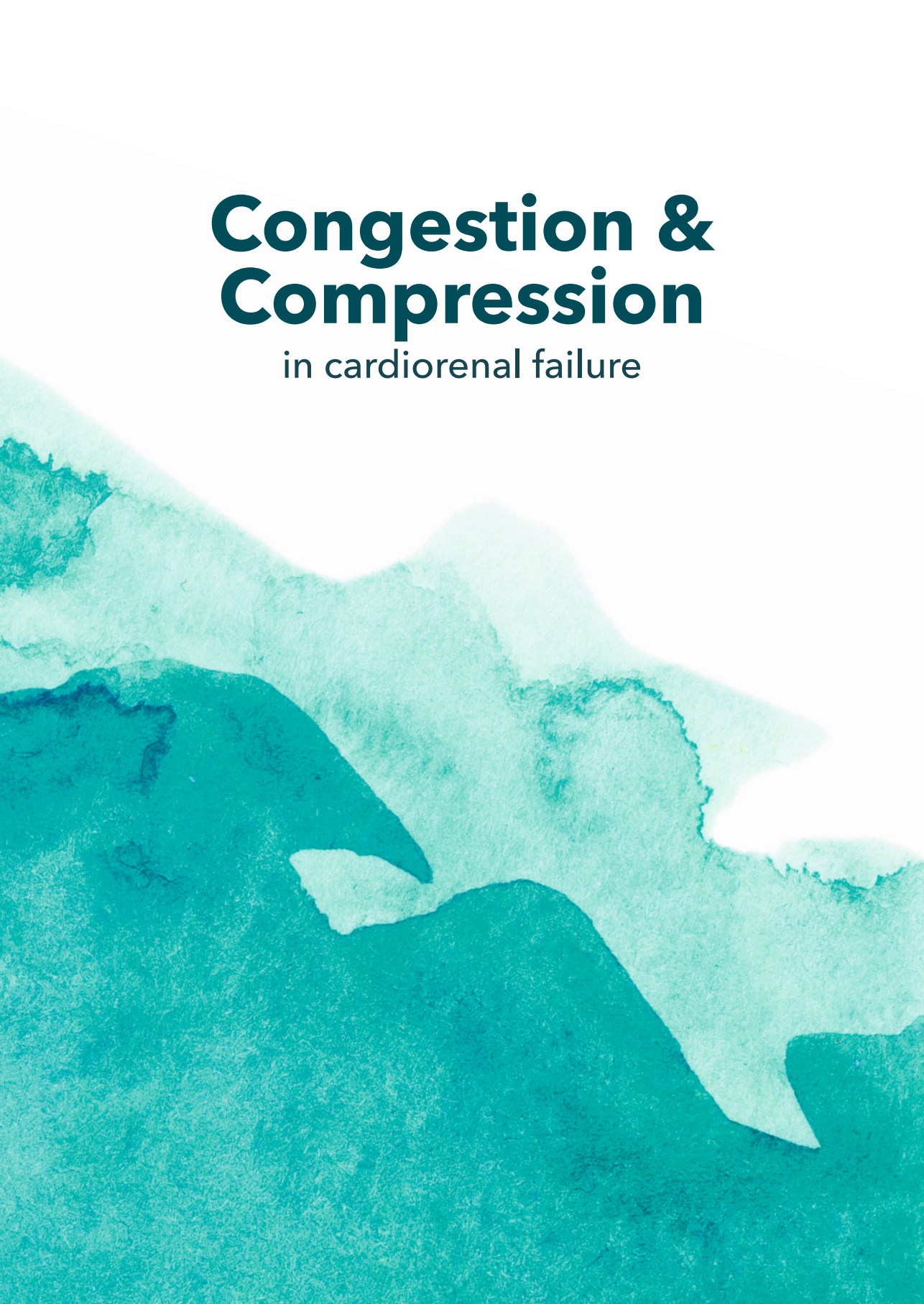
Congestion & Compression

in cardiorenal failure



Congestion and Compression in Cardiorenal Failure

Eva M. Boorsma

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Chapter 1

General introduction and aims

Heart Failure

Heart failure is a clinical syndrome consisting of cardinal symptoms such as breathlessness, ankle swelling, and fatigue that are accompanied by typical signs (e.g., elevated jugular venous pressure, pulmonary rales, and peripheral edema). The clinical syndrome is attributable to a structural or functional abnormality of the heart resulting in elevated intracardiac pressures and/or inadequate cardiac output at rest or during exercise.[1]

Heart failure is associated with high hospitalization and mortality rates as well as a poor reported quality of life.[2, 3] The incidence of heart failure is increasing and as much as 10% of the population in Europe above 70 years of age are currently diagnosed with heart failure.[4] The combination of the high incidence and high morbidity makes heart failure an extraordinary burden on health care systems, both in the Netherlands and worldwide.[5, 6]

The definition of heart failure implies that symptoms are mandatory for the diagnosis of heart failure. Despite important differences in the etiology of heart failure, all cases present with key signs and symptoms, in most cases driven by congestion[7]. Congestion is caused by increased intracardiac pressures and diminished cardiac output, causing activation of the renin-angiotensin-aldosterone (RAAS) and catecholamine axes. This activation then leads to sodium and water retention, in turn giving rise to fluid accumulation in the vasculature and the interstitial space, perpetuating the vicious cycle of congestion by further increasing intravascular and intracardiac pressures (Figure 1).

