteers n=75	**CRT patients with myocardial recovery n=75**	**CRT patients with reduced LVEF n=75**	**p-value (CRT pts)**
Demographic characteristics				
Age (years)	43±15	67±23	69±11	0.570
Male gender	49%	49%	71%	**<0.001**

Medical History				
History of hypertension	/	36%	36%	0.939
History of Diabetes	/	12%	26%	**0.046**
History of COPD	/	15%	15%	0.957
Heart Failure characteristics				
Ischemic cardiomyopathy	/	7%	56%	**<0.001**
Left ventricular ejection fraction (%)	65±6	55±3	31±8	**<0.001**
NYHA functional class				**<0.001**
I or II	100%	100%	71%	
III or IV	0%	0%	29%	
Medical therapy				
ACE-i/ARB use (%)	/	90%	93%	0.625
≤50% of target dose		42%	58%	
>50% of target dose		48%	36%	
Beta-blocker use (%)	/	94%	97%	0.299
≤50% of target dose		47%	52%	
>50% of target dose		47%	45%	
MRA use (%)	/	64%	90%	0.265
Loop diuretic use (%)	/	14%	47%	**<0.001**
Clinical/laboratory measures				
Heart rate (bpm)	69±10	65±8	67±9	0.163
Mean arterial pressure (mmHg)	94±11	84±21	85±11	0.375
Creatinine (mg/dl)	0.9±0.2	1.1±0.3	1.4±0.6	**<0.001**
eGFR (ml/min/1.73m2)	97±15	66±16	56±22	**0.001**

Table 1: Baseline characteristics of the study population

Demographics, clinical measures and medical history

Healthy subjects were on average 43±15 years old, half were male. Compared to CRT-HFrEF patients (69±11 years), among CRT-HFrecEF patients (67±23 years) there was a higher percentages of females. Healthy subjects had a significantly higher blood pressure compared to CRT patients but there was no difference between both groups of CRT patients regarding mean arterial pressure (85±11 vs 84±21 mmHg, p=0.375). Patients with myocardial recovery after CRT had a significant better renal function (66±16 vs 56±22 ml/min/1.73m^2; p=0.001). The prevalence of diabetes was significantly lower in HFrecEF

patients (12 vs 26%;p=0.046) but the prevalence of hypertension and chronic obstructive pulmonary disease (COPD) was comparable between both CRT groups.

Heart Failure characteristics

All healthy subjects had a normal LVEF, and none took neurohormonal blockers or loop diuretics.

More CRT-HFrEF patients (mean LVEF 31±8%) had an ischemic cardiomyopathy compared to CRT-HFrecEF patients (mean LVEF 55±3%) (56 vs 7 %; p<0.001). No patients with myocardial recovery were functioning in NYHA class III or IV compared to 29% of CRT-HFrEF patients. Intake of neurohormonal blockers was comparable between groups and HFrecEF patients took significantly less loop diuretics (14% vs 47%; p<0.01)

Biochemical profile

Table 2 shows the biochemical profile of the different cohorts. In CRT-HFrecEF patients PRA and NT-proBNP were significantly lower than in CRT-HFrEF (3.3 (1.0;12.5) vs 7.7 (2.4;17.3) ng/ml/h and 213 (129;431) vs 973 (346;1903) ng/L respectively, both p< 0.05). However, these markers of the neurohormonal system were significantly higher than in healthy subjects. Thirthy-five % (26 out of 75) still had NT-proBNP values above 300 ng/L (considered as the cutoff of normal for patients >65 years of age) and 56 out of 75 (87%) of CRT-HFrecEF patients had values above the cutoff of strictly normal (135 ng/L). Serum aldosterone was not significantly different between both groups of CRT patients nor between CRT-HFrecEF patients and healthy controls. (Table 2, Figure 1). Uric acid was within the range of normal (95th percentile 2.6-6 mg/dl for woman and 3.5-7.2 mg/dl for men) in HF patients with myocardial recovery. HsTroponin was significantly lower in patients with HFrecEF compared to HFrEF patients but higher than normal if compared to healthy subjects. Based on univariate analyses, PRA was significantly associated with lower arterial blood pressure and age (Table 3).

Clinical course

In the subgroup with CRT-HFrEF patients, 2 HF related-deaths and 5 heart failure readmissions occurred over a follow-up period of 624±191 days. In the cohort of CRT-HFrecEF patients one death (non-cardiac) occurred but no hospitalization for HF related symptoms or signs were registered during a follow up of 580±218 days. Significantly less

events occurred in HFrecEF patients compared to HFrEF patients (log rank: 0.04; RR: 0.15, CI (0.01;0.84); p=0.028) (Figure 2).

	Healthy volunteers n=75	CRT patients with myocardial recovery n=75	P *	CRT patients with reduced LVEF n=75	p **
Biomarkers					
Uric acid (mg/dl)	5.0±1.1	6.1±1.4	<0.001	7.2±1.9	<0.001
NT-proBNP (ng/l)	47 (30;73)	213 (129;431)	<0.001	973 (346;1903)	<0.001
PRA (ng/ml/h)	1.4 (0.6;2.2)	3.3 (1.0;12.5)	<0.001	7.7 (2.4;17.2)	0.044
Plasma aldosterone (ng/l)	248 (167;346)	256 (198;371)	0.927	259 (154;431)	0.448
hsTroponin (ng/L)	3 (3;3)	9 (6;14)	0.004	14 (9;21)	0.001

Table 2: Biomarkers in Healthy volunteers, CRT patients with myocardial recovery and CRT patients with reduced LVEF *NT-proBNP: N terminal of the prohormone of brain natriuretic peptide; PRA: plasma renin activity * Between healthy subjects and HFrecEF patients ** Between both groups of CRT patients*

	Beta	S.E.	p
Age (years)	-0.11	0.03	**<0.001**
Male gender	0.01	0.01	0.351
Left ventricular ejection fraction (%)	-0.04	0.04	0.348
Heart rate (bpm)	0.03	0.03	0.331
Systolic blood pressure (mmHg)	-0.14	0.06	**0.011**
Diastolic blood pressure (mmHg)	-0.06	0.04	0.182
Mean arterial pressure (mmHg)	-0.09	0.04	**0.032**
ACE-i/ARB dose (% of guideline recommended dose)	-0.01	0.01	0.173
Beta-blocker dose (% of guideline recommended dose)	0.00	0.01	0.956
MRA dose (% of guideline recommended dose)	-0.03	0.02	0.062
Loop diuretic dose (mg bumetanide equivalents)	-0.01	0.01	0.279
eGFR (ml/min/1.73m^2)	0.03	0.06	0.617
NT-proBNP (ng/l)	1.47	4.32	0.734

Table 3: Determinants of plasma renin activity in CRT patients

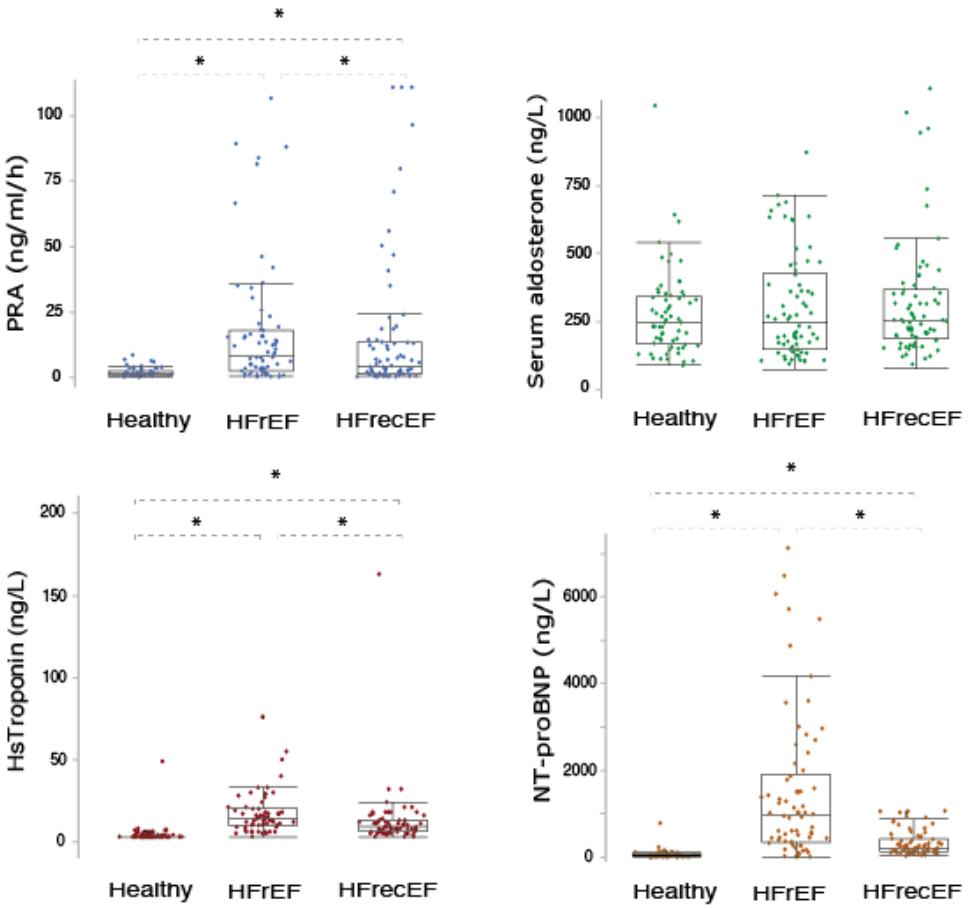
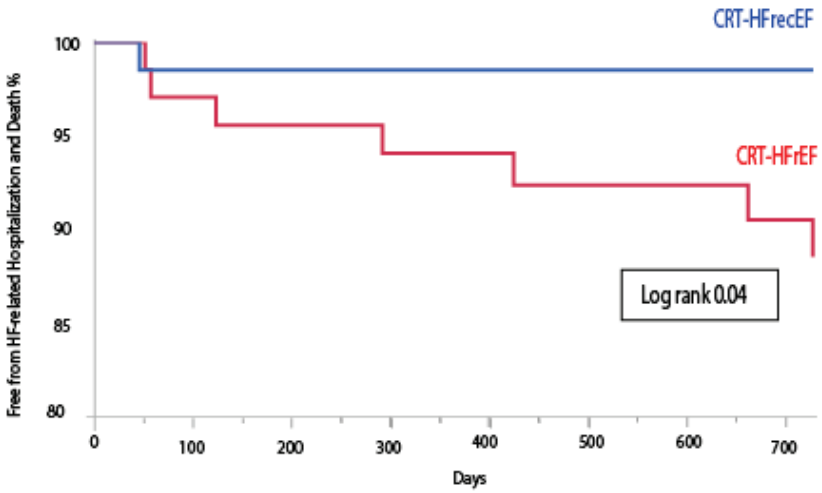


Figure 1: A) PRA, serum aldosterone, hsTroponin and NT-proBNP levels in healthy subjects, HFrecEF patients after CRT and patients with persistent reduced LVEF after CRT. *HFrEF: Heart failure with reduced ejection fraction, HFrecEF Heart failure with recovered ejection fraction; hsTroponin: High sensitivity Troponin; NT-proBNP: N-terminal pro Brain natriuretic peptide; PRA: Plasma renin activity, ULN: Upper limit of normal . * indicates p<0.05*



Nr at risk	Baseline	1 month	6 months	12 months	18 months	24 months
CRT-HFrecEF	75	75	67	59	49	42
CRT-HFrEF	70	69	67	57	56	47

Figure 2: Kaplan Meier curve of HFrEF and HFrecEF patients after CRT for the combined endpoint of all cause death and HF-related hospitalizations

DISCUSSION

To our knowledge, this is the first study investigating the neurohormonal profile of HF-recovered patients after cardiac resynchronization therapy. The primary findings of this study are that: 1) HFrecEF patients after CRT have significantly less neurohormonal stimulation and levels of myocardial injury compared to CRT-HFrEF patients, 2) levels of neurohormonall activation and natriuretic peptides are not strictly normal in HFrecEF after CRT, and 3) the clinical course of CRT-HFrecEF patients is excellent.

Myocardial recovery after cardiac resynchronization therapy

All HF patients in our study received CRT for a class I indication implying that their LVEF was <35% despite optimal medical therapy with the highest tolerated dose of NH blockers. In 10% of patients with dyssynchrony and a persistent LVEF<35% despite optimal neurohormonal blockade, cardiac resynchronization therapy (CRT) can result in

normalization of LVEF (384). The effect of resynchronization on improvement in LVEF (≥15%) in this subgroup is spectacular compared to the average improvement in LVEF caused by CRT (+2.7% (1.9 to 2.4) or pharmacological therapy such as beta-blockers (bisoprolol +12%, CI (4.4 to 19.6) ; carvedilol +6.9%, CI (5.8 to 8.0), angiotensin converting enzyme-inhibitors (Enalapril +3.7% CI(1.5 to 5.9)), angiotensin receptor blockers (candesartan +4.0%, CI (0.5 to 7.5) or spironolactone (+3%, CI (1.9-4.1)) (386). In HFrecEF patients after CRT, it is likely that dyssynchrony is the prevailing factor in the etiology of their cardiomyopathy. Indeed, HFrecEF patients in our cohort had few other comorbidities or characteristics of other causes of a cardiomyopathy: patients were more likely to be having a non-ischemic pathology and had less renal insufficiency and diabetes as well as less pronounced negative cardiac remodeling.

Neurohormonal profile of HFrecEF patients after CRT

Heart failure (HF) is considered a multifactorial, systemic disease, in which neurohormonal and cellular mechanisms are activated. Neurohormonal activation is often perceived as the key driver in HFrEF disease progression. However, markers of neurohormonal activation have never been studied in HFrecEF patients in general. In our cohort, PRA was significantly lower in HFrecEF patients compared to CRT-patients with a persistent reduced LVEF. Levels of serum aldosterone were not different between CRT patients and healthy subjects. The lower PRA level might reflect downregulation or even normalization of neurohormonal activation coinciding with myocardial recovery. However, PRA levels in CRT-HFrecEF patients were still significantly higher than normal subjects.

PRA is the rate-limiting step of the RAAS-cascade and is released from the renal afferent arteriole in response to 3 main stimuli: 1) decreased arterial blood pressure sensed by baroreceptor cells in the afferent arteriolar vessel wall; 2) decreased chloride concentrations in macula densa cells lining the renal tubules at the end of Henle's loop; and 3) sympathetic nervous system activation (9, 225). Importantly, all CRT patients were on comparable medical therapy, had comparable blood pressure (MAP respectively 84±21 vs 85±11 mmHg, p=0.35) and plasma chloride concentration (103±3 vs 103±4 mmol/L, p=0.781). Therefore, differences in intake in neurohormonal blockers and blood pressure are unlikely to account for the observed differences in neurohormonal stimulation.

Levels of PRA were significantly higher in CRT-HFrecEF patients compared to normal subjects. Importantly, blood pressure differences (lower in CRT-HFrecEF) and intake of

neurohormonal blockers both might lead to increased PRA (372-374). Indeed, angiotensin converting enzyme (ACE) -inhibitors and angiotensin receptor blockers (ARB) tend to lower aldosterone concentrations, but increase PRA, while beta-blockers might lower both, and mineralocorticoid receptor antagonist increase both (365-371). Yet, the individual response to medication varies greatly due to genetic polymorphisms (347, 375-379). Our data corroborate this as we observed a wide spread in both PRA and plasma aldosterone levels among patients with HF-recovered. Secondly, PRA levels in CRT patients were significantly correlated with age and blood pressure. Indeed, a decrease in arterial blood pressure sensed by the baroreceptor cells in the afferent arteriolar wall of the renal vasculature is a strong stimulus for renin release (183). Therefore, the measured residual increase might be purely secondary to neurohormonal blocker therapy.

Biochemical markers of myocardial injury and oxidative stress in CRT-HFrecEF patients

HFrecEF patients after CRT have significantly lower levers of NT-proBNP, hsTroponin (a marker of myocardial injury) and Uric acid (a marker of oxidative stress) compared to CRT patients with reduced LVEF but significantly higher than healthy subjects. This is consistent with a previous study of Basuray and coauthors in which they demonstrated that HFrecEF patients (not necessarily after CRT) have higher than normal levels of these markers (383). They concluded that this suggested ongoing abnormalities of myocyte biology and hemodynamic disturbances. Notably, in our cohort the median levels of NT-proBNP, hsTroponin and uric acid were lower than described in the study of Basuray and in each case near the expected upper limit of normal for this cohort with a mean age approaching 70 years and mild kidney dysfunction. Therefore, HFrecEF patients specifically after CRT seem to have even a more extensive recovery from heart failure. This group probably consist of a spectrum of patients in whom heart failure may be "cured" due to resynchronization versus patients exhibiting only normalization of LVEF without true cure of HF.

Clinical course

Previous studies focused on recovery due to medical therapy observed a better event-free survival, though there remained to be a risk for HF (383, 387, 388). However, these patients constituted a different group of myocardial recovery patients with mainly improvement in LVEF after medical therapy. Event-free survival in HF-recovered patients

was excellent and significantly better than CRT patients with persistent reduced LVEF in our study. The event rate was much lower than described in previous studies. Only one non-cardiac death occurred which is even in line with expected for healthy subjects of this age group. This might indicate that even among HFrecEF patients, HFrecEF patients *after CRT* is a distinct subgroup with an underlying reversible cause and beneficial etiology of HF.

FUTURE PERSPECTIVES

Since in daily practice clinicians encounter more and more HFrecEF patients, there is much interest in the best treatment strategy for this subgroup. However, no data nor guidelines exists on this subject. Previous studies have demonstrated that if CRT is withheld and dyssynchrony reoccurs, LV systolic function and geometry rapidly declines (389, 390). However, if the primary cause of the cardiomyopathy is reversed and neurohormonal activation seems to almost have disappeared, neurohormonal blockers might not be necessary or even be the best treatment strategy for this subgroup. Indeed, neurohormonal blockers can have important side effects such as renal impairment, electrolyte disturbances, hypotension, bradycardia, etc. Moreover, patients often dislike lifelong intake of medication. A prospective randomized trial – "The Systematic withdrawal of neurohormonal blocker Therapy in Optimally responding Patients to Cardiac Resynchronization Therapy- trial (NCT02200822)" - is currently investigating the necessity of neurohormonal blocker therapy in HFrecEF patients after CRT.

STUDY LIMITATIONS

This was a small-sample single center study making our observations hypothesis-generating. First, the number of patients included was not based on a power analysis but decided based on the availability and logistic restraints in our center. In our center 710 CRT patients are in chronic follow up and are at least once scheduled for an appointment in the outpatient clinic, 75 patients fulfilled criteria of CRT-HFrEF patients (Biplane simpson LVEF ≥50%). To construct equivalent cohorts, the first 75 consecutive HFrEF patients and healthy subjects were included. Second, normal subjects were significantly younger than CRT patients. It has been described that with increasing age PRA and serum aldosterone levels drop (391). However, even with this limitation the principal observations from this study remain valid. Finally, our follow-up was limited to 2 years, the expected event rate was low and this study was not powered for outcome. Based on our observations the clinical course

was excellent but larger studies with longer follow up are necessary to determine if the clinical course of HFrecEF patients is similar to a normal population of that age.

CONCLUSION

Patients with myocardial recovery (LVEF $\geq$ 50%) after CRT have a different biochemical and neurohormonal profile compared to patients with persistent reduced LVEF after resynchronization. Although levels of neurohormonal activation, oxidative stress and myocardial injury are near the upper limit of normal, these remain significantly higher than healthy subjects. Nevertheless, the 2-year clinical course of this group is excellent.

CHAPTER 12

Heart failure with myocardial recovery

The patient whose heart failure has improved: What next?

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ABSTRACT

In an important number of heart failure (HF) patients substantial or complete myocardial recovery occurs. In the strictest sense, *myocardial recovery* is a return to both normal structure and function of the heart. HF patients with myocardial recovery (HFrecEF) are a distinct population of HF patients with different underlying etiologies, demographics, comorbidities, response to therapies and outcomes compared to HF patients with persistent reduced (HFrEF) or preserved ejection fraction (HFpEF). Improvement of left ventricular ejection fraction has been systematically linked to improved quality of life, lower rehospitalization rates and mortality. However, mortality and morbidity in HFrecEF patients remain higher than in the normal population. Also, persistent abnormalities in biomarker and gene expression profiles in these patients lends weight to the hypothesis that pathological processes are ongoing. Currently, there remains a lack of data to guide the management of HFrecEF patients. This review will discuss specific characteristics, pathophysiology, clinical implications and future needs for HFrecEF.

INTRODUCTION

Four to five decades ago, heart failure (HF) was a deadly disease with few options to stabilize the disease process, let alone improve or cure HF. However, due to the success of neurohumoral blockers and implantable devices, HF has become treatable. Only a minority of patients will rapidly decline despite therapy to end-stage HF warranting heart transplantation or mechanical circulatory support. In contrast, in a large part of HF patients, stabilization and often improvement of symptoms and cardiac dysfunction is possible. Moreover, in an important number of HF patients substantial or complete myocardial recovery occurs, and it is expected that this number of HF patients will further increase in the future. These patients differ from HF patients with persistent reduced ejection fraction (HFrEF) as well as preserved ejection fraction (HFpEF) in underlying mechanisms of cardiac dysfunction, comorbidities and prognosis. In this review we will discuss specific characteristics, pathophysiological and clinical implications and future needs for HF patients with myocardial recovery.

MYOCARDIAL RECOVERY AND DEFINITIONS

The main terminology used to describe HF is historical and based on clinical signs and symptoms as well as measurements of left ventricular ejection fraction (LVEF) (Table 1). Three distinct categories are defined: those with normal LVEF (considered as ≥50%; HF with preserved EF (HFpEF)), those with reduced LVEF (<40%) and recently, patients with an EF of 40-49% defined as HF with mid range ejection fraction (HFmrEF) or in the 2013 American guidelines defined as "HFpEF, improved" (41-49%) (13, 392). However, this group is probably a heterogeneous population consisting of patients with mild systolic heart failure and patients with improved HFrEF (392). It is well recognized that ejection fraction is dynamic over time with 39% of HFpEF patients progressing to an LVEF<50% and 39% of HFrEF patients progressing to an LVEF ≥ 50% at some point after diagnosis over a mean 5-year follow up (Figure 1) (393).

Reverse remodeling , the opposite of (negative or maladaptive) remodeling, is the process associated with a decrease in left ventricular volume and mass leading to a (more) normal elliptical shape of the ventricle which can occur spontaneously or due to medical or device therapy (394). In the strictest sense, *myocardial recovery* is a return to both normal structure and normal function of the heart (382). Due to the absence of a strict definition of patients with improved or recovered ejection fraction (HFrecEF) there remains to be

heterogeneity regarding the cut-off value for LVEF (≥40% to ≥50%) in the literature. However, a correct differentiation between HFrEF, HFpEF, HFmrEF and HFrecEF is important. All these patient categories often have different underlying etiologies, demographics, comorbidities, response to therapies and outcomes; which is crucial information for the patient as well as the treating physician. Moreover, correct definitions are necessary to stimulate research which can lead to the development of successful individualized management strategies in HF.

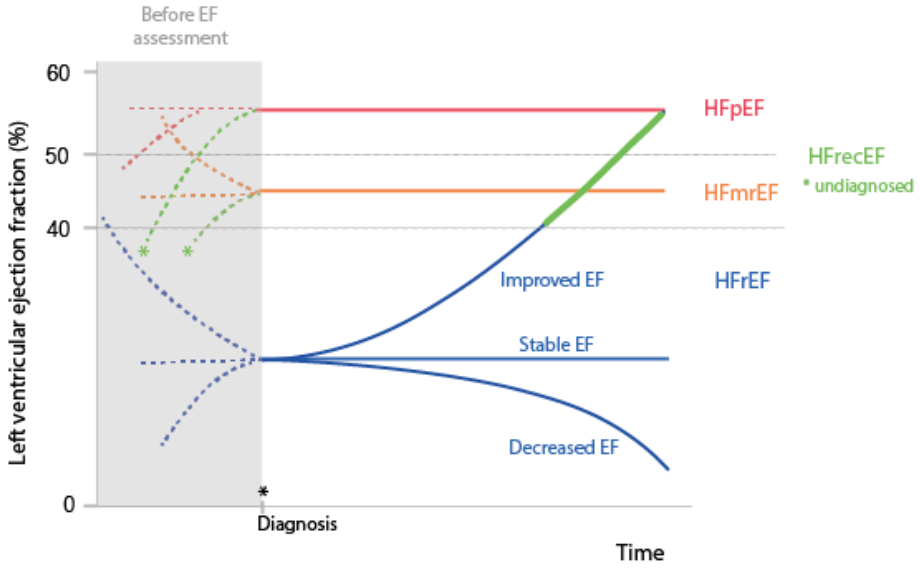

Figure 1. Heart failure definitions based on left ventricular ejection fraction and evolution over time. *EF: ejection fraction HFmrEF: Heart failure with mid range ejection fraction; HFpEF: Heart failure with preserved ejection fraction; HFrEF: Heart failure with reduced ejection fraction; HFrecEF: Heart failure with recovered ejection fraction.*

	Acronym	Criteria	LVEF cut-off	Definition adopted by current guidelines
Heart failure with reduced ejection fraction (Synonym: systolic HF)	**HFrEF**	Symptoms and signs of HF + LVEF </≤40%	- LVEF <40% (395) - LVEF ≤40% (234)	ACCF/AHA ` ESC
Heart failure with preserved ejection fraction (synonym: diastolic HF, Heart failure with normal ejection fraction)	**HFpEF** HFNEF	Symptoms and signs of HF + LVEF ≥50 % + elevated levels of natriuretic peptides + at least one of the following 1) relevant structural heart disease (left ventricular hypertrophy or left atrial enlargement) 2) diastolic dysfunction	LVEF ≥50 %(234, 395)	ACCF/AHA + ESC
Heart failure with mid range ejection fraction **Heart failure with preserved ejection fraction, borderline (234)**	**HFmrEF** HFpEF borderline	Symptoms and signs of HF + LVEF 40-49 % + elevated levels of natriuretic peptides + at least one of the following 1) relevant structureal heart disease (left ventricular hypertrophy or left atrial enlargement) 2) diastolic dysfunction	LVEF 40-49% (395) LVEF 41-49% (234)	ESC ACCF/AHA
Heart failure with recovered ejection fraction **(Synonym: HF with improved ejection fraction)**	**HFrecEF** HFpEF Improved (234)	Recovery is a substantial or complete improvement of left ventricular systolic function (395)	- Previous LVEF <40% but current LVEF ≥40% (234) or - Previous LVEF<40% but current LVEF ≥50%	ACCF/AHA

Table 1. Overview of definitions of heart failure. *The acronyms in bold are used throughout the manuscript*

MYOCARDIAL PROCESSES ASSOCIATED WITH REVERSE REMODELING

The progression of HF is associated with LV remodeling, which manifests as gradual increases in left ventricular end-diastolic and end-systolic volumes, wall thinning, and a change in chamber geometry to a more spherical, less elongated shape. This process is usually associated with a progressive decline in LVEF. Different triggers can lead to a decline in LVEF and the process of remodeling (Figure 2). The process is influenced by hemodynamic load, neurohumoral activation and other factors. Due to continuous maladaptive remodeling, myocardial dysfunction is usually a progressive condition. In contrast, the biology of myocardial recovery is not well understood. It is likely a spectrum of improvement with (partial) reversal of biological processes which occur in the failing heart. These may be categorized into those that occur in cardiac myocyte versus changes within the extracellular matrix of the myocardium (Figure 2) (394). During the process of reverse remodeling several studies showed that changes within cardiac myocytes, extracellular matrix but also genetic and proteomic alterations (partly) reverses (394). Recovery of structure and function probably occurs easier in hearts with fewer pre-existing myocyte and extracellular matrix derangements (396). Perhaps the purest example is a Takotsubo cardiomyopathy, in which an acute stressor leads to severe regional LV dysfunction with return to normal LV structure and function once the insult resolves (397).

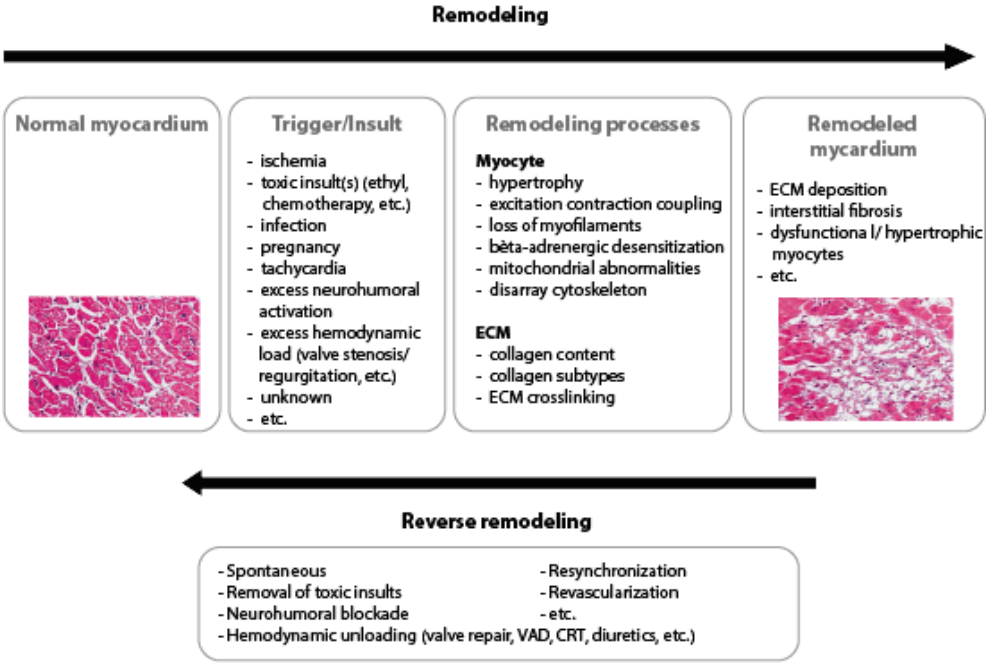

Figure 2. Factors involved in the process of remodeling and reverse remodeling. *The changes that occur in the biology of the failing cardiac myocyte include 1) cell hypertrophy 2) changes in excitation-contraction coupling leading to alteration in the contractile properties of the myocyte 3) progressive loss of myofilaments 4) beta-adrenergic desensitization 5) abnormal myocardial energetics secondary to mitochondrial abnormalities and altered substrate metabolism and 6) progressive loss and/or disarray of the cytoskeleton. Changes within the ECM constitute the second important myocardial adaptation that occurs during cardiac remodeling and include changes in collagen content, the relative contents of different collagen subtypes, collagen cross-linking, and connections between cells and the extracellular matrix via integrins (394).*

IMPROVED EJECTION FRACTION OR CURED HF?