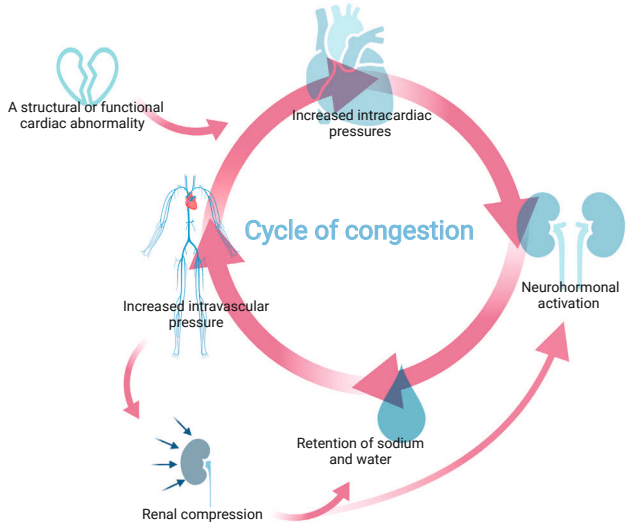

Figure 1 | the cycle of congestion, created with biorender.com

Congestion

Congestion is not only the main driver for symptoms in heart failure, but also the main reason for acute heart failure hospitalization.[8] While treatment for chronic heart failure has improved tremendously over the last decades, treatment for acute heart failure is still comparable to that of 50 years ago. Loop diuretics remain the mainstay of treatment[1]. Although the great majority of patients admitted for acute heart failure are treated with loop diuretics, a large number of these patients do not adequately respond to loop diuretics. This poor diuretic response results in incomplete decongestion at discharge, which in turn is associated with high mortality and even higher rehospitalization rates.[2, 9] Several clinical trials investigating new decongestive drugs in the setting of acute heart failure have been conducted, although none of them have succeeded in improving rehospitalization rates in this high-risk population.[10-13] A potential explanation for the fact that we have not yet succeeded in improving these outcomes, could be the fact that to date all patients with acute heart failure are treated the same, while the underlying pathophysiology of their congestion might not be the same. Recognition of different congestive profiles is therefore of the utmost importance to improve future acute heart failure treatment.

Renal dysfunction and compression

Another important goal in heart failure research is to better understand the pivotal role of the kidneys in the development and progression of congestion. First, RAAS activity is initiated by secretion of renin by the juxtaglomerular apparatus of the kidney. Secretion of renin is elicited by either a low arterial blood pressure, high epinephrine concentrations following activation of baroreceptors or low chloride delivery to the macula densa. All of these pathophysiological processes are typically found in heart failure. Secondly, RAAS activation leads to retention of sodium and consequently water by the renal tubules, giving rise to congestion. Additionally, all diuretic treatments are targeted at receptors located within the kidney. Lastly, diuretic resistance occurs frequently in those with both cardiac and renal dysfunction.[9] The central role of the kidney in onset, progression and treatment of congestion, make understanding the connection between the heart and the kidney a crucial goal in heart failure research.

Renal dysfunction in heart failure is common and associated with worse outcomes. Several hypotheses on this relationship exist. First, forward failure leading to reduced perfusion of the kidney might be a factor responsible for renal dysfunction in heart failure.[14] While this finding might explain renal dysfunction in the setting of diminished perfusion, it is important to note that only a minority of patients with chronic heart failure, or even acute heart failure, present with a significantly reduced cardiac

output.[15] Secondly, renal dysfunction might result from central venous congestion, as higher central venous pressures have been shown to correlate to lower glomerular filtration across many different patient categories.[16] Thirdly, several non-hemodynamic mechanisms to explain the cardiorenal interaction in heart failure have been proposed, including fibrosis, inflammation and (microvascular) endothelial dysfunction.[17, 18] Hypothetically, there may be another factor responsible for renal dysfunction in heart failure. Compression of the renal parenchyma and vasculature would complement our current understanding of renal dysfunction in heart failure. The kidney is a vulnerable organ and is therefore well protected within the body. In the first place by its anatomical location behind the rib cage, followed by a layer of fat surrounding the kidney, and finally by a thick fibrous capsule surrounding the renal parenchyma itself. While these protective mechanisms are effective in protecting the kidney from outside damage in the healthy individual, they can become counterproductive in those with heart failure. In the occurrence of congestion, the capsule prohibits the renal parenchyma from expansion, deflecting the pressures inwards and worsening renal function. Moreover, this increased pressure will likely also lead to further activation of the RAAS-system and even more retention of sodium and water (Figure 1). Similarly, in the case of perirenal adiposity, the renal fascia prohibits the perirenal space from expanding, leading to compression of mainly the renal vasculature, exacerbating intrarenal congestion. While the concept of renal compression resulting from perirenal adiposity has been described in diabetes and hypertension, no data on its prevalence and effects on renal hemodynamics in heart failure are available.

Aims of this thesis

Combining all these insights leads to the conclusion that both congestion and renal dysfunction in heart failure are of great pathophysiological importance. Both are highly prevalent in (acute) heart failure, and both present significant therapeutic challenges.

Incomplete decongestion occurs frequently, and is associated with recurrent hospitalizations, usually already at the very short term. However, it remains difficult to recognize incomplete decongestion, and possibly even more difficult to treat incomplete residual congestion, despite our best efforts in conducting clinical trials aiming to find new diuretic regimens.

One possible explanation for the difficulty to find better decongestive treatments, might be the fact that currently all congested patients are treated equally, while their congestive profile is likely not equal. One of the aims of this thesis is to phenotype different congestive subtypes, in order to tailor diuretic treatment to the congestive

profile. In **Chapter 2** we aim to provide an overview of the pathophysiology of congestion based on literature review. Next, we differentiate decongestive treatments, based on their potential to excrete either sodium (natriuresis) or free water (aquaresis). From this literature review we suggest two congestive subtypes: intravascular and tissue congestion. While many patients will present with a combination of both, one subtype may be more pronounced.

In **Chapter 3** we aim to study the effects of the use of the sodium glucose cotransporter-2 (SGLT-2) inhibitor *empagliflozin* on clinical outcomes in patients with acute decompensated heart failure. SGLT-2 inhibitors are drugs designed to treat type 2 diabetes mellitus through reduction of glucose resorption in the proximal tubule. This resorption is sodium-dependent and blockage therefore also reduces sodium resorption in the proximal tubule. The high tubular glucose and sodium concentration draw fluid from the renal interstitium to the tubular space, explaining the diuretic properties. While SGLT-2 inhibitors have repeatedly proven to reduce mortality and heart failure hospitalizations in patients with chronic heart failure, no data on the effects of empagliflozin in acute heart failure are available, though its diuretic properties theoretically provide a distinct potential to increase diuretic response.

Combining the insights gained in **Chapters 2 and 3**, in **Chapter 4** we aim to investigate the diuretic potential of SGLT-2 inhibitors and to determine whether SGLT-2 inhibitors are mainly natriuretic or aquaretic drugs. In order to do so, we use the data acquired in **Chapter 3** and measure urinary excretion of sodium, glucose and chloride in those who received empagliflozin versus those who received placebo. Additionally, we measure plasma and urinary osmolality to investigate the potential for treatment of residual congestion.

As RAAS-activation is one of the key mechanisms in the cycle of congestion this is an attractive system to investigate further. One biomarker is of particular interest, as an antagonistic antibody, and thus a treatment possibility, for it is available. **Chapter 5** aims to identify the role of this peptide called dipeptidyl peptidase 3 (DPP3), a member of the antagonistic pathway of the RAAS-axis, in chronic heart failure. **Chapter 5** correlates DPP3 to markers of congestion and disease severity and investigates its treatment potential in chronic heart failure.