Phenotype versus genotype

Observational studies suggest that improved hearts, even those with normal LVEF ("phenotype"), are not truly normal despite parallel improvements at organ, tissue, and cellular level ("genotype"). As such, HFrecEF patients have higher than normal levels of brain natriuretic protein (BNP), Troponin I, soluble fms-like tyrosine kinase receptor and uric acid, although significantly lower than patients with HFrEF and HFpEF (383). Furthermore, subtle left ventricular systolic changes such as global longitudinal strain often remain decreased despite a recovery in LVEF (398). This suggest the ongoing of abnormalities in the salt and water homeostasis and abnormal myocyte biology in at least a subset of patients (383). Moreover, studies using microarrays to profile myocardial gene expression revealed that the reverse-remodeled heart is different from a normal or non-failing heart (399, 400).

PREVALENCE, PREDICTORS AND PROGNOSIS OF MYOCARDIAL RECOVERY

Improvement of LVEF has been increasingly observed in a variety of clinical settings over the past 10-15 years. In some etiologies of HF such as acute lymphocytic myocarditis, peripartum cardiomyopathy, some forms of toxic cardiomyopahties (e.g. ethanol or anthracyclines), tachycardia or hyperthyroidism associated cardiomyophaties; recovery and normalization of LV structure and function can occur spontaneously in up to 40-50% of patients (386, 401-403). HF with myocardial recovery (defined as a previous LVEF <40 but ≥40% at the time of study inclusion) was relatively prevalent in a single tertiary HF clinic setting (404). Within a sample of 358 HF patients, 56 were defined as HFpEF, 181 as HFrEF and 121 (34%) as HFrecEF. Another report on chronic HF patients identified 10% as HF

patients with myocardial recovery, however in this study HFrecEF was defined as a LVEF ≥ 50% and previously <50% (383).

Patients with myocardial recovery are typically younger than patients with HFpEF and HFrEF, and have a lower prevalence of comorbidities such as hypertension, diabetes and atrial fibrillation (404). Nonischemic origin of HF and no prior myocardial infarction were associated with improvement in LVEF in the large IMPROVE-HF cohort, a finding that supports the experience of many HF physicians (405). Also, myocardial recovery is more likely in patients with a shorter duration and less myocardial fibrosis (406)(43).

Improvement of LVEF has been systematically linked to improved quality of life and lower rehospitalization rates and mortality, regardless of how it is achieved (407). HFrecEF patients have often milder symptoms, with patients mainly functioning in New York Heart association (NYHA) class I or II (404). All-cause mortality, the need for cardiac transplantation or mechanical circulatory supports is lower in patients with HFrecEF (LVEF ≥50%) when compared to HFrEF and HFpEF (HR for HFrEF compared to HF recovered 4.1 (2.4-6.8); HR for HFpEF compared to HF recovered 2.3 (1.2-4.5)). However, patients with myocardial recovery still experienced a significant number of hospitalization for HF, with approximately 50% of this group being hospitalized by 6 years hinting towards ongoing subclinical alterations driving residual heart failure morbidity (383).

THE CONTRIBUTION OF DIFFERENT THERAPIES TO LVEF IMPROVEMENT

Renin-angiotensin-aldosterone inhibitors, beta-blockers, cardiac resynchronization therapy and ventricular assist devices have the potential to achieve reverse remodeling and to date, every therapy with mortality benefits (except for a cardioverter defibrillator) in HFrEF is capable of inducing reverse remodeling (386). Moreover, reverse remodeling strategies, whether medical or device-based therapies, seem to exhibit a dose-response relationship (408). Indeed, the higher the intake of neurohumoral blockers or effective biventricular pacing, the higher the chance of recovery. However, not every patient exposed to these therapies achieves myocardial recovery and reverse remodeling is not always durable.

Neurohumoral blockers

Optimal medical therapy appears to be a key component of achieving myocardial recovery. In the IMPROVE-HF (Registry to improve the Use of Evidence-Based Heart Failure Therapies in the Outpatient Setting) study, a large observational cohort of outpatients enrolled in a

performance measure intervention, almost one-third of patients experienced meaningful recovery of myocardial function with nearly doubling of LVEF (from 25% to 46%) (405). Beta-blockers are the medical therapy most strongly linked to reverse remodeling. In the magnetic resonance imaging substudy of the MERIT-HF trial, treatment with metoprolol for 6 months significantly increased LVEF by an average of 28% (409). Animal and human studies have revealed that sustained beta-blocker treatment improves cardiac myocyte contractility, contractile reserve and calcium handling in myopathic hearts (410-414). Several trials demonstrated early and sustained effects on LVEF and LV dimensions after initiation of angiotensin-converting-enzyme inhibtors (ACE-inhibitors) or angiotensin receptor blockers (ARB) (415, 416). Additionally, even when aldosterone receptor antagonists are added to background therapy with angiotensin receptor blockade or angiotensin converting enzyme inhibitors and beta blockers, significant beneficial effects on LV volume and function occur (417-420). Limited data regarding the reverse remodeling response following Sacubitril/valsartan exists as the PARADIGM-HF trial did not collect follow-up echocardiography data. However, in a rat model of myocardial infarction, 4 weeks of therapy with Sacubitril/valsartan resulted in significant improvement of ejection fraction and reduction of left ventricular end diastolic diameter. Undoubtedly, data regarding the effect of reverse remodeling and sacubitril/valsartan will arise in the future (414). Table 2 summarizes the effect of different pharmacotherapies on LVEF in large randomized trials.

Intervention	**Change in LVEF (95%CI)**	**Mean weeks follow up (range)**
Beta Blockers		
- Bisoprolol **(421)**	12.0(4.4 to 19.6)	52
- Carvedilol **(422-443)**	6.9(5.8 to 8.0)	30(13-52)
- Metoprolol **(444-447)**	4.5(1.8 to 7.1)	26(24-26)
ACE-inhibitors/ARB		
- Captopril **(448-452)**	3.3(0.3 to 6.4)	37(12-52)
- Enalapril **(415, 453-457)**	3.7 (1.5 to 5.9)	24(4-52)
- Valsartan **(416)**	1.3 (0.7 to 1.9)	78
- Candesartan**(458)**	4.0 (0.5 to 7.5)	26
Aldosterone receptor antagonist (418-420)	3.0 (1.9 to 4.1)	26(8-52)

Cardiac resynchronization therapy (CRT) (459-462)	2.7 (1.9 to 3.5)	21 (6-26)
- MIRACLE-ICD **(463)**	2.1	
- CARE-HF **(463)**	6.9	
- REVERSE **(464, 465)**	3.8	
- MADIT CRT **(466)**	11	
- CONTAK CD **(461)**	5.1	
- PATH CHF **(467)**	24	

Table 2. Average change in LVEF in response to Heart Failure therapies compared to placebo

Cardiac resynchronization therapy

Among HFrecEF patients, a very intriguing group are those with improved ejection fraction after cardiac resynchronization therapy (CRT). Indeed, patients eligible for CRT had persistent reduced ejection fraction ≤35% despite maximally tolerated neurohumoral blockers. Yet, systolic function often improves after implantation of a CRT. The average increase in LVEF in the large CRT trials (MIRACLE-ICD, CARE-HF, REVERSE and MADIT-CRT) ranged from +2 to +11% (463-466, 468) (Table 2). Substantial regression of myocardial dilatation and improvement of ejection fraction is likely the effect of resynchronization which initiates a cascade of positive effects such as improved contractility and filling of the ventricles, reduction of mitral regurgitation, decreased sympathetic nerve activity, and reduction of LV wall stress. (469, 470). Moreover, dog-models of CRT actually indicate that CRT is capable of targeting the molecular underpinnings of progressive left ventricular remodeling, hereby inducing reverse remodeling on a cellular level. CRT has been shown to improve calcium, sodium and potassium channel function, hereby improving calcium cycling and abbreviating action potential duration, with the latter associated with a lower pro-arrhythmogenic risk. Furthermore, CRT induces mitochondrial genome expression hereby improving myocardial substrate utilization and decreasing apoptotic signaling. (471-475).

Ventricular assist devices

Since hemodynamic overload is regarded as one of the most prominent stimuli for maladaptive remodeling, VADs are an effective mean to induce reverse remodeling. It has been demonstrated that the powerful mechanical unloading of the LV offered by VADs, accompanied by increments in dosages of HF medication which can then be tolerated by the HF patients, can be a major contribution towards the process of myocardial recovery

during mechanical support (476, 477). A number of reports have demonstrated the effect of VAD therapy on cellular and subcellular reverse remodeling. One prominent feature of reverse remodeling in VAD-supported hearts is regression of myocyte hypertrophy and cell lengths and this was associated with echocardiographically documented reduction in LV dimensions and mass (478, 479). Furthermore, LVADs are capable of enhancing long chain acylcarnitines use in the failing heart, which is indicative of a recovering myocardial metabolism (480). However, this improvement only allows for successful explanation of the LVAD in 1-2% of patients (481-483).

Revascularization

There is extensive clinical trial-based evidence supporting the potential for reverse remodeling in patients with chronic heart failure who have received surgical interventions (408). Significant improvement in systolic function in the days and weeks after myocardial infarction, as well as the potential for recovery after revascularization for patients with myocardial stunning or hibernation is observed (484, 485). Both medical and catheter-based revascularization techniques have been associated with significant rates of reverse remodeling following acute myocardial ischemia in patients with chronic ischemic heart disease (486-488). More viable myocardial segments indicate a greater likelihood of improved LV function following revascularization (489).

Life style

Relatively high rates of myocardial recovery and improved ejection fraction have also been associated with the discontinuation of ethanol. One of the largest series indicated a >50% rate of myocardial recovery following cessation of ethanol use ad a 6-fold better survival with abstinence compared with ongoing heavy ethanol use (490). Additionally, long-term moderate exercise training has been shown to induce reverse remodeling in patients with stable chronic heart failure (491).

Additionally, there are indications that several other (less or more experimental) therapies can influence left ventricular remodeling such as mitral clipping, intravenous iron substitution, kidney transplantation, etc (492-495).

Current literature on management strategies of patients with HFrecEF

There remains to be a lack of prospective data to guide the management of patients with improved ejection fraction or myocardial recovery. There is a paucity of evidence on treatment strategies for patients with an LVEF in the grey zone of 40-50% (HFmrEF) or full recovery (LVEF ≥50%).

Pharmocotherapy

The persistent abnormalities in biomarker profile in these patients lends weight to the hypothesis that HF pharmacotherapy should be continued. However, there are no prospective data to support this approach and data on long-term use of neurohormonal medications in HF recovered patients are lacking. Only small studies studied the effects of withdrawal of medication in stable heart failure patients with recovered LVEF (496). Swedberg et al withdrew beta-blockers from 15 patients whose HF had improved (mean LVEF 46±3%) (497). Echocardiography demonstrated an overall reduction in LVEF from 46±3% to 35±3% ($p<0.01$) after a mean of 72 days following beta-blocker withdrawal. Clinical features of HF recurred in 9 of the 15, with 1 sudden death. In a retrospective cohort study of 42 patients with dilated cardiomyopathy and improved LVEF (LVEF ≥40%), medication cessation was the only identified predictor of recurrence (498). A retrospective cohort study of 85 patients with LVEF recovery >45% evaluated outcome after LV recovery with no changes in baseline medical pharmacotherapy (499). Thirty-three patients (39%) patients developed a recurrence of LV systolic dysfunction. When divided by the presence or absence of recurrence of systolic dysfunction, both groups had comparable angiotensin converting enzyme inhibitors/angiotensin receptor blockers but a trend towards lower beta blockers and mineralocorticoid receptor antagonists. Therefore, in the absence of more robust prospective data, these studies suggest potential benefit of continuation of standard guideline recommended HF medications in patients with HF and myocardial recovery.

Cardiac device therapy

Discontinuation of biventricular pacing in patients with improved or even normalized ejection fractions is contraindicated. One study in patients with mean LVEF 40±15% showed that discontinuation of CRT during 4 weeks resulted in a rapid and progressive decline in ejection fraction (after 1 week 33±14% and after 4 weeks 30±12%) and increases in LV dimensions (500). Regarding the indication for CRT implantation, a large gap in evidence exists in patients with an LVEF of 35-50%. One small pilot study looked at the effects of CRT in patients with only mildly reduced LVEF beyond the current indications for CRT (n=15, LVEF

40±2%) (501). Biventricular pacing resulted in significant increase in LVEF and decrease in dyssynchrony. Patients reported a significant reduction in NYHA class, however if this results in an improvement of mortality and hospitalization rates is unclear. The MIRACLE EF (clinicaltrials.gov identifier: NCT01735916) and MADIT-ASIA (ClinicalTrials.gov. Identifier NCT01872234) trials were designed to address patients with HFmrEF, but both trials have been halted due to difficulties with enrolment. Therefore, up till now, for new implantation of cardiac devices current guidelines should be followed and these exclude HFmrEF with LBBB patients as well as patients with complete myocardial recovery if there is no additional pacing indication.

Patients with complete myocardial recovery after CRT are a specific subset of HFrecEF patients since these patients only demonstrated full recovery after resynchronization and not under optimal medical therapy. Currently a randomized prospective cohort study is investigating if neurohumoral blockers can safely be withdrawn in CRT patients with fully recovered heart function (clinical trial.gov identifier NCT02200822).

Additionally, many HF patients with implantable cardioverter-defibrillator (ICD) have an improved LVEF at the moment of battery change and therefore do not fulfill anymore guideline criteria for an ICD. In one study among 91 patients undergoing ICD generator exchange, 25 had LVEF improvement of at least 10% greater than 35%. The incidence of appropriate ICD shocks was the same between individual with or without recovery (502) In a cohort of 231 Veterans affairs patients, 26% no longer met guideline indications for ICD therapy at the time of generator exchange (503). Subjects without ongoing ICD indication received a smaller number of appropriate ICD therapies than patients with indications (2.8 vs 10.7% annually, $p<0.001$), but again, appropriate shocks were delivered in HF patients with myocardial recovery. Other studies report similar observations (504, 505) (503). However, only a substudy of the MADIT-CRT trial investigated the prevalence of arrhythmias in HF patients with myocardial recovery to an LVEF ≥50%. The investigators observed that only one ventricular arrhythmia event among 55 subjects occurred and therefore suggested that these patients could be considered for downgrade from CRT-defibrillator to CRT-pacemaker at the time of battery depletion, if the device was placed in primary prevention and ventricular arrhythmias have not been detected during the life-span of the device (384).

CLINICAL IMPLICATIONS AND MANAGEMENT STRATEGIES OF PATIENTS WITH MYOCARDIAL RECOVERY

Many patients with heart failure and reduced ejection fraction have some degree of myocardial recovery or improvement in LVEF. In our daily clinical practice, we will encounter more and more patients with HFrecEF. Althoug prognosis of these patients is better than HFrEF and HFpEF patients, outcome is not normal and periodic follow up with echocardiographic assessment of LV function remains necessary. Until prospective data is available, pharmacotherapy and device therapy as for HFrEF patients should be continued/applied. Should there be a reason to withdrawal medication following recovery of function, periodic screening of LV function remains necessary to ensure stability of cardiac function. In case of battery depletion of a ICD, a limited amount of evidence suggests that in patients with a LVEF≥50% a downgrade is justified if no prior arrhythmias have been detected.

Future research should focus at identifying the best diagnostic and treatment strategies. A one-size-fits-all approach for drug development and management strategies in HF patients, where patients with persistent reduced ejection fraction and myocardial recovery are combined, should be replaced by individualized strategies. Therefore, prospective randomized studies looking at the potential of therapy withdrawal or therapy selection and specific (laboratory or imaging) biomarkers of recovery are urgently warranted.

CHAPTER 13

Cardiac resynchronization therapy significantly improves contractility after myocardial recovery in heart failure patients

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Submitted

ABSTRACT

Background: Heart failure (HF) is associated with a reduction in left ventricular (LV) contractility and contractility reserve during exercise as evidenced by a blunted force-frequency response (FFR). It remains unclear if cardiac resynchronization therapy (CRT) still augments LV contractility once patients exhibited myocardial recovery (HFrecEF).

Objectives: This study sought to determine effects of CRT on contractile performance in HFrecEF compared to patients with persistent reduced LVEF (<50%).

Methods: 50 heart failure patients with implantation of CRT for a class I indication at least 6 months before, underwent echocardiographic examination during incremental AAI and DDD-CRT pacing at 70,90 and 110 beats/min. Contractility was determined by constructing the FFR from the ratio of the LV systolic pressure/end systolic volume index (contractility index, CI) at given heart rates.

Results: Myocardial recovery (HFrecEF patients, n=28, mean LVEF 60±5%) was associated with an upward shift of FFR during AAI pacing compared to patients with a LVEF <50% (n=22, mean LVEF 34±9 %) ($p<0.05$). DDD-CRT pacing shifted FFR even more upward at each heart rate in both HFrecEF patients and patients with LVEF<50% (all $p<0.05$). However, force-frequency amplification was only noticed during incremental CRT pacing in patients with HFrecEF ($p<0.001$ vs $p=0.300$).

Conclusion: HFrecEF after CRT is associated with an increased contractile performance compared to patients with persistent reduced LVEF. However, resynchronization remains necessary to induce significant force-frequency amplification despite apparent myocardial recovery.

INTRODUCTION

Heart failure is associated with a reduction in left ventricular (LV) contractility and exercise intolerance. Impaired contractility deficits secondary to myocyte contractile properties, dyssynchrony, changes in cardiac structure (dilation), abnormal energy utilization and neurohumoral disturbance is an important factor for the initiation and progression of heart failure (506-509). The ratio of Systolic blood pressure/left ventricular end systolic volume index (SBP/LVESVI) – *or contractility index (CI)*- has been demonstrated to be a non-invasive reproducible and reliable marker for LV contractile performance (474, 510-514). The CI at incremental heart rates *or force-frequency relationship (FFR,* represented by the line connecting the CI at different heart rates) is a measure of contractile reserve. Loss of *force-frequency amplification* (absence of a rising FFR or neutral/negative slope) can contribute to LV dysfunction and exercise intolerance (511).

Cardiac resynchronization therapy (CRT) leads to a restored coordination of LV contraction and increased diastolic filling time (DFT) improving LV contractile performance. Moreover, this ultimately leads to upregulation of contractility genes (474). Indeed, the benefit of CRT on contractility has been convincingly demonstrated (389, 390, 475, 515). Additionally, a substantial subset of patients (approximately 10%) demonstrate complete myocardial recovery (HF with recovered ejection fraction, HFrecEF) after CRT implantation in combination with optimal medical therapy (384). However, it is unclear if this represents a genuine normalization of myocardial function or only an improvement of EF (516). Further insights into LV contractile performance in HFrecEF patients during incremental heart rates with and without CRT might help to assess if CRT still has additional effects on contractility after LV systolic function has recuperated. Moreover, this may give also insights in the effect of cardiac resynchronization in patients currently not eligible for CRT such as those with a LVEF >35%.

METHODS

This study was carried out in a single tertiary care center (Ziekenhuis Oost-Limburg, Genk, Belgium) between September 2014 and September 2016. The study complies with the Declaration of Helsinki and the institutional review board approved the study protocol. Written informed consent was obtained from every patient before any study-specific action was performed.

Study population

In the outpatient clinic, CRT patients were screened by two certified cardiac device specialist. Consecutive patients were included. Inclusion criteria for HFrecEF were 1). Implantation for a class I indication for CRT at least 6 months before: LVEF <35%, a ventricular conduction delay with a left bundle branch block (LBBB) morphology and a QRS complex ≥130 msec. 2) positive remodeling 6 months after implantation defined as a left ventricular ejection fraction (LVEF) of ≥50% 3) on optimal medical therapy including maximal tolerated dosages of beta-blockers, angiotensin-converting enzyme inhibitors or angiotensin receptor blockers and spironolactone. Similarly, HFrEF patients were screened in the outpatient clinic in order to reach a total population of 50 patients. Inclusion criteria were identical except that there was no cutoff-level for LVEF. Exclusion criteria for both HFrEF and HFrecEF were: 1) atrial fibrillation 2) continuous or intermittent grade 2 or 3 atrio-ventricular block 3) symptoms suspicious for angina pectoris or known coronary stenotic lesions 4) clinical signs or symptoms of congestion or volume overload (elevated jugular venous pressure, rales, peripheral edema, NYHA class III or IV) 4) hospitalization or necessity of IV diuretics or medication adjustments for HF symptoms within the previous month.

Pacing protocol

In each patient the pacing protocol was performed in AAI and DDD-CRT or BIV pacing mode at predefined heart rates in the left decubitus position. The pacing protocol was performed in an identical manner in each patient. First, pacing was initiated in the AAI-mode at 70 bpm with a stepwise increase with 20 bpm every 4 minutes until the target heart rate of 110 bpm was reached. After 4 minutes of recovery, BIV pacing mode was initiated in the optimal AV and VV interval at rest, starting at 70 bpm with 20-beat increments every 4 minutes until a heart rate of 110 bpm. Heart rate increase was achieved through incremental pacing. During each step 3 blood pressure measurement (right arm) were recorded using an electronic upper-arm cuff sphygmomanometer.

Echocardiography protocol

During the pacing protocol two-dimensional echocardiography was performed with a commercially available system (Philips Healthcare, iE33w Androver, Massachusetts). Images were acquired in the left lateral decubitus position in the standard parasternal and apical views during each pacing step. Standard 2-dimensional and Doppler data, triggered to the QRS complex were analyzed by 2 independent experienced echocardiographers

blinded for each other study results. Measurements were averaged from 3 consecutive cycles. A comprehensive baseline echocardiography was performed. LV volumes were calculated from the apical 4 chamber view using the Simpson formula, and the LV end-systolic volume index (LVESVI) was calculated as the LVESV divided by the body surface are.

Contractility index (CI) and force-frequency relationship (FFR)

The contractility index (CI) is the ratio of the systolic blood pressure (average of 3 measurements) over LVESVI. To build the FFR, the CI at incremental heart rates was determined in AAI pacing and during DDD-CRT pacing and the slope of the linear curve was calculated. In addition, diastolic filling time (DFT) was assessed during each step (474, 511).

Statistical analyses

Continuous variables are expressed as mean ± standard deviation, if normally distributed, or otherwise by median [interquartile range]. Normality was assessed by the Shapiro-Wilk statistic. Categorical data are expressed as percentages and compared with the Pearson χ^2-test. Student's t test or the Wilcoxon-Mann-Whitney test were used as indicated. A paired t test for continuous data, Fisher exact test for categorical data were used for appropriate comparisons. Significance between the slope of FFR of different groups was calculated by a mixed model regression analyses. Statistical significance was always set at a 2-tailed probability level of <0.05. All statistics were performed using SAS JMP Pro (version 11.2 for Windows).

RESULTS

Study population

Fifty patients were enrolled at least 6 months after CRT implantation for a class I indication. All patients were functioning in NYHA class I or II, had no clinical signs of congestion or volume overload and relatively low levels of NTproBNP (281(145;597) ng/L). The average age was 64±22 years and mean ejection fraction 50±14%. In 27 % of patients an ischemic etiology was the underlying cause of the cardiomyopathy. The average QRS width at the moment of inclusion was 166±16.Medical treatment with renin-angiotensin-aldosterone inhibitors and beta-blockers was high.

Twenty-two (44%) patients had an ejection fraction <50% (34±9%) and 28 (66%) had a recovered ejection fraction ≥ 50% (60±5%) after CRT implantation. Patients with HFrecEF were more likely female, functioning in NYHA class I, suffering from a non-ischemic cause and had a significantly shorter intrinsic QRS width at the moment of CRT implant (157±18 vs 176±12, p<0.001) compared to patients with an LVEF <50%. Moreover, in the group with recovered ejection fraction, end-systolic and end-diastolic dimensions after CRT implantation were significantly smaller (both p<0.001) and time for ventricular filling was significantly longer (both p<0.001). Baseline characteristics are summarized in Table 1 and echocardiographic data in Table 2.

Variable	All Patients (n=50)	LVEF ≥ 50% (n=28)	LVEF<50% (n=22)	p
Age (years)	64±22	69±8	62±23	0.197
Male gender (%)	64%	46%	82%	0.011
BSA (kg/m2)	1.9±0.2	1.9±0.3	1.9±0.2	0.740
NYHA functional class	1(1;2)	1(1;2)	2(1;2)	0.009
Etiology				<0.001
Ischemic	27%	7%	50%	
Idiopatic dilated	73%	93%	50%	
Medication use (%)				
ACE-inhibitor/ARB	92%	93%	91%	0.801
Beta-blocker	96%	100%	91%	0.104
Spironolacton	80%	82%	74%	0.669
CRT-P/CRT-D (%)	50/50	60/40%	36/64%	0.087
Clinical/laboratory parameters				
SBP (mmHg)	126±17	121±17	132±17	0.045
HR (bpm)	62±8	61±10	64±6	0.104
NTproBNP (ng/L)	281(145;597)	261(148;497)	426(75;1244)	0.296

Table 1: Baseline patient characteristics

Variable	All Patients (n=50)	LVEF ≥ 50% (n=28)	LVEF<50% (n=22)	p
Echocardiography				
LVEDV (ml)	163±83	117±35	232±86	<0.001
LVESV (ml)	91±72	46±14	158±72	<0.001
LVEF (%)	50±14	60±5	34±9	<0.001
MR grade				
0/1-2/3-4(%)	76/24/0%	86%/14%/0	60/40/0%	0.038
DFT	483±134	532±134	401±95	0.002
ET	318±90	346±103	275±35	0.001
E/A	1.0±0.4	1.1±0.4	0.8±0.4	0.019
E/E'	9±4	11±4	6±2	<0.001
Intrinsic ECG				
Heart rate (Bpm)	62±8	62±8	64±6	0.104
Intrinsic PR-interval (msec)	180±38	182±32	178±42	0.586
Intrinsic QRS width (msec)*				
QRS <130 msec (n)	166±16	157±18	176±12	<0.001
	5	4	1	

Table 2: Baseline echocardiographic and clinical parameters. * QRS width measured on surface EC during AAI pacing at the lower rate of 40 bpm.

Intrinsic contractile performance and the effect of CRT pacing at heart rate of 70 beats per minute

Figure 1 illustrates the CI during AAI pacing at 70 bpm in the total population, HFrecEF patients and patients with a LVEF <50%. The intrinsic CI was significantly higher in HF patients with myocardial recovery vs <50%.

Additionally, DDD-CRT pacing was associated with a significant upward shift of the CI compared to AAI pacing in all groups (Figure 2 and supplemental material table 1). Compared to AAI pacing, CRT pacing resulted in an average increase of the CI of 10 (0;28) % (Total cohort, Table 3). The relative increase was higher in patients with an LVEF<50% than patients with a recovered ejection fraction (23 (9;28) vs 6 (-5;28) %; p= 0.059). The better contractile performance during DDD-CRT pacing compared to AAI was associated with a significant prolongation of DFT in both HFrecEF patients as patients with an LVEF<50% (Figure 2 and supplemental material Table 1).

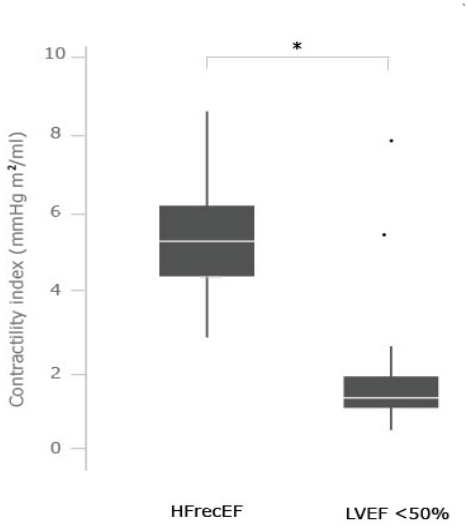

Figure 1: Intrinsic contractility index (AAI 70) in patients with recovered ejection fraction (HFrecEF, LVEF≥50%) versus LVEF <50%

	Relative CI increase 70 (%)	**p**	**Relative CI increase 90 (%)**	**p**	**Relative CI increase 110 (%)**	**p**
All	10 (0;28)		10 (-4;32)		35 (13;58)	
LVEF≥50	6 (-5;28)		12 (-3;32)		38 (10;58)	
LVEF<50	23 (9;28)	0.059	10 (-15; 36)	0.537	35 (18; 57)	0.726

Table 3: Relative (%) increase in contractility from AAI to CRT pacing at incremental heart rates. *p represents the p-value between the cohort of patients with LVEF ≥ vs < 50%*

Force-frequency relationship.

No single patient complained of angina during the pacing protocol or presented with evolutive ECG changes compatible with ischemia.

Diastolic filling time (DFT) during incremental pacing was significantly higher in DDD-CRT versus AAI pacing at intermediate heart rates and non-significantly higher at 110 bpm.

Overall incremental AAI pacing did not result in an amplification of the FFR (all $p>0.05$; Table 3). In contrast, incremental CRT pacing did result in amplification of FFR ($p<0.001$) .