Another biomarker interesting to both the cardiorenal axis and the cycle of congestion could be urinary albumin excretion, or albuminuria. Albuminuria has been consistently associated with worse outcomes, however the reason for this relationship ure with preserved ejection fraction have more perirenal and renal sinus adipose tissue than

age-, sex- and BMI matched controls and that this adipose tissue contributes to worse renal function and worse exercise hemodynamics. In order to do so, we will measure perirenal and renal sinus fat on computed tomography scans in a cohort of patients who previously underwent exercise right heart catheterization. Figure 2 summarizes the different aims of this thesis.

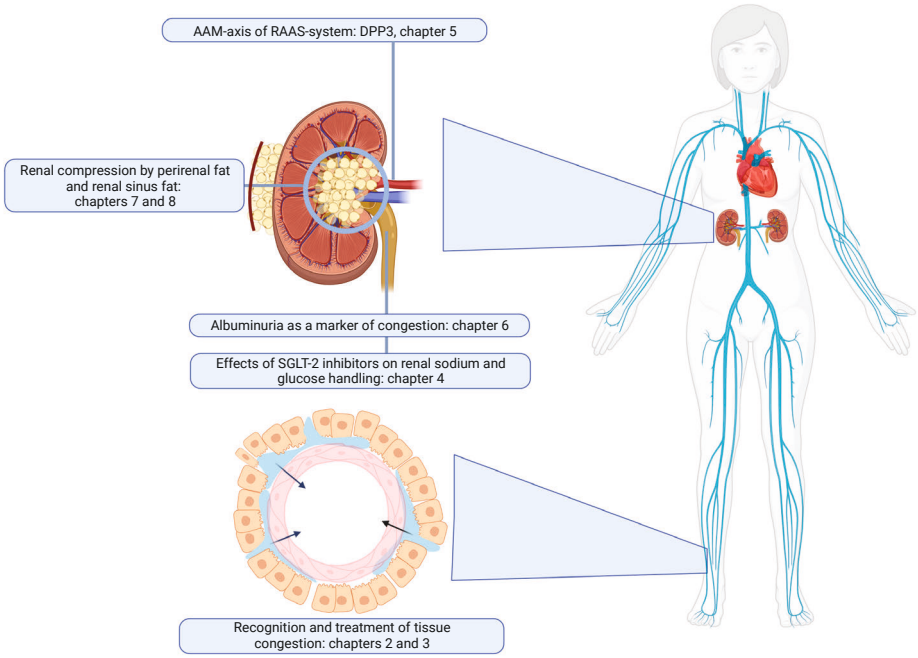

Figure 2 | summary of aims, created with biorender.com

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Part 1 **Congestion**

Chapter 2

Congestion in heart failure: a contemporary look at physiology, diagnosis and treatment

Eva M. Boorsma, *Jozine M. ter Maaten, Kevin Damman, Wilfried Dinh, Finn Gustafsson, Steven Goldsmith, Daniel Burkhoff, Faiez Zannad, James E. Udelson and Adriaan A. Voors*

Nat Rev Cardiol **17,** *641–655 (2020).*

Abstract

Congestion is the main reason for hospitalization in patients with acute decompensated heart failure and is a critical target for therapy. However, achieving complete decongestion can pose a difficult clinical challenge. Furthermore, residual congestion before discharge from hospital is associated with a high risk of early rehospitalization and death. An improved understanding of the pathophysiology of congestion is of great importance to find better and more personalized therapies. In this Review, we describe the two different forms of congestion — intravascular congestion and tissue congestion — and hypothesize that differentiating between and specifically treating these two different forms of congestion could improve the outcomes of patients with acute decompensated heart failure. Although the majority of these patients have a combination of both intravascular and tissue congestion, one phenotype can dominate. Each of these two forms of congestion has a different pathophysiology and requires a different diagnostic approach. We provide an overview of novel and established biomarkers, imaging modalities and mechanical techniques for identifying both types of congestion. Treatment with loop diuretics, the current cornerstone of decongestive treatment, reduces circulating blood volume, thereby reducing intravascular congestion. However, the osmolality of the circulating blood decreases with the use of loop diuretics, which might result in less immediate translocation of fluid from the tissues (lungs, abdomen and periphery) to the circulation when the plasma refill rate is exceeded. By contrast, aquaretic drugs (such as vasopressin antagonists) predominantly cause water excretion, which increases the osmolality of the circulating blood, potentially improving translocation of fluid from the tissues to the circulation and thereby relieving tissue congestion.

Key points

- Congestion is the main reason for hospitalization in patients with acute decompensated heart failure.
- Residual congestion at hospital discharge is associated with higher rates of death and hospital readmission for heart failure.
- Congestion can be predominantly present in the vascular system (intravascular congestion) or in the interstitium (tissue congestion), although the majority of patients have a combination of both intravascular and tissue congestion.
- Intravascular congestion and tissue congestion can be identified and differentiated with the use of specific diagnostic assessments, such as physical examination, biomarkers and imaging techniques.

- Loop diuretic therapy reduces circulating blood volume, thereby improving intravascular congestion; however, these therapies lower plasma osmolality, which might impede translocation of fluid from the tissues to the circulation.
- Aquaretic drugs, such as vasopressin antagonists, reduce plasma volume and increase plasma osmolality, which might stimulate translocation of fluid from the tissues to the circulation.

Introduction

The detection and treatment of residual congestion is one of the greatest unmet needs in heart failure. Treatment with loop diuretics, the current cornerstone of decongestive treatment, reduces circulating blood volume, thereby reducing intravascular congestion. However, compared with the use of aquaretic drugs, loop diuretics cause the osmolality of the circulating blood to decrease, which might result in less immediate translocation of fluid from the tissues (lungs, abdomen and periphery) to the circulation when the plasma refill rate is exceeded. A reduced circulating blood volume without the translocation of a similar volume of fluid from the tissues results in neurohormonal activation and possibly worsening of renal function, while patients experience persistent clinical signs and symptoms of (tissue) congestion, such as dyspnoea, rales and peripheral oedema. We hypothesize that the distinction between tissue congestion and intravascular congestion is of importance in our understanding and treatment of patients with decompensated heart failure. In this Review, we focus on the important differences between the two forms of congestion in the intravascular and the tissue interstitial compartments. In current literature this distinction is not yet clearly made, but difference can be noted at bedside. In particular, we discuss the diagnosis and treatment of tissue congestion versus intravascular congestion in patients with heart failure and the role of aquaresis versus natriuresis.

Definition, epidemiology and grading

Congestion in heart failure is defined as fluid accumulation in the intravascular compartment as well as in the interstitial space, resulting from increased cardiac filling pressures as a result of maladaptive sodium and water retention by the kidneys.[1] Congestion is the main reason for hospitalization in patients with acute (decompensated) heart failure, but the severity of congestion varies widely between patients.

Table 1 | Commonly used congestion scores in acute heart failure trials.

	0	1	2	3	Maximum points	Refs
Rales	None	Basal	<50%	>50%	18	74, 138, 139
	None	<1/3	1/3 - 2/3	>2/3	16	140
Peripheral oedema	Absent/Trace	Slight	Moderate	Marked	18	74, 138, 139
	Absent	1+	2+	3+	16	140
	Absent	Until shins	Until knees	Until sacrum	8	141
	Trace	Moderate	Severe		4	142
Jugular venous distention	<6 cmH2O	6-9 cmH2O	10-15 cmH2O	>15 cmH2O	16	140
	<6 cmH2O	6-10 cmH20		>10 cmH2O	18	74, 138, 139
	<6 cmH2O	6-9 cmH2O	10-15 cmH2O	>15 cmH2O	9	5
Orthopnoea	No		Yes		16	140
	No		Yes		8	141
	None	1 pillow	2 pillows	>30 degrees	18	74, 138, 139
	None	2 pillow	3 pillows	>30 degrees	9	5
	≤2 pillows		>2 pillows		4	142
Dyspnoea	None	Seldom	Frequent	Continuous	18	74, 138, 139
	No		On exertion	In rest	16	140
	None	Seldom	Frequent	Continuous	9	5
Fatigue	None	Seldom	Frequent	Continuous	18	74, 138, 139
	No		Yes		16	140
NTproBNP	First tertile	Second tertile	Third tertile		8	141

In a large, long-term European registry, 83% of all patients admitted to hospital with acute heart failure showed clinical signs or symptoms of congestion[2]. One large global cohort study on patients hospitalized for heart failure showed incidences of peripheral oedema ranging from 39.2 % (South East Asia) to 75.2 % (Eastern Europe) and incidences of rales ranging from 23.9% (North America) to 80.6 % (Africa)[3].

Clinical congestion scores can be used to assess the degree of congestion. Most clinical congestion scores are a composite of the severity of orthopnoea, jugular vein distention and rales. A summary of the most commonly used congestion scores is reflected in **Table 1**.

Tissue versus intravascular congestion

Fluid accumulation leading to decompensated heart failure starts in the intravascular compartment[8]. Continuously increased hydrostatic pressures in the capillary vessels subsequently lead to tissue congestion (Figure 1). The majority of patients with decompensated heart failure have a combination of both intravascular and tissue congestion as displayed by the composites of congestion scores, however we postulate that one phenotype can dominate. A typical patient with predominant intravascular congestion presents with an acute-onset, high blood pressure leading to suddenly increased pulmonary and cardiac filling pressures[9, 10]. These patients generally respond well to treatment with vasodilators[11, 12]. In the heart failure guidelines, these patients are referred to as having the vascular type of congestion[13]. By contrast, a patient with predominant tissue congestion presents with a gradual increase in cardiac filling pressures and slowly progressive pulmonary, abdominal and peripheral oedema[14]. Moreover, as a result of diuretic treatment, the congestive phenotype can change (that is, intravascular fluid depletion, with residual tissue congestion). We postulate that these different mechanisms of origin of the congestion require different criteria for diagnosis and different approaches to treatment.

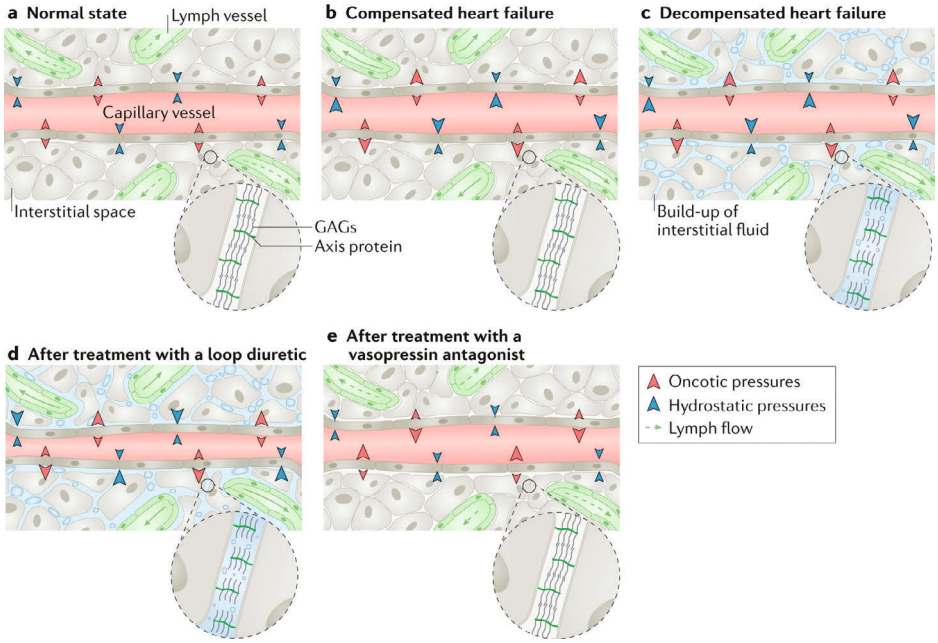


Figure 1 a | Under normal healthy conditions, hydrostatic pressures and oncotic pressures in the capillary vessel and interstitium are in equilibrium. **b** | During compensated heart failure, hydrostatic pressure in the capillary vessel rises, and oncotic pressure in the capillary vessel decreases. Compensatory mechanisms maintain equilibrium. **c** | During decompensated heart failure, the compensatory mechanisms become insufficient to maintain equilibrium. Hydrostatic pressures in the capillary vessel and oncotic pressures in the interstitium keep increasing. Fluid starts to build up in the interstitial space. The glycosaminoglycan (GAG) networks are no longer bound together. **d** | After treatment with a loop diuretic, hydrostatic pressures in the capillary vessel return to normal, allowing some fluid to re-enter the bloodstream. As a result of natriuresis, osmotic pressure in the capillary vessel also decreases. **e** | After treatment with a vasopressin antagonist, osmotic pressure in the capillary vessel decreases, and residual interstitial fluid re-enters the bloodstream.