However, if patients were divided based on LVEF < or ≥ , incremental CRT pacing, only resulted in a significantly better FFR in the HFrecEF (slope of FFR in LVEF<50% = 0.01 vs 0.06 in HFrecEF, p= 0.001) (Figure 2 and Supplemental Material Table 1). Thus, the contribution of CRT to the FFR was more pronounced in HFrecEF patients compared to patients with LVEF <50%.

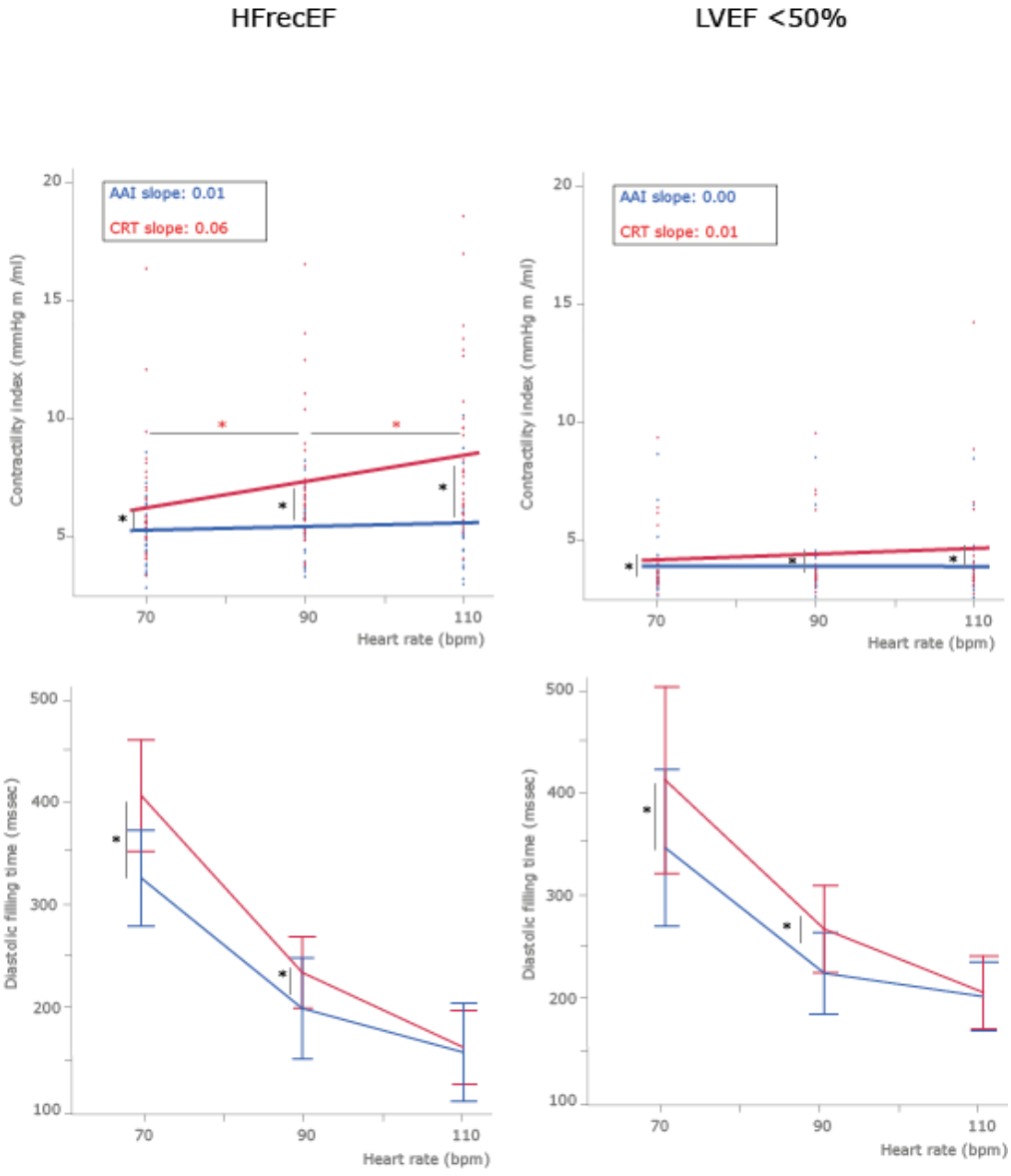

Figure 2: A. FFR and B. DFT during incremental heart rates in AAI and DDD-CRT pacing in patients with LVEF < and >50%.

DISCUSSION

This mechanistic study compared - in vivo- the acute effects of withholding CRT pacing on contractile performance in HFrecEF > 6 months after CRT implantation versus HF patients with persistent LVEF <50%. Our main observations are 1) HFrecEF patients demonstrate a significantly better contractile performance (without resynchronization) compared to HF patients with a LVEF<50% 2) Cardiac resynchronization remains to significantly contribute to force frequency amplification (i.e. contractility) at higher heart rates of patients with recovered ejection fraction after CRT.

Myocardial contractility and recovery

Contractility is the ability of the heart to eject a stroke volume at a given afterload and preload, which is particularly influenced by exercise. In clinical practice, contractility is often (erroneously) oversimplified and represented as the ejection fraction of the left ventricle. The normal heart demonstrates an intrinsic positive ventricular contractility phenomenon with increasing heart rates which is termed the "positive force-frequency relation" or "Bowditch-Treppe" phenomenon (517). In vivo and in vitro studies have demonstrated that in failing myocardium the FFR becomes greatly impaired and lead to impairment in exercise tolerance (518, 519). Increased left ventricular contractility is characterized by a smaller end-systolic volume and higher end-systolic pressure and previous studies have validated the ratio of SBP/LVESVI as a reproducible and reliable marker for LV contractile performance (474, 510-514) We corroborate previous findings that long-term biventricular pacing remains to induce an acute upward shift of LV contractile performance compared to RV-pacing and atrial pacing in patients warranting CRT (474, 519, 520). Re-coordination of left ventricular contraction and upregulation of contractile genes both contribute to the functional improvement after CRT (473, 474). Additionally, chronic CRT pacing still improved diastolic filling time. Importantly, optimizing of the DFT also contributes to myocardial contractile performance through several mechanisms: 1) increasing ventricular preload, 2) reducing "wasted time "of the cardiac cycle and increasing time for filling and ejection 3) preventing diastolic mitral regurgitation (382, 521).

The effect of CRT in patients with myocardial recovery after CRT

10% of CRT patients will demonstrate complete myocardial recovery (LVEF ≥50%) after CRT implantation (384). In the strictest sense, *myocardial recovery* is a return to both normal structure and normal function of the heart. It has been proposed that if myocardial recovery is restored, CRT may even be discontinued. However, for this idea to be true, myocardial recovery should indicate genuine "cure" and restoration of myocardial contractility (516). This study tries to fill the gap in current knowledge regarding contractile performance and the effects of resynchronization in patients with LVEF ≥50% after CRT by analyzing the myocardial performance with increasing heart rates or force-frequency relation. First, we observed that intrinsic myocardial contractile performance (assessed by the CI during AAI pacing) is significantly better in patients with HFrecEF compared to HF patients with LVEF. This likely indicates that more pronounced remodeling of geometric indices accompanies better recovery of functional indices such as myocardial contractility. Similar to CRT patients with a LVEF <50%, CRT pacing significantly increased the CI and DFT at incremental heart rates in HFrecEF patients. However, force-frequency amplification was only noted during DDD-CRT pacing in HFrecEF patients. These observations indicate that resynchronization of a heart with normal systolic function as assessed by LVEF, still importantly contributes to a better cardiac performance. Indeed, most patients (24/28) remained to exhibit mechanical dyssynchrony which was evident from the increased (>130 msec) intrinsic QRS width. In contrast to what one might logically think, the contribution of CRT to contractility was largest in patients with a normal LVEF. It has been demonstrated that intraventricular and atrio-ventriuclar dyssynchrony importantly influences myocardial performance and increases with incremental heart rates (522). Therefore, it appears that the continuous re-coordination of LV contraction in combination with an underlying better contractility can optimally be exploited in those with the best LVEF.

Is there an indication for CRT in patients with LVEF >35% or preserved ejection fraction?

The current European Society of Cardiology (ESC) guideline for HF patients recommend that CRT is indicated for patients with a LVEF ≤ 35% and a QRS duration of ≥130 msec with left bundle branch block QRS morphology (Class I, Level of evidence A for QRS≥150 and Class I level B for QRS 130-149) or a QRS duration of ≥150 msec irrespective of QRS morphology (Class IIa, level of evidence: B). Although HF patients with preserved ejection fraction (HFpEF) have a normal baseline ejection fraction, it has been demonstrated that HFpEF

patients with LBBB have increased mechanical dyssynchrony and impaired contractile reserve which may contribute to exertional intolerance (523, 524). Moreover, the prevalence of a QRS width ≥120 msec in HFpEF patients is 18% and is significantly associated with all-cause mortality (525). We demonstrated that resynchronization of a heart with normal systolic function but with persistent dyssynchrony can importantly contribute to a better cardiac performance, particularly during increased heart rates also observed during exercise. These observations might stimulate future research to see if the indication for CRT can be expanded to HF patients with LBBB and LVEF>35% or even in HFpEF patients.

STUDY LIMITATIONS

First, this was a small single-center mechanistic study, meaning that results should be interpret as hypothesis-generating. Second, the number of subjects included was not based on a power analysis, but determined by logistic limitations and participation of particularly HF with fully recovered ejection fraction in our center. Third, we did not measure LV contractility invasively. However, previous studies have shown that the ratio of SBP/LVESVI is a reliable and reproducible parameter of LV contractility. (474, 510-514) Fourth, we did not have a normal control group to compare with and see if the observed CI and FFR in HFrecEF patients can be interpret as normal. Fifth, we used atrial pacing to increase heart rate, which could, in theory, have introduced a rate-dependent and pacing mode dependent delay between atrial excitation and contraction. However, other ways to increase heart rate such as medication would influence adrenergic stimulation and therefore the inotropic response through a mechanism different from the Treppe effect.

CONCLUSION

HFrecEF after CRT is associated with increased myocardial performance compared to patients with persistent reduced LVEF. However, resynchronization remains to contribute significantly to force amplification in patients with recovered systolic function. Therefore, resynchronization therapy might also be meaningful in patients with LBBB and LVEF>35% and even within the normal range

SUPPLEMENTAL MATERIAL

	CI AAI-70	CI AAI-90	CI AAI-110	P (AAI 70 vs 90 vs 110)	CI CRT-70	CI CRT-90	CI CRT-110	P (CRT 70 vs 90 vs 110)	P (AAI-70 vs CRT 70)
All	4.0±2.2	4.2±2.3	4.2±2.4	0.078	4.5±2.4	4.8±2.9	5.7±3.5	<0.001	0.002
LVEF≥50	5.3±1.3	5.6±1.3	5.6±1.6	0.079	5.8±1.5	6.4±1.7	7.5±2.2	<0.001	0.022
LVEF<50	2.0±1.8	2.0±1.8	2.0±1.8	0.271	2.3±2.2	2.1±2.3	2.9±3.4	0.300	0.005

	DFT AAI-70	DFT CRT-70	p	DFT AAI 90	DFT CRT90	p	DFT AAI 110	DFT CRT 110	P
All	337±65	411±77	**<0.001**	212±46	252±42	**<0.001**	180±46	185±42	0.809
LVEF≥50	326±47	406±54	**<0.001**	199±49	234±34	**0.033**	156±47	161±36	0.861
LVEF<50	347±78	415±96	**0.023**	223±41	266±43	**0.002**	200±34	204±36	0.875

Table 1: Contractility index and diastolic filling time during AAI and CRT pacing at incremental heart rates

CHAPTER 14

Rationale and Design of the STOP-CRT Trial

Systematic Withdrawal of Neurohumoral Blocker **T**herapy in **O**ptimally responding **P**atients to **C**ardiac **R**esynchronization **T**herapy

Petra Nijst, Matthias Dupont, Wilfried Mullens

ABSTRACT

It remains unclear if in patients with myocardial recovery (HF-recovered) neurohumoral system activation normalizes and if neurohumoral blockers remain indicated. Previous observations demonstrated that levels of NT-proBNP and troponins are still elevated in HF-recoverd patients which might suggests ongoing abnormalities of salt-and water homeostasis and abnormal myocyte biology in at least a subgroup of HF-recovered patients. The objective of this study is to determine if patients with recuperated/normalized left ventricular function after implantation of cardiac resynchronization therapy, device treatment is sufficient and neurohumoral blocker therapy can safely be withdrawn. The STOP CRT - trial is a 2 by 2 randomized, open-label, controlled trial. HFrEF patients with a class I indication for CRT and completely normalized left ventricular ejection fraction (>50%) are randomized to withdrawal of beta blockers (STOP BB-arm) versus non-intervention and withdrawal of renin-angiotensin-aldosterone blockers (STOP RAAS-arm) versus non-intervention. Subjects are evaluated throughout a 2-year follow-up period after inclusion. *S*tudy endpoints are the occurrence of an increase of >15% in left ventricular end systolic volume, HF-related hospitalizations and all cause after 12 months of neurohumoral blocker withdrawal. This study will provide for the first timeprospective data to guide the management of HF patients who experience myocardial recovery.

INTRODUCTION

Heart failure (HF) is considered as a multifactorial, systemic disease, in which – after cardiac injury or malfunctioning- structural neurohumoral, cellular and molecular mechanisms are activated. Activation of neurohumoral systems – renine-angiotenin-aldosterone system (RAAS) and sympathetic nervous system (SNS) – plays a key role in the pathophysiological progression of HF. Indeed, neurohormonal antagonists: angiotensin converting enzyme inhibitors (ACE-inhibior) or angiotensin receptor blocker, beta blocker and mineralocorticoid receptor antagonists (MRA) are proven to be fundamentally important in modifying the course of HF with reduced ejection fraction (HFrEF) and reduce HF-related morbidity and mortality (13).

HF patients with recovered or normalized myocardial function (HF-recovered; LVEF≥50%) is a distinct entity from HFrEF and HF with preserved ejection fraction. In general, HF-recovered patients are younger, have a lower prevalence of comorbidities and have milder symptoms as compared to patients with HFrEF and HFpEF (404). It remains unclear if neurohormonal system activation normalizes when cardiac function recovers. However, these patients remain to have higher than normal levels of brain natriuretic peptide (BNP), troponine I and uric acid (383). Also, HF-recovered patients still experience a significant number of hospitalizations for HF (11).

Among HF-recovered patients, a very intriguing group are those with fully recovered ejection fraction after cardiac resynchronization therapy (CRT). Indeed, these patients had persistent reduced ejection fraction ≤35% despite maximally tolerated neurohumoral blockers. Yet, systolic function recuperated after implantation of a CRT. It remains unclear if after the primary culprit for cardiac dysfunction (bundle branch block delay with asynchrony) is neutralized by implantation of a CRT, neurohumoral system activation normalizes and device therapy alone would be sufficient. CRT appears to energetically advantage the failing heart enabling it to achieve improved cardiac output for a lower substrate consumption. 'Reverse remodeling' – the process of cardiac chamber volume decrease is present in …% of CRT patients and is often accompanied by improved beta-adrenergic responsiveness (ref). Similar to the study of Basuray, our group observed that HF-recovered patients after CRT have on average borderline elevated levels of NT-proBNP and troponins, which suggests ongoing abnormalities of salt-and water homeostasis and abnormal myocyte biology in at least a subgroup of HF-recovered patients.