Differences in pathophysiology

Intravascular congestion

During an episode of acute decompensated heart failure, a combination of haemodynamic and neurohormonal factors lead to sodium and water retention by the kidneys.[1] These factors are variably present during different stages and severities of heart failure and include low cardiac output, activation of the renin–angiotensin–aldosterone and natriuretic peptide axes, the sympatho-sympathetic reflex as a result of cardiac stretch, and probably other unknown

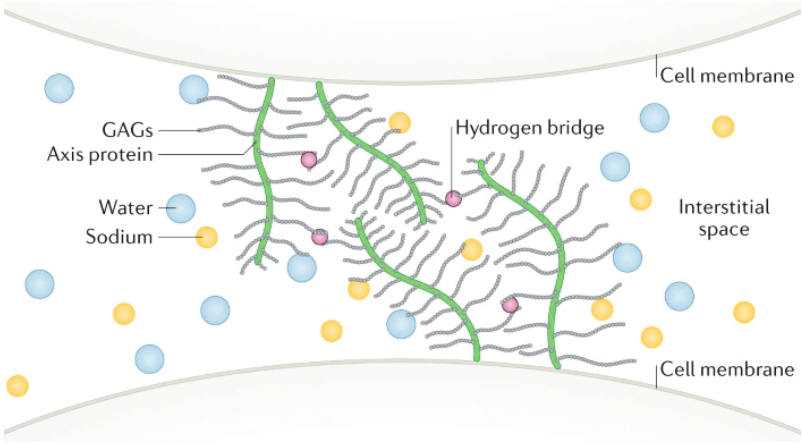
contributors[15]. The relative contributions of these mechanisms varies between patients, but most patients with acute heart failure have little or no evidence of cardiogenic shock or low output[16]. Regardless of the cause, the end point of heart failure decompensation is congestion.

Although plasma volume expansion underlies many if not most cases of congestion in acute heart failure, endogenous fluid reservoirs can also make an important contribution, particularly in patients in whom heart failure decompensation develops quickly — that is, in the vascular type of congestion referred to above. The splanchnic veins (the abdominal compartment of the venous circulation) are characterized by a much larger capacity than that of other veins. The splanchnic veins contain anywhere from 20% to 50% of the total blood volume.[17, 18] These veins function as a functionally sequestered circulation and act as a blood reservoir that can be recruited in the event of hypovolaemia. Another characteristic of splanchnic veins is a high density of α-adrenergic receptors.[19] In acute heart failure — which is characterized by neurohormonal overactivation — stimulation of α-adrenergic receptors leads to potent venoconstriction and the movement of blood from the abdominal compartment to the circulating compartment.[19] This dysregulation of blood distribution has been suggested to have an important role in acute intravascular congestion.[20] Administration of nitroglycerin produces venodilatation and increases the capacity of the splanchnic system, restoring the balance of blood distribution in acute heart failure.[19, 21] Therefore, much of the intravascular congestion seen in acute heart failure is likely to be a mixture of both gradual plasma volume expansion and the shifting of fluid from venous reservoirs, particularly as sympathetic activity increases with symptomatic deterioration.

Tissue congestion

The development of tissue oedema is the result of an imbalance between hydrostatic and oncotic pressures at the level of the interstitium. A schematic representation of the factors that are involved in tissue congestion is depicted in Figure 1. In brief, the interstitium contains many glycosaminoglycans (GAGs) branching from one central protein, alongside collagen and elastin fibres. All the GAGs together form a strong network, giving structure to the interstitium. The potential clinical relevance and implications of the GAG networks are discussed in Box 1.

Box 1 | The role of glycosaminoglycans in tissue congestion



The composition of the interstitium, the space between the cells, differs according to the tissue. Nevertheless, the interstitium always contains a so-called ground substance: a gel-like structure composed of proteoglycans. These proteoglycans consist of one axis protein to which many glycosaminoglycans (GAGs) are attached (see Figure box 1). The GAGs from different proteoglycans are in turn connected through various hydrogen bridges. Most of the water molecules in the interstitial space are bound to these GAGs. Small vesicles of free water are present within the gel. Pitting oedema arises when the free water vesicles become larger and can be mobilized between the cells and the interstitial gel. All the GAGs together form a strong network, giving structure to the interstitium. These GAGs are polyanionic and can, therefore, bind large quantities of cations, namely sodium. In doing so, the GAG network has been hypothesized to have an important role in sodium homeostasis and to protect against overt hypernatraemia in heart failure[162]. Furthermore, increases in sodium concentration stimulate gene and protein expression of GAGs[163], increasing the capacity of the GAG network to bind sodium. However, long-term saturation of GAGs with sodium might change their form and function, thereby reducing the integrity of the network as a whole[162,164]. In this situation, a slight increase in capillary blood pressure would be sufficient to induce oedema.

Tissue congestion (continued)

Three protective mechanisms need to be overcome before interstitial fluid can accumulate.[8]. First, a slight vacuum relative to atmospheric pressure keeps the interstitial GAG networks together. As long as these networks are bound together, a small increase in interstitial pressure will lead to a large increase in hydrostatic pressure in the capillary

vessels. At a neutral pressure, when the vacuum is lost, the force that keeps the GAG networks together no longer exists, providing room for the accumulation of free fluid (that is, water not bound to GAGs. Second, the lymphatic system is highly sensitive to pressure and can increase fluid removal by 10-fold to 50-fold when hydrostatic pressure rises[22]. Third, the lymphatic system drains large amounts of protein, thereby reducing colloid osmotic pressure in the interstitium.

Similar mechanisms are in place in the alveoli. A pulmonary capillary pressure of approximately 28 mmHg, which is 21 mmHg above normal pulmonary capillary pressure, is enough to overcome colloid osmotic pressure in the perialveolar interstitium[23]. In acute left-sided heart failure, minimal surpassing of this threshold is enough to cause life-threatening pulmonary oedema. However, when pulmonary capillary and artery pressures are increased chronically, the diameter and flow in the lymphatic system can gradually increase, and pulmonary artery pressures as high as 45 mmHg without pulmonary oedema have been measured[24].