The persistent abnormalities in biomarker profile in HF-recovered patients lends weight to the hypothesis that HF pharmacotherapy should be continued to maximize the likelihood of sustained myocardial recovery and clinical response. However, the data to support this approach is extremely limited, does not prove causality and may be confounded (476). Therefore, there is a need for prospective data to guide the management of HF patients who experience myocardial recovery.

PREVIOUS STUDIES ON WITHDRAWAL OF MEDICATION IN STABLE HF PATIENTS

Only a handful small studies studied the effects of withdrawal of medication in stable heart failure patients (496). To our knowledge, no trials were performed in patients with HF-recovered and a LVEF≥50%.

Raas-inhibitors

Withdrawal of captopril was associated with a sharp increase in RAAS activity within 1 day in 5 patients with HF (mean LVEF 22±2%) (526, 527). However, no worsening in clinical status was observed during the 2 days of observation. A second study found higher rates of worsening HF in a quinapril withdrawal group compared with the continuation group (33% vs 19%; P= 0.003) in 224 participants with stable chronic HF (mean LVEF 25±7%) (528). Clinical status deteriorated gradually over a 4 to 6-week period. The Carvedilol and ACE-Inhibitor Remodeling Mild Heart Failure Evaluation Trial (CARMEN) study compared enalapril, carvedilol, and enalapril/carvedilol combination in mild HF (mean LVEF 30±7%) (529). Participants randomized to the carvedilol group (n = 191) had their ACE inhibitor ceased before the trial (62% were taking an ACE inhibitor). Compared with the enalapril group, the carvedilol group showed a nonsignificant reduction in left ventricular end systolic volume index determined by transthoracic echo and an improvement in LVEF at 6 and 12 months, which, however, was not present at 18 months. The enalapril-carvedilol combination was superior to either alone.

Beta-blockers

Beta-blockers were withdrawn in 3 small uncontrolled open-label observational trials in idiopathic dilated cardiomyopathy (IDCM) with stable HF, with all trials observing deterioration in clinical signs of HF and reduction in LVEF. Swedberg et al withdrew beta-

blockers from 15 patients whose HF had improved (mean LVEF 46±3%) after 6 to 50 months of betablocker use, with continuation of background digitalis and diuretics (497). Clinical features of HF recurred in 9 of the 15, with 1 sudden death. Echocardiography demonstrated an overall reduction in LVEF from 46±3% to 35 6 3% (P<.01) after a mean of 72 (range 7-119) days following beta-blocker withdrawal. In another trial metoprolol was withdrawn in 24 patients, with 16 deteriorating (4 of whom died) after an average of 5.8 (range 1e12) months (530). Mean LVEF on echocardiography before withdrawal was 41±12%, decreasing to 32±13% after withdrawal (P <.01). The remaining 8 patients remained stable for a follow-up period ranging from 2.5 to 6.5 years. Morimoto et al withdrew metoprolol (mean dose of 61.5±34.1 mg/d) in a stepwise fashion over a period of 14 weeks in 13 patients, 10 of whom were in NYHA functional class I (531). Seven patients deteriorated (2 sudden deaths and 2 deaths from worsening HF) during the 4-month follow-up period. Six patients remained stable. Overall, LVEF fell from 38±14% to 34±14% (P < .05). Medication cessation was the only identified predictor of recurrence of HF in a retrospective study by Moon et al of 42 patients with IDCM and improved LVEF (498). All patients were receiving ACE inhibitors and about one-half receiving beta-blockers. Improvement was considered to be LVEF ≥40% and a net increase in LVEF of ≥10%. Of the 42 patients, 8 experienced recurrence of HF, defined as left ventricular systolic dysfunction with LVEF<40%. Of the 8 with recurrence, 5 had ceased HF medications. Amos et al retrospectively studied the outcomes of 55 patients with peripartum cardiomyopathy, 22 (45%) of whom had recovery of normal LVEF during a mean follow-up of 38± 28 months (532). Baseline LVEF was 23± 10% at initial presentation, and improved to 43% (no SD available) by 2 months. The authors stated that this group of 22 patients thereafter normalized their LVEF (however actual values not available). Fifteen patients then had further echocardiography, and of them, 11 had ceased either ACE inhibitor or beta-blocker and 5 had ceased both. No deterioration in LVEF was observed an average of 29 (range 5 to 63) months after recovery.

THE STOP CRT-TRIAL

The STOP CRT - trial is a 2 by 2 randomized, open-label, controlled trial assessing the necessity of neurohumoral blockers in patients with fully recovered ejection fraction after CRT. Subjects are evaluated throughout a 2-year follow-up period after inclusion. The study

is conceptualized as a non-inferiority study compared to current clinical guideline recommended practice.

Objective

The objective of this study is to determine if patients with recuperated/normalized left ventricular function, defined as an ejection fraction (EF) ≥ 50%, after implantation of cardiac resynchronization therapy, device treatment is sufficient and neurohumoral blocker therapy can safely be withdrawn.

Pre-defined Outcome measurements

	Description
Primary Outcome	a > 15% increase in left ventricular end systolic volume (LVESV) after 12 months of neurohumoral blocker withdrawal.
Secondary outcome	HF related hospitalizations defined as admission to hospital / presentation to emergency room with need for parental therapy after 12 months of neurohumoral withdrawal
	All cause mortality after 12 months of neurohumoral withdrawal
	Change in VO_2 max from baseline after 12 months of neurohumoral withdrawal.
Other outcome measurements	>15% increase in LVESV at 6 and 24 months after withdrawal of neurohumoral blocker therapy
	> 15% decrease in LVEF volume at 6 and 24 moths after withdrawal of neurohumoral blocker therapy
	mean blood pressure change volume at 6 and 24 moths after withdrawal of neurohumoral blocker therapy
	HF symptoms change based on New York Heart Association (NYHA) class at 6, 12 and 24 moths after withdrawal of neurohumoral blocker therapy
	incidence of heart rhythm events at 6, 12 and 24 moths after withdrawal of neurohumoral blocker therapy. Arrythmia detection was documented form device interrogation every 3-60 months and through home monitoring. o Pre-specified ventricular arrythmic end points are sustained VT with a ventricular tachycardia with rate >120 bpm, lasting >30 sec, ventricular fibriilation, appropriate anti-tachycardia pacing therapy, appropriate

	defibrillator shock therapy or sudden cardiac arrest, VES>3% of the time resulting in <97% of BIV pacing, or VT requiring restart of beta blockade decided by the treating cardiologist. o Pre-speficied supraventricular arrhythmic end points are sustained supraeventricular tachycardia with a rate >150 bpm lasting >30 sec, or SVT requiring restart of beta blockade decided by the treating cardiologist.
	Change in plasma concentrations of plasma renin activity and aldosterone at 6,12 and 24 moths after withdrawal of neurohumoral blocker therapy
	Change in NT-prBNP level at 6,12 and 24 moths after withdrawal of neurohumoral blocker therapy

Pre-defined interventions

1) In case of sinustachycardia ivabradine twice daily can be started
2) In case of hypertension one or two antihypertensive therapies (calciumantagonist, beta blocker if not in BB STOP arm, thiazide-type diuretic) can be started
3) Recommended tachycardia settings for the defibrillator are:
    a. VT1 zone starting at 160 bpm – monitorzone
    b. VT2 zone starting at 180-290 bpm – monitorzone
    c. VF zone starting at 222 bpm – ATP while charging, 6x36J

Pre-defined safety-endpoints requiring determination of 1 or both study arms

Pre-defined safety-endpoints requiring restart of RAAS-blockers and BB blockers

1) HF-related hospitalization
2) Increase of >15% in LVESV, LVEDV or LVEF

Pre-defined safety-endpoints requiring restart of RAAS-blockers

1) Presence of severe proteinuria requiring RAAS-inhibition
2) Inadequate tension control with the combination of two antihypertensive classes including a calciumantagonist, thiazide-type diuretic or beta-blocker

Pre-defined safety-endpoints requiring restart of BB blockers

1) Occurrence of one or more episodes of ventricular tachycardia (Non-sustained VT, VT or Vfib) lasting cumulatively 10 seconds or more.
2) Occurence of one or more episodes of SVT (sinustachycardia, VKF) lasting 30 seconds or more.

3) Occurrence of supraventricular or ventricular extrasystoles leading to a loss of BIV pacing of 3% or more of the time.
4) Occurrence of sinustachycardia with a mean heart rate >100 bpm under therapy with ivabradine
5) Inappropriate tachy-therapy (anti tachypacing or shock) insuperable with conservative tachycardia settings.

Sample size and power calculation

Based on the experience in our own center, it was anticipated that at most 5% of patients in a general population of CRT patients with normalized ejection fraction after implantation will show an increase of more than 15% in LVESV. With a 2:2 randomization, a 2-sided type 1 error rate of 0.10 and a non-inferiority limit of 12%, a total sample size of 80 subjects will provide 80 % power to exclude a significant difference between the control group and intervention groups (533, 534).

Study design

Eligibility

All patients were screened for eligibility in the outpatient clinic of 2 centers by dedicated heart failure specialists. (Ziekenhuis Oost Limburg, Genk and UZLeuven, Leuven) Patients were eligible for study inclusion if ≥18 years of age and able to give informed consent. All patients received CRT for a class I indication at least 6 months before inclusion (385) and fulfilled all of the following inclusion criteria; 1) left ventricular ejection fraction >50% on baseline transthoracic echocardiography; 2) no hospital admission for worsening heart failure signs or symptoms within the past 6 months 3) unchanged pharmacological therapy with ACE-i, ARB, beta-blockers, MRA and diuretics during the last 3 months 4) optimal medical therapy according to current guideline recommendations including the maximally tolerated dose for neurohormonal blockers (234, 235) 5) euvolemic state and functioning in NYHA class I or II.

Exclusion criteria for withdrawal of beta blockers were 1) severe ventricular arrythmia (sustained entricular tachycardia or ventricular fibrillation) occuring at the time LV function was normalized and 2) intermittent supraventricular tachycardia. Exclusion critera for withdrawal of RAAS-inhibitors were 1) diabetic nephropathy 2) proteinuria > 1g / 24 h. Exclusion criteria for all study-arms were: 1) ischemic cardiomyopathy with evidence

of scarring (scarring on MRI or severe hypokinesia/akinesia in >1 LV wall segment on echocardiography) 2) known severe coronary atherosclerosis (stenosis ≥ 80%).

Baseline evaluation

Baseline evaluation includes history, physical examination, vital signs and body weight assessment, review of medications, dyspnea assessment (NYHA class), transthoracic echocardiography, device interrogation, cardio-pulmonary exercise testing and collection of blood for measurements of renal function, electrolytes, NT-proBNP levels.

Randomization

Randomization was done by electronic block randomization to ensure equal sample size. All patients were randomized to both

- withdrawal of beta blockers (STOP BB-arm) versus non-intervention
- withdrawal of renin-angiotensin-aldosterone blockers (STOP RAAS-arm) versus non-intervention

Intervention and follow-up

Patients in the non-intervention arm continued neurohumoral blocker therapy at the maximum tolerated guideline recommended dose. Patients were seen at baseline, after 6, 12, 18 and 24 months in the outpatient clinic for clinical investigation, vital parameters, transthoracic echocardiogrphy, device analyses, cardiopulmonary exercise testing and venous blood sampling.

In Patients in the STOP BB-arm, beta-blockers were downtitrated in the reverse sequence of guideline recommended uptitration. Similarly, in patients in the STOP RAAS-arm, ACE-inhibitors/ARB and spironolactone were downtitrated in the reverse sequence of guideline recommended uptitration. Patients in the intervention-arms were seen at 6, 12, 18 and 24 months after cessation of therapy in the outpatient clinic for clinical investigation, vtial parameters, transthoracic echocardiogrphy, device analyses, cardiopulmonary exercise testing and venous blood sampling. Aditionnaly, patients were seen in the outpatient clinic directly after cessation of theray and after 3 and 9 months after cessation of therapy for safety concerns. At safety-visits clinical investigation, vital parameters, a transthoracic echocardiograpy, device check-up and venous blood sample were obtained.

Echocardiographic evaluation of LVESV and other parameters.

Two-dimensional echocardiographic exam was performed with a commercially available system (Philips Healthcare, iE33w Androver, MA) according to published guidelines. All analyzes were performed blinded and offline.

Left ventricular end systolic volume, left ventricular end diastolic volume and left ventricular ejection fraction was obtained by Simpson's biplane formula (194). All measurements were averaged over 3 consecutive beats. Diastolic function was assessed by use of the transmitral pulsed wave doppler signal (E and A wave), tissue velocity of the lateral and septal side (lateral and septal E') of the mitral annulus and the isovolumetric contraction and relaxation time (195, 196). Vena cava inferior diameter (IVC) and collapsibility during respiration were assessed from a subcostal view. CVP was estimated based on the guidelines provided by the American Society of Echocardiography (198). A IVC diameter > 2.1 cm that collapses <50% with a sniff was defined as an elevated CVP corresponding with a CVP of 15 mmHg. A IVC dimeter ≤2.1 cm that collapses >50% with a sniff suggests a normal CVP pressure of 3 mm Hg. Indeterminate cases in which the IVC diameter and collapse do not fit this definition were given an intermediate value of 8 mmHg. Right ventricular systolic pressure was calculated as the sum of the maximal trans-tricuspid continuous wave Doppler velocity and estimated CVP (198).

Laboratory analyses

All blood samples were obtained in the semi-supine position.

Plasma N-terminal of the prohormone of B-type natriuretic peptide (NT-proBNP) levels were measured by the Roche Diagnostics Assay (Roche, Rotkreuz, Switzerland). Plasma renin activity (PRA) was determined using the Gamma-coat*radio immunoassay (DiaSorin, Sallugia, Italy). Plasma aldosterone levels were assessed by the Aldosterone Maia radioimmunoassay (Adaltis, Rome, Italy). Estimated glomerular filtration rate was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula (161).

Device analyses

A comprehensive device interrogation was performed at each visit. The following variables were systematically recorded: % of biventricular and atrial pacing, programmed atrioventricular (AV) and VV-delays, measured paced AV- and sensed AV-delay. Events

were systematically analyzed from stored intra-cardiac electograms (EGM). All EGMs of stored events were discussed with an electrophysiologist and/or heart failure specialist with CRT-training, with final adjudication of the event at that time (ventricular versus supraventricular tachyarrhythmia). The appropriateness of device therapy (anti-tachypacing and shocks) if any occurred were also evaluated with final adjudication to appropriate or inappropriate therapy.

TRIAL STATUS

The first patient in the STOP CRT trial was enrolled on 24 of September 2014 and the last patient will be enrolled in the summer of 2017 . Follow up of patients is ongoing.