Conversely, two factors can lower the threshold for the development of oedema. First, long-term sodium saturation of interstitial GAGs changes the form and function of the GAG networks (BOX 1). These changes reduce the integrity of the GAG network so that a slight increase in capillary blood pressure is sufficient to induce oedema. Second, certain comorbidities increase vascular permeability. One condition is diabetes mellitus, probably as a result of the destruction of vascular tissue through the formation of advanced glycation end-products[25, 26]. Another situation in which vascular permeability increases is when cytokines are released, such as during inflammation, sepsis or ischaemia[27-29].

The factors described above explain why certain patients with heart failure can have extremely high pulmonary capillary pressures without alveolar congestion, and other patients can develop clinically significant congestion with only slightly increased pulmonary capillary pressures.

Assessment of intravascular congestion

Currently, the gold standard for assessing intravascular congestion is to measure right atrial pressure (normally 2–6 mmHg) and pulmonary capillary wedge pressure (PCWP; normally 3–8 mmHg) through right heart catheterization[30, 31]. However, right heart catheterization is an invasive method and, therefore, routinely performing this procedure is not attractive. To assess changes in congestion on a day-to-day basis, noninvasive measurements are needed, such as bedside evaluated changes in jugular

venous pressures, patient reported symptoms or admission-to-discharge changes in natriuretic peptides (Figure 2).

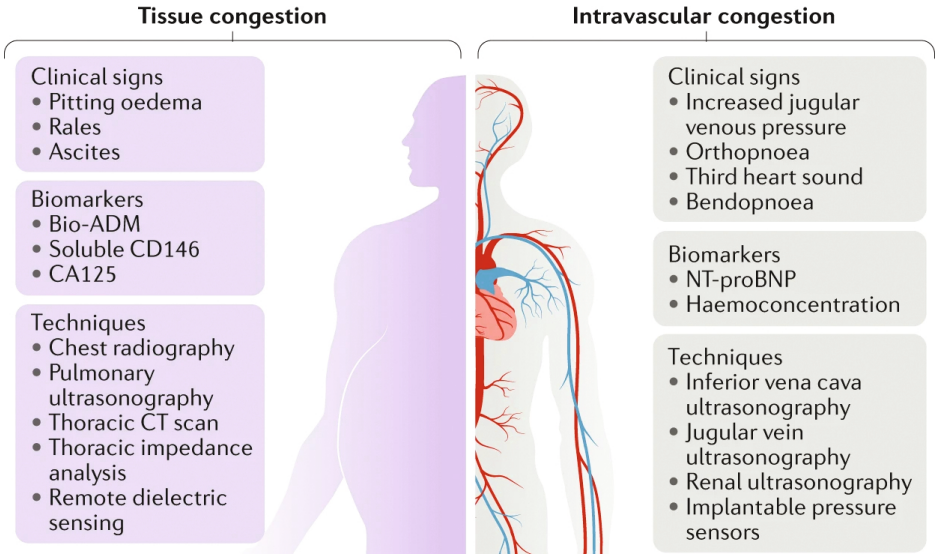


Figure 2 | The figure shows the clinical signs and biomarkers that have been established as markers of congestion and the techniques used for diagnosis. Some signs and biomarkers are more indicative of tissue congestion, whereas others are more indicative of intravascular congestion. bio-ADM, biologically active adrenomedullin; CA125, carbohydrate antigen 125; NT-proBNP, N-terminal pro-B-type natriuretic peptide.

Clinical signs and symptoms

Jugular venous pressure

Distension of the jugular vein provides an indication of right atrial pressure. Classically this can be done with a venous arch, to measure the distance between the sternal notch and the collapse of the venous column[32]. In current clinical practice however, assessment of jugular venous pressure is usually performed by inspection of the patients jugular veins and estimating in the degree of extension[32]. However, precise estimation is difficult, and the sensitivity and specificity for estimating central venous pressure are poor (57.3% and 43.6%, respectively)[33]. We postulate increased jugular venous pressure to be a finding specific of intravascular congestion, as it reflects jugular intravascular overfilling.

Orthopnoea

Orthopnoea is the result of increased venous blood flow from the lower extremities in a supine or semi-supine position, which increases cardiac preload that cannot be processed by the failing heart. This sudden, additional preload promotes an increase in the symptoms of dyspnoea. Since orthopnoea results from increased preload rather than alveolar oedema, we hypothesize it to be a symptom of intravascular congestion rather than tissue congestion.

Third heart sound

The third heart sound is the result of rapid filling of the ventricle in the early part of diastole and rapid deceleration of blood flow in an already-filled ventricle[34]. Therefore we consider it to be mainly a sign of intravascular (or rather intracardiac) congestion. The sound can occur in healthy children and young adults as a sign of quick heart relaxation. However, the third heart sound can also be heard in situations of cardiac volume overload and systolic dysfunction[35].

Bendopnoea

Bendopnoea is the sensation of increased dyspnoea when bending forwards and is associated with increased right atrial pressure and PCWP[36]. The presence of bendopnoea correlates with the presence of orthopnoea, exercise intolerance and increased jugular venous pressure but not with rales or peripheral oedema[36, 37]. Therefore, bendopnoea seems to be indicative of intravascular congestion, rather than of tissue oedema. This notion is supported by the observation that in patients with pulmonary arterial hypertension, the presence of bendopnoea correlates with more advanced disease[38].

Biomarkers

Natriuretic peptides

Release of natriuretic peptides into the circulation is induced by increased stretch and/or pressure of the atria and ventricles. Different natriuretic peptides can be distinguished, some are specific to ventricular stretch, while others are more reflective of atrial stretch[39]. Therefore, elevated circulating levels of natriuretic peptides are likely an indication of intravascular and intracardiac congestion, rather than of tissue congestion. A reduction in the circulating level of natriuretic peptides by ≥30% from hospital admission is generally considered to be an indicator of successful intravascular decongestion and is associated with reductions in jugular vein distention, vena cava diameter and wedge pressure, and mortality[40-42]. Although these findings suggest a role for in-hospital therapy guided by plasma levels of natriuretic peptides, several studies comparing this approach with standard of care found no significant differences

in the combined end point of rehospitalization and all-cause death, despite a greater reduction in plasma levels of Nterminal pro-B-type natriuretic peptide (NT-proBNP) in patients in the natriuretic-peptide-guided treatment group[43, 44].

Haemoconcentration

Haemoconcentration, the relative increase in haemoglobin levels in the blood as a result of reduction in plasma volume, has also been proposed as a marker of decongestion[45, 46]. In patients with acute heart failure, haemoconcentration has been associated with clinical signs and symptoms of more aggressive decongestion and improved outcomes, including a lower risk of readmission to hospital for heart failure[47-50]. In one study, patients showing haemodilution after initial treatment for acute heart failure had more alveolar oedema and higher body weight at baseline, compared to patients showing haemoconcentration, which indicates translocation from the tissues to the intravascular space[51]. Based on these associations we conclude haemoconcentration to be a sign of intravascular volume status. Changes in estimated plasma volume can also be used as a proxy for the plasma refill rate (the rate at which fluid can be transported from the tissue interstitium into the vessels). Given that residual congestion is a predictor of worse outcome, irrespective of the degree of haemoconcentration[50], aiming maintain haematocrit at a constant level during decongestive treatment has been suggested in order to decongest at a rate equal to the plasma refill rate[45]. For patients undergoing haemodialysis, continuous monitoring of haematocrit levels has been shown to prevent episodes of intradialytic hypotension[52]. To date, this approach has not been examined in patients with heart failure during decongestion treatment.

Imaging and monitoring techniques

Inferior vena cava ultrasonography

Collapse of the inferior vena cava measured by ultrasonography is an easy tool to estimate right atrial pressure. More specifically, a decrease of <50% in the diameter of the inferior vena cava (caval index) during inspiration correlates with a right atrial pressure of ≥10 mmHg[53]. Increasing inferior vena cava diameters measured in the outpatient setting are predictive of an increased risk of hospitalization and death in patients admitted to hospital for heart failure and are associated with the presence of clinical signs and symptoms of both tissue and intravascular congestion[54-56].

Jugular vein ultrasonography

Jugular vein ultrasonography might be a more accurate and reproducible measurement than visual inspection of jugular vein distension. This approach has been shown to

correlate well with congestive status and to be a predictor of rehospitalization for heart failure[57, 58].

Renal ultrasonography

Under normal circumstances, patterns of renal venous flow assessed by ultrasonography are rarely altered by changes in cardiac output[59]. However, patterns of renal venous flow are altered by changes in renal interstitial compliance and increased renal venous pressures[60]. In patients with heart failure, two types of discontinuous renal venous flow patterns can be distinguished: monophasic and biphasic, with patients with monophasic flow having worse outcomes[60]. Patients with discontinuous renal venous flow at baseline, as assessed by ultrasonography, have higher plasma levels of NT-proBNP, higher E/A ratio (a marker of left ventricular function) and more tricuspid regurgitation than patients with continuous renal venous flow.[59, 60]. However, no data are available on the use of renal ultrasonography to assess the presence of residual congestion.

Implantable pressure sensors

Implantable pressure sensors are monitoring devices that can be implanted in the pulmonary artery, where the device continuously monitors pulmonary diastolic pressures. Pulmonary diastolic pressure is used to estimate PCWP, although different formulae to estimate PCWP have been reported[61]. In this way, early increases in pulmonary artery pressure can be detected days before clinical signs and symptoms are present, enabling clinicians to make early adjustments to the treatment regimen and thereby prevent hospitalizations. Monitoring pulmonary artery pressures in patients with heart failure with the use of an implantable pressure sensor results in significant reductions in hospital admissions[62, 63].

Assessment of tissue congestion

Clinical signs and symptoms

Tissue congestion can be assessed by symptoms and physical examination, and the established indicators are the presence of rales, ascites and peripheral oedema. While pitting oedema is highly specific for presence of interstitial oedema[65], most clinical signs and symptoms have moderate specificity but a poor sensitivity to diagnose heart failure as cause for interstitial oedema[64]. Tissue congestion can be assessed also with the use of biomarkers and imaging methods.

Biomarkers

Biologically active adrenomedullin

Adrenomedullin has a prominent role in maintaining the barrier function of the endothelium[66]. The loss of this barrier function results in vascular leakage and subsequently pulmonary and systemic oedema[66]. Accordingly, higher plasma levels of biologically active adrenomedullin are indicative of increased accumulation of interstitial fluid, and circulating levels of biologically active adrenomedullin are elevated in patients with heart failure[67, 68] and particularly in patients with sepsis[69] (another condition characterized by massive vascular leakage) compared with healthy individuals. High plasma levels of biologically active adrenomedullin are associated with more severe peripheral oedema and higher jugular vein pressure, the presence of orthopnoea and hepatomegaly, and increased length of hospital stay and all-cause mortality[70, 71]. In patients with acute decompensated heart failure, high levels of biologically active adrenomedullin after 7 days of decongestive therapy correlate well with the presence of other clinical signs of residual congestion[72-74].

Soluble CD146

Soluble CD146 (also known as cell surface glycoprotein MUC18) is a protein secreted by the vein wall tissue in response to stretch[75]. Plasma levels of soluble CD146 were found to be higher in patients with heart failure than in healthy controls or patients with noncardiac dyspnoea[76]. In patients with acute heart failure, higher plasma levels of soluble CD146 correlated with the presence of more clinical signs of congestion and a higher degree of congestion as assessed by chest radiography[77]. The role of circulating levels of soluble CD146 in predicting hospitalization and assessing decongestion remains to be established.

Carbohydrate antigen 125

Up to two-thirds of patients admitted to hospital for heart failure have elevated plasma levels of carbohydrate antigen 125 (CA125; also known as mucin 16), and elevated levels of CA125 correlate with increased morbidity and mortality[78]. CA125 is released by serous tissue (such as the pericardium and pleurae) as a result of mechanical and/or inflammatory stimuli triggered by oedema[79]. Plasma levels of CA125 are higher in patients with peripheral and/or pulmonary oedema and are further elevated in patients with serosal effusion compared with patients with acute heart failure without pronounced serosal effusion.[80, 81]. In patients with myocardial infarction, increased plasma levels of CA125 predict the onset of heart failure[82]. The CHANCE-HF study[83] examined the use of CA125-guided therapy versus standard of care in patients with acute heart failure and found a reduction in rehospitalizations for acute decompensated heart failure in

the CA125-guided group. Of note, patients allocated to the CA125 group were more frequently visited than patients in the standard-care group and received intravenous loop diuretics at home depending on their CA125 levels.