CONCLUSION

There is growing interest for prospective data on the necessity of treatment with neurohumoral blockers in patients with HF-recoverd. If the STOP CRT trial shows no difference between groups with neurohumoral blocker withdrawal and patients treated with guideline recommended therapy, this will challenge current understanding on neurohumoral activation in HF patients with recovered cardiac function. Furhtermore, this study can stimulate further investigation of individualized treatment strategies in patients with HF and recovered ejection fraction different from patients with heart failure and reduced or preserved ejection fraction.

CHAPTER 15

Leadless Left Ventricular Pacing: Another step towards improved CRT response

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CRT: WHERE DO WE COME FROM?

In 1986 it was observed in a canine pacing experiment that left ventricular pressure decreased linearly as the QRS duration increased (535). A decade later it was demonstrated in patients undergoing elective coronary artery revascularization surgery that epicardial biventricular pacing improved hemodynamics compared to right ventricular pacing (536). The Multisite Stimulation in Cardiomyopathy (MUSTIC) Trial, published in 2001, was the first of many large randomized clinical trials demonstrating clinical benefits of CRT in symptomatic heart failure patients with reduced left ventricular ejection fraction (LVEF) and increased QRS width (537). By coordinating left and right ventricular contraction as well as atrio-ventricular timing, CRT improves LV function, reverses adverse cellular remodeling and reduces HF hospitalizations and mortality (466, 538). However, a suboptimal response based on echocardiographic criteria of remodeling, still occurs in one-third of patients (539). The response to CRT depends on multiple factors such as patient selection, device programming, comorbidities and underlying HF therapy (4). Several lead-related issues including unsuitable coronary venous anatomy, LV scar tissue, and phrenic nerve capture also contribute to a suboptimal response to CRT which might be overcome by leadless pacing (6).

LEADLESS LV PACING: THE FUTURE?

In this issue of the *Journal*, Reddy et al present a prospective study with data of a wireless left endocardial pacing system (the WiSE-CRT system, EBR Systems, Sunnyvale, California) in conjunction with a co-implanted standard right ventricular pacing system in 35 patients (540). The system consists of a LV endocardial electrode, a subcutaneous battery and a subcutaneous pulse transmitter. The implantation takes place over two consecutive days with surgical subcutaneous implantation of the pulse generator system (two incisions; 1 for the battery, 1 for the transmitter requiring an acoustic parasternal window of 3 cm^2) followed by catheter placement of the LV endocardial pacing electrode. Biventricular pacing is achieved by sensing right ventricular (RV) pacing, followed by the immediate transmission of acoustic energy to the LV electrode, thereby achieving nearly simultaneous (5 milliseconds time difference) pacing of both ventricles. Procedural success was very high with 97% of successful implants within an acceptable period of time. After 6 months, 94% of patients received biventricular pacing. Additionally, two-thirds of patients had an increase in quality of life and/or reduction in New York Heart Association score and half of patients demonstrated a positive echocardiographic response at 6 months. Importantly, these

benefits were achieved in patients who were classified as non-responders to conventional CRT or in whom conventional CRT could not be deployed due to anatomical considerations. Therefore, the prospect of endocardial LV pacing with more flexibility with regards to LV pacing site selection away from scar tissue and phrenic nerve stimulation looks promising. Moreover, it has been demonstrated that endocardial pacing results in a more physiological myocardial activation pattern, and therefore improved acute hemodynamic function and a narrower QRS complex compared with epicardial pacing (541). Finally, endocardial pacing might be less pro-arrhythmogenic than epicardial pacing due to reduced dispersion of ventricular repolarization (542). Nevertheless, specific technological concerns regarding the ultrasound-based leadless LV pacing system and the high incidence of serious adverse events remain to be solved. First, almost 1 out of 10 patient was not suitable due to the absence of a good parasternal acoustic window for the transducer to stimulate the LV electrode. Additionally, 3 out of 35 patients encountered a defective transmitter circuitry within the first months. Moreover, it is unclear what the interference of for example exercise (as the relation between electrode and transducer may alter during forced breathing), pulmonary pathology or external radiation might be with the acoustic window and the systems' sensing and pacing performance. Also, energy transfer of ultra-sound mediated pacing systems is rather inefficient and might result in a short battery life with the need for frequent battery replacements and higher infectious risk compared to conventional systems (543). Second, the initial study of WiSE-CRT system in 2013 was stopped for safety reasons: 3 patients (18%) developed pericardial effusions associated with LV electrode delivery (544). While this problem was no longer observed in this study after redesign of the delivery system, serious adverse events still occurred in 1 out of 4 patients. The minority of events related to the procedure (femoral artery pseudo-aneurysm or fistula and catheter induced ventricular fibrillation) but 6 patients experienced a serious complication (1 embolization of the LV electrode, 3 pocket infections, 1 stroke and 1 death following catheter-induced ventricular fibrillation). Since placement and presence of pacing components in the endocardium of the left side of the heart will always carry a certain risk and the potential for thromboembolic complications, important improvements need to be made in the design and applicability of the system. Additionally, it remains unclear if sufficient % of biventricular pacing can be reached and efficient AV as well as VV optimization could be performed with this system. Finally, other leadless pacing systems such as single-component leadless pacing systems eliminating the need for pockets *and* leads (e.g. the Nanostim leadless cardiac pacemaker St Jude Medical, St. Paul Minnesota; and the MICRA transcatheter pacing

system, Medtronic) and the combination of a subcutaneous intracardiac defibrillator (ICD) and a leadless pacemaker allowing antibradycardia and –tachycardia pacing are currently being investigated (12,13,(545). Additionally, the subset of patients in which a conventional endovascular CRT implantation is not possible due to inadequate CS anatomy or CRT nonresponse is present due to suboptimal LV lead position/ phrenical nerve stimulation is rapidly declining as a result of technological advances in both delivery systems and LV lead technology (e.g. quadripolar leads).

OVERCOMING CRT NONRESPONSE: HOW TO GET THE BEST OUT OF THE MACHINE

Importantly, most often the ventricular dyssynchrony is only one of many contributing factors to HF and even an optimal resynchronization technology will not 'cure' HF. Indeed, the average increase in LVEF in the large CRT trials (MIRACLE-ICD, CARE-HF, REVERSE and MADIT-CRT) ranged only from +2 to +11% (386). It has been demonstrated that a variety of HF related factors contribute to a great extent to a suboptimal response to CRT (Figure 1) (546). Also, most attention has focused on pre-implantation characteristics to optimize CRT response, a variety of post-implant issues should be addressed in order to get the maximum benefit out of CRT. Most of these issues can be relatively easy, in a short period of time, with conventional equipment be diagnosed and treated (546). For instance, despite the convincing literature regarding the benefits in morbidity and mortality of higher dosages of neurohumoral blockers, the "simple" uptitration of these therapies after CRT implantation is still often overlooked but of great value to increase the response to CRT (547-549). Indeed, uptitration of neurohumoral blockers after CRT implantation is possible in the majority of patients, and associated with an improved clinical outcome, similar to patients treated with the guideline-recommended target dose at the time of CRT-implant *(550).* Second, AV optimization, resulting in improved LV filling, can also lead to improved CRT response in a subset of nonresponders with out-of–the-box timing intervals (546). Additionally, the medical or electrophysiological treatment of arrhythmias preventing efficient biventricular pacing will substantially impact response to CRT. Therefore, it has been demonstrated and advocated that a protocol driven and multidisciplinary approach including input of an electrophysiological and cardiac imaging expert, coupled with a heart failure disease management strategy, can provide insights in the reason(s) for a suboptimal response above and beyond the standard of care (546).

In conclusion, while many reasons for suboptimal response to CRT exist, leadless pacing may overcome problems related to LV lead placement, LV scarring or CS anatomy and will

most certainly expand the ability to improve the success of CRT. The WiSE CRT-system may be the first step towards a complete leadless CRT. However, once technical issues have been solved with these systems, randomized clinical trials will be necessary to definitively determine whether leadless systems will be superior to conventional pacemakers.

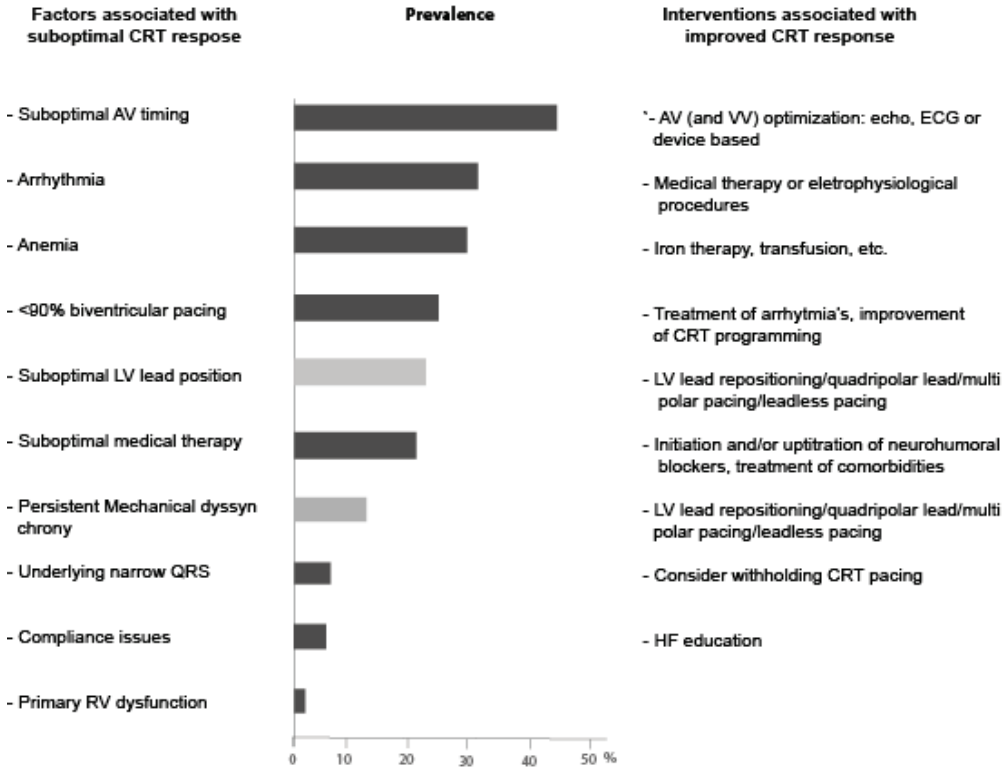

Figure 1: Factors associated with suboptimal CRT response and possible interventions. *Leadless left ventricular pacing might improve CRT response in patients with a suboptimal lead position, impossibility to place the left ventricular lead or persistent mechanical dyssynchrony. However, the majority of factors associated with suboptimal response to CRT should be addressed by other interventions. Adopted from Mullens et al. J Am Coll Card vol 53 No 9 2009 with permission*

GENERAL DISCUSSION

AND

SUMMARY

GENERAL DISCUSSION

The aim of this PhD thesis was to critically (re-)investigate the neurohumoral and hemodynamic alterations deemed responsible for typical HF symptoms and disease progression.

PART I | INTERSTITIAL SALT HANDLING IN HEART FAILURE

The objective of the first part of this thesis was to explore the role of interstitial sodium and fluid handling in the pathophysiology, clinical presentation and prognosis of HF.

In the past, the key abnormality in HF was understood to be neurohumoral upregulation promoting renal sodium retention, eventually leading to fluid overload. Generally, it is assumed that edema occurs when central venous pressures rises with coincident increases in Starling forces favoring transudation over the capillary membrane into the interstitial compartment exceeding lymphatic drainage. However, the occurrence of pulmonary and peripheral edema is poorly correlated with cardiac filling pressures. Moreover, total body sodium levels were found to be increased in observational studies of HF from more than sixty years ago. Interestingly, this increase was found in patients both with overt peripheral edema and without edema. Therefore, other factors might determine the occurrence of extravascular fluid overload and raised cardiac filling pressures.

The first chapter of this PhD thesis is the results of a comprehensive literature study. We were the first to hypothesize an important role for local interstitial factors in the presentation of HF. Recent insights suggest that sodium is not distributed in the body solely as free cations, but is also bound to large interstitial glycosaminoglycan (GAG) networks in different tissues. Interstitial glycosaminoglycan networks can function as sodium buffers, regulating interstitial fluid accumulation, lymphatic vessel formation, and endothelial function. GAGs are linear polymers of disaccharide units with variable lengths that are modified by sulfation and/or acetylation/deacetylation. The extremely polyanionic nature of these macromolecules leads to electrostatic interactions between, among others, sodium cations, but without accumulating excessive interstitial fluid due to the low compliance state of the network. Therefore, the classic idea of simultaneous sodium and fluid retention may not

always hold true as an explanation for fluid overload and increased cardiac filling pressures in HF. Additionally, we hypothesized that factors often present in patients with HF, such as chronic sodium overload and neurohumoral up-regulation can cause dysfunction of interstitial GAG networks, resulting in interstitial edema.

In the second chapter, we were able to demonstrate the previous hypothesis in an original in vivo study. We obtained skin biopsies and analyzed interstitial content as well as tissue water content in healthy subjects and HFrEF patients. Interstitial GAG content was significantly elevated in HF patients compared to normal controls and strongly correlated with tissue water content. Moreover, there appears to be a relationship between GAG function and the neurohumoral system. The latter observation is rather associative, but it may not be surprising since the main function of the RAAS is regulation of total body sodium which does certainly include extracellular fluid in the interstitial compartment.

In the third chapter we tried to study the role of the endothelial glycocalyx (eGC), a network of glycosaminoglycans at the surface of the endothelium. The eGC has multiple vasoprotective functions, and shields the underlying apical side of the endothelium from the plasma. It was recently demonstrated that the eGC also acts as an endothelial sodium buffer by binding positively charged sodium cations and therefore regulating sodium passage into the interstitium and endothelial cells, influencing endothelial stiffness and nitric oxide production. We hypothesized that increased plasma levels of sodium and natriuretic peptides can damage the eGC, which may be another pathway for endothelial and vascular dysfunction in HF. Based on these insights, we tried to study eGC with side darkfield video-microscopy in several patients. However, we were not able to accurately study this structure in vivo. It was however possible to obtain plasma shedding products of the glycocalyx in a group of healthy subjects and HFrEF patients. We observed increased levels of glycocalyx shedding products in HF patients compared to healthy controls, suggestive for glycocalyx disruption in a subset of HF patients. Moreover, shedding products were an independent predictor for worse clinical outcome independent of other established risk factors as well as HF related processes such as neurohumoral activation, myocardial injury and inflammation in our cohort.

Unfortunately, tools to evaluate interstitial function or glycocalyx integrity are non-existing in daily clinical practice. However, the knowledge that volume status and volume handling in HF patients goes beyond our classical thinking of Starling forces and renal salt retention might help to better understand the process of HF decompensation. Based on our insights

obtained within this PhD project, we strongly believe that a better understanding of the contributory role of the interstitium may help further unravel the pathophysiology of volume and salt handling in HF. Therefore, I hope that this preliminary work can stimulate further research regarding the function of the interstitial compartment, and subsequently lead to novel diagnostic techniques and therapeutic targets n HF patients. The hypothesis made in these first three chapters may have the potential to change the way clinicians will manage HF in the future.

PART II | VOLUME HANDLING IN HEART FAILURE

The objective of the second part of this thesis was to investigate the homeostasis of intravascular volume and to identify the effects of intravascular volume changes on central hemodynamics and renal function in Heart Failure.

It is assumed that sodium and water are retained in patients with HF, second to the disturbed cardio-renal interaction, with a corresponding increase in intravascular volume eventually causing increased cardiac filling pressures, pulmonary congestion and peripheral edema. However, whereas decompensated HF patients always present with elevated filling pressures, the occurrence of pulmonary and peripheral edema is poorly correlated with cardiac filling pressures. Moreover, recent evidence showed that the average increase in total body weight (total body water) at admission is around 1 kg and rather limited. It has been hypothesized that small amounts of intravascular volume, below the limit of detection of body weight change, contribute significantly to these pressure increases. Few studies have investigated intravascular volume status at the moment of decompensation, but no studies have provided insight in intravascular volume status in stable euvolemic HF patients or studied volume handling during the transition from euvolemia to intravascular volume expansion.

In chapter 4, we measured intravascular volume based on ^{99}Tc labeled RBC, the gold standard, in a group of stable, clinically euvolemic HFrEF patients and found that intravascular volume was on average normal but heterogeneously distributed. Moreover, we found that intravascular volume seems independent of HF therapy, neurohumoral activation and renal function. Second, to elucidate the relationship between intravascular volume and cardiac filling pressures we demonstrated in chapter 5 how the cardiovascular

system of stable HFrEF patients handles significant increases in intravascular volume of approximately 1 liter. Importantly, we observed that HFrEF patients with moderate to severely depressed LVEF can handle important increases in volume similar to healthy subjects and demonstrate a "cardiovascular volume reserve". Both studies provide important insights for current practice: 1) While intravascular volume has gained interest to serve as a potential therapeutic surrogate to assess euvolemia in HF patients, it seems that intravascular volume in patients with stable chronic HF is variable. Therefore, more studies are needed to evaluate plasma volume changes during decongestive therapy in clinical practice. 2) Moreover, our observations contradict the current mindset of many physicians that small increases in intravascular volume are the direct cause of rises in cardiac filling pressures and rapidly lead to clinical decompensation in HFrEF patients. Importantly, measurement of intracardiac pressures (by echocardiography or right heart catheterization) is frequently used to decide on further therapies in HF patients. Very often an increase in cardiac filling pressures leads to the administration of diuretics in an attempt to decrease intravascular volume. Hopefully our data can stimulate current HF specialists to use cardiac filling pressures together with other variables in their assessment of volume status. As such, a more tailored decongestive therapy of volume reduction (in case of volume overload with edema) and/or creating volume reserve by vasodilation (in case of high intracardiac pressures without volume overload) might be possible. 3) Additionally, the assessment of volume status in patients with HF is mainly deducted from cardiac pressure measurements (clinical signs and symptoms, echocardiographic estimations or invasive measurements). Based on our observations the predictive value of pressure(-estimates) to diagnose 1 liter of extra intravascular volume is low.

An important question to these observations might be: is intravascular volume expansion of 1 liter, not resulting in an acute rise in cardiac filling pressures, of any prognostic significance for HF patients? In Chapter 6 we demonstrated that even HFrEF patients on optimal medical therapy have an impaired ability to alleviate this excess of intravascular volume. The renal response relates to filtration function and tubular sodium handling. Additional to filtration function and tubular sodium handling, we investigated intrarenal flow patterns during the transition of intravascular euvolemia to intravascular volume expansion in chapter 7. We observed that after a mean increase of 0.6 Liter of intravascular volume there was a significant blunting of venous – not arterial - flow in HFrEF patients before a change in cardiac filling pressures could be demonstrated. Moreover, the impairment of venous flow was significantly correlated with less diuretic efficiency independent of

underlying renal function. In conclusion, 1) HF patients might accommodate plasma volume expansion without an immediate increase in cardiac filling pressures but they fail to elicit an appropriate natriuretic response to such an expansion. 2) diagnostic tools that evaluate intrarenal venous flow might help to detect early renal alteration as a result of changes in plasma volume before cardiac filling pressures rise. Importantly, this technique is easily applicable in clinical practice with standard echocardiographic equipment. In patients where the interpretation of volume status is difficult, the visualization of a discontinuous renal flow pattern might be an additional argument for intravascular (subclinical) volume overload.

These observations from chapter 7 are in line with previous research from our group showing that venous congestion and intrarenal hemodynamics are more important determinants for renal function in acute decompensated HF patients than cardiac output. In chapter 8 and 9 we had the opportunity to discuss the importance of different hemodynamic contributing determinants for renal function and volume handling in acute decompensated HF patients in an editorial and review paper.

PART III | NEUROHUMORAL ACTIVATION IN HEART FAILURE PATIENTS ON OPTIMAL MEDICAL THERAPY AND PATIENTS WITH HEART FAILURE AND RECOVERED EJECTION FRACTION

In the third part of this PhD thesis we tried to study neurohumoral activation and explore the contribution of different (medical and device-related) therapies to myocardial recovery in heart failure.

Unrestrained neurohumoral activation due to cardiac dysfunction is up till today considered as one of the key drivers of disease progression in HF with reduced ejection fraction. Blockers of the neurohumoral systems have consequently been established as the backbone of pharmacological treatment for his condition. It has been demonstrated many years ago that the degree of increase of activity of the neurohumoral systems are directly associated with prognosis in HF patients. However, this was long before the current era of combination therapy of neurohumoral blockers. Moreover, due to the success of neurohumoral blockers and device therapy, in a substantial number of HF patients complete myocardial recovery occurs. However, it is unclear to what degree the neurohumoral system remains activated in patients once recovery of cardiac function has occurred. No previous trials "re-studied"

neurohumoral activity in patients with HF under optimal medical therapy or in patients with myocardial recovery.

In the first chapter (chapter 10) of the third part of this thesis we measured plasma levels of neurohormones in distinct patient populations with HFrEF under optimal medical therapy. In contrast to previous observations before the era of neurohumoral blockers, we observed that patients with stable chronic HFrEF have higher levels of plasma neurohormones than acute decompensated HFrEF patients. Higher PRA levels were significantly associated with lower blood pressure and higher dosages of neurohumoral blockers. Moreover, congestion and volume overload caused an important decrease in levels of plasma renin activity. Since all these associative factors are rather positive it seems logic that high levels of neurohormones in stable chronic HFrEF patients are not associated with a negative prognosis. Based on a literature review on HF patients with myocardial recovery (Chapter 12) we found that no data exist on underlying neurohumoral activation in patients with myocardial recovery. Therefore, in a similar cohort study, we compared neurohormones in stable HFrEF patients with and without complete myocardial recovery after cardiac resynchronization therapy (Chapter 11). Interestingly, patients with complete myocardial recovery after CRT are rather a specific subset of HFrecEF patients since these patients only demonstrated full recovery after resynchronization and through optimal medical therapy. We found a limited but significant lower level of PRA in patients with complete myocardial recovery. This might indicate that the intrinsic neurohumoral activation is reduced together with recuperation of myocardial function. However, the presence of higher than normal levels of brain natriuretic peptide, troponin I and other markers in this patient group suggests ongoing abnormalities in the salt and water homeostasis, and abnormal myocyte biology in at least a subset of patients.

Therefore, we believe that plasma levels of neurohormones should not be obtained in current clinical practice to determine the prognosis of the individual HF patient since this depends on many influencing factors such as neurohumoral blocker therapy, blood pressure and underlying genetic polymorphisms. Since no data on neurohumoral activation in HF recovered patients exist nor has the necessity of neurohumoral blockers ever been evaluated in a prospective randomized manner, we set up the STOP CRT (Systematic withdrawal of neurohumoral blocker therapy in optimally responding patients to cardiac resynchronization therapy) trial. This double randomized, multicenter prospective interventional cohort study tries to determine if in patients with recuperated cardiac function,

neurohumoral blocker therapy can safely be withdrawn. Moreover, this trial gives the opportunity to study intrinsic neurohumoral activity in former HFrEF patients without interference of neurohumoral blocker intake. Eighty patients are currently included and followed during 2 years. The study methodology is described in Chapter 14. We hope to add further insights to current knowledge regarding the evolution of the neurohumoral activity and neurohumoral blocker necessity in patients with myocardial recovery based on the outcome of this study.

SUMMARY

Heart Failure is a prevalent disease with an important morbidity and mortality. The most important reasons for HF-related death and hospitalization are associated with increased cardiac filling pressures and volume overload. Traditionally, it is thought that unrestrained neurohumoral activation due to cardiac dysfunction stimulates renal sodium and water retention increasing intravascular volume and eventually causing increased cardiac filling pressures and extravascular edema.

In this thesis we add novel pathophysiological insights to current understanding of the disease processes associated with heart failure. First, we demonstrated that local interstitial factors contribute to the occurrence of interstitial edema. Moreover, we observed that increased markers of shedding of the endothelial glycocalyx are related to worse prognosis in HF patients independent of classic prognosticators. Second, we critically evaluated the contribution of intravascular volume alterations in HF through several mechanistic studies trying to improve the current diagnostic and therapeutical strategies. Importantly, limited but significant increases in intravascular volume do not rapidly lead to an increase of cardiovascular filling pressures since HFrEF patients have cardiovascular volume reserve. However, the increase of the intravascular volume rapidly blunts renal venous flow thereby reducing the capacity of the kidneys to excrete water and sodium. Additionally, the echographic assessment of intrarenal flow seems to be a better technique to evaluate intravascular volume than pressure estimates. Finally, measured plasma values of the neurohumoral system do not relate to intrinsic neurohumoral activation and prognosis in the current era of combination of different neurohumoral blocker therapies. There are indications that in patients with recovered cardiac function a reduction of the neurohumoral activitation occurs. Currently, we're conduction a prospective randomized clinical trial, in which we're investigating the necessity of continuation of neurohumoral blockers once cardiac function has normalized.

SAMENVATTING

Hartfalen is een zeer frequente aandoening met een belangrijke morbiditeit en mortaliteit. De belangrijkste oorzaken voor hospitalisatie en hartfalen-gerelateerde dood zijn geassocieerd met verhoogde vullingsdrukken en volume overbelasting. Traditioneel neemt men aan dat neurohormonale stimulatie ten gevolge van cardiale dysfunctie zout en water retentie ter hoogte van de nieren stimuleert waardoor intravasculair volume toeneemt en uiteindelijk vullingsdrukken stijgen en extravasculair oedeem optreedt.

In deze thesis hebben we getracht om nieuwe pathofysiologische inzichten te vinden in de huidige kennis van het ziekteproces binnen hartfalen. Eerst hebben we aangetoond dat lokale interstitiële factoren deels verantwoordelijk zijn voor het ontstaan van oedeem. Daarnaast hebben we geobserveerd dat toegenomen markers voor afbraak van de endotheliale glycocalyx geassocieerd zijn met een slechtere prognose in hartfalen patiënten en dit onafhankelijk van de klassieke prognosticatoren.

Vervolgens hebben we kritisch de bijdrage bestudeerd van intravasculaire volume veranderingen aan verschillende pathofysiologische factoren in hartfalen. Dit in een poging om de huidige diagnostiek en therapeutische strategieën voor hartfalen te verbeteren. We observeerden dat een beperkte maar significante toename in intravasculaire volume niet snel tot een toename in cardiovasculaire vullingsdrukken leidt, gezien HFrEF patiënten een cardiovasculaire volumereserve vertonen. Echter, deze toename in intravasculair volume hindert reeds de veneuze bloedstroom ter hoogte van de nieren en beïnvloedt de capaciteit van de nier voor water en zoutexcretie. Het echografisch onderzoek van renale veneuze flow lijkt een betere techniek voor het evalueren van beperkte intravasculaire volume veranderingen dan het volgen van intracardiale vullingsdrukken.

Tot slot toonden we aan dat gemeten plasmawaarden van het neurohormonale systeem niet relateren aan intrinsieke neurohormonale activatie en prognose in het huidige tijdperk van optimale neurohormonale blokkade. Er zijn indicaties dat bij patiënten met een gerecupereerde myocardiale functie ook een (gedeeltelijke) recuperatie van het neurohormonale systeem optreedt. In een prospectief gerandomiseerde studie proberen we de ontbrekende kennis in de literatuur aan te vullen betreffende de noodzaak tot inname van neurohormonale blokkers bij patiënten met een genormaliseerde cardiale functie.

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SCIENTIFIC ACHIEVEMENTS

Papers published in international peer-reviewed journals

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34. Stroobants S, **Nijst P**, Dens J. Slaapapneu: Sleutelrol van de cardioloog in de multidisciplinaire aanpak. TvC 2015; 27(4):183-189
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1. **Nijst P.** Verbrugge FH, Martens P, Bertrand PB, et al. Plasma volume in patients with stable heart failure is normal but heterogeneously distributed and anemia is highly prevalent. (Poster, BSC 2016, Brussels)
2. **Nijst P.** Verbrugge FH, Martens P, Bertrand PB, Dupont M, Penders J, Tang WH, Mullens W. Plasma Renin Activity in Distinct Patient Populations with Heart Failure and Reduced Ejection Fraction. (Poster, Annual congress of the European Heart Failure Association– HFA Florence 2016)
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6. **Nijst P,** Martens P, Verbrugge H, Dupont M, Tang W, Mullens W. Patients with Heart Failure and reduced ejection fraction can handle intravascular volume expansion (Annual congress of the American college of Cardiologie - ACC 2017 Washington)
7. **Nijst P,** J. Cops, P. Martens, Q. Swennen, M. Dupont, W. Tang, W. Mullens. Endovascular shedding markers in patients with heart failure with reduced ejection fraction. (Annual congress of the European Heart Failure Association - HFA 2017 Paris)

Oral presentations

8. **Nijst P,** Martens P, Verbrugge H, Dupont M, Tang W, Mullens W. Patients with Heart Failure and reduced ejection fraction can handle intravascular volume

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9. **Nijst P,** Martens P, Dupont M, Tang W, Mullens W Intrarenal venous flow alterations during transition from euvolemia to intravascular volume expansion in heart failure patients with reduced ejection fraction (Annual congress of the European Heart Failure Association - Moderated poster presentation - HFA Paris 2017)
10. **Nijst, P.** Martens, P. Hilkens, M. Olinevic, M. Dupont, W. Tang, I. Lambrichts, JP. Noben, W. Mullens. Dermal interstitial alterations in patients with heart failure and reduced ejection fraction are associated with volume status (Annual congress of the European Heart Failure Association - HFA Paris 2017 Young Investigator Award)

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- Research Grant Biotronik 2015
- Young Cardiologist in Training Award 2015 – Tijdschrift Van Cardiologie
- Finalist of the Young Investigator Award, Annual Meeting of the Belgian Society of Cardiology, Brussels 2017
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