Imaging and monitoring techniques

Chest radiography

Chest radiography can be used to assess the degree of congestion. Radiographic congestion scores correlate well with directly measured lung weight in lungs obtained from organ donors[84]. Patients with heart failure discharged from hospital with higher radiographic congestion scores have higher rates of rehospitalization for heart failure[85]. Interestingly, radiographic congestion scores at hospital admission were not related to patient outcomes in this study[85]. Chest radiography is mostly specific to pulmonary tissue congestion, however increases in pulmonary vascular width on chest radiography are indicative of intravascular congestion, as increased vascular width correlates well to total blood volume ($r = 0.80$)[86].

Pulmonary ultrasonography

Ultrasonography of the lungs is becoming a generally accepted tool for the evaluation of pulmonary oedema[6, 87]. The quantity of water in the lungs corresponds to the degree of echogenicity found with ultrasonography[88]. In the case of interstitial pulmonary oedema, the ultrasound beam is reflected by the oedematous interlobar septa. This situation produces comet-tail reverberation artefacts called B-lines. The number of B-lines is indicative of the degree of pulmonary oedema, with <5 B-lines in the complete anterolateral scan (28 regions across the chest) indicating no pulmonary oedema and >30 B-lines indicating severe pulmonary oedema[89]. The number of these B-lines correlates moderately well with both wedge pressure (PCWP) ($r = 0.48$) and the radiographic congestion score ($r = 0.60$)[90]. Moreover, lung ultrasonography can also be used to predict hospitalizations for pulmonary oedema in the outpatient setting, with a higher sensitivity than clinical congestion scores, E/e' ratio and plasma levels of NT-proBNP[91, 92]. Thoracic ultrasonography can also be used to identify existing pleural effusion.

Thoracic CT scans

Increased density on high-resolution pulmonary CT scans correlates well with lung weights and has been suggested as a gold standard for the assessment of pulmonary interstitial oedema[93, 94].

Thoracic impedance analysis

Bioelectrical impedance analysis is a technique that uses conductance (the inverse of resistance) to estimate the degree of fluid in a body compartment. Transthoracic conduction is measured to estimate pulmonary congestion. In patients with acute heart failure, bioelectrical impedance analysis is a good predictor of length of hospital stay and correlates well with PCWP, plasma levels of natriuretic peptides, E/e' ratio and number of B-lines on thoracic ultrasonography[95-97].

Remote dielectric sensing

Remote dielectric sensing is another technique to determine intrathoracic fluid content noninvasively. Unlike the electric currents used in bioelectrical impedance analysis, remote dielectric sensing uses electromagnetic signals, which are less dependent on body habitus and electrode placement. The lungs are predominantly composed of air and water, and dielectric values are highly dependent on the ratio between these two components. No data exists on the degree in which remote dielectric sensing might also be used in establishing intravascular congestion. Remote dielectric sensing has been shown to correlate with the degree of pulmonary oedema measured by chest CT and with pulmonary pressures measured during right heart catheterization[98]. Moreover, medical therapy guided by remote dielectric sensing in the months after hospitalisation significantly reduced rehospitalization in an uncontrolled observational study in patients with heart failure[99].

Treatment of congestion

Natriuresis leads to fluid loss because free water passively follows excreted ions in the tubule of the kidney. Loop diuretics make use of this property by blocking the reabsorption of sodium, leading to increased sodium excretion and urine output (Figure 3). Inhibition of the $Na^+/K^+/2Cl^-$ symporter in the thick ascending limb of the nephron stops the kidney from either diluting or concentrating the urine. Therefore, natriuresis induced by the use of loop diuretics creates tubular fluid that is isosmotic to plasma[100]. In some instances, the loss of sodium, potassium and chloride might even decrease the osmolality of the plasma, which prevents interstitial fluid from fully re-entering the bloodstream. Additionally, loop diuretics have a direct effect on the macula densa, leading to renin secretion and a state of further increased neurohormonal activation[101].

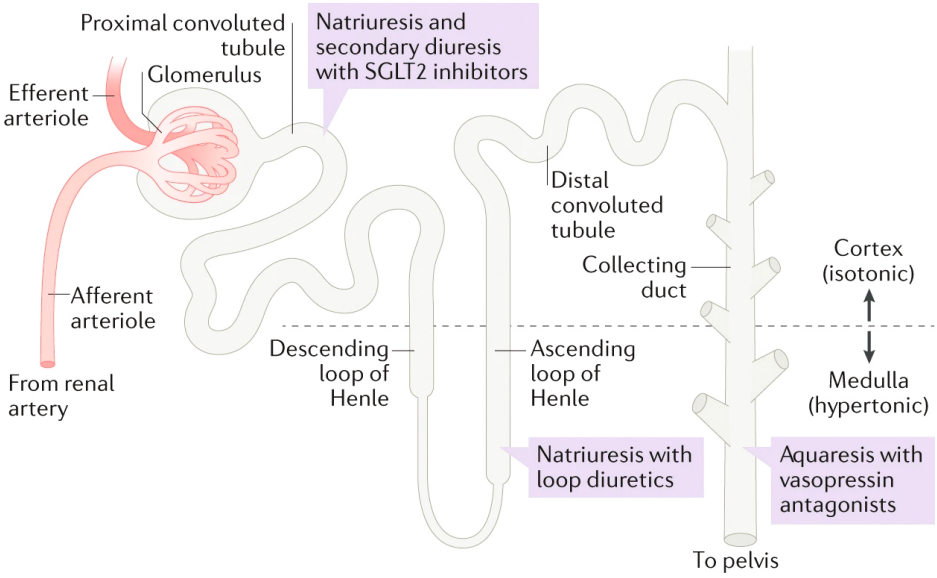


Figure 3 | Inhibitors of the sodium–glucose cotransporter 2 (SGLT2) in the first two-thirds of the proximal convoluted tubule increase the excretion of sodium and glucose and thereby cause secondary diuresis. Loop diuretics inhibit sodium reabsorption via sodium–potassium–chloride cotransporters in the thick ascending limb of the loop of Henle, causing natriuresis. Antagonists of vasopressin V2 receptors in the collecting duct promote aquaresis.

An alternative mechanism of producing fluid loss is through direct promotion of the excretion of free water — that is, aquaresis (Figure 3). Aquaresis decreases urine osmolality and increases blood osmolality and the concentrations of all blood ions. As a result, translocation of fluid from the interstitium to the intravascular space is promoted. The prototypical aquaretic drugs are antagonists of the V2 receptor for arginine vasopressin (also known as antidiuretic hormone). In contrast to loop diuretics, V2 receptor antagonists do not promote neurohormonal activation and do not lead to worsening renal function[102-104].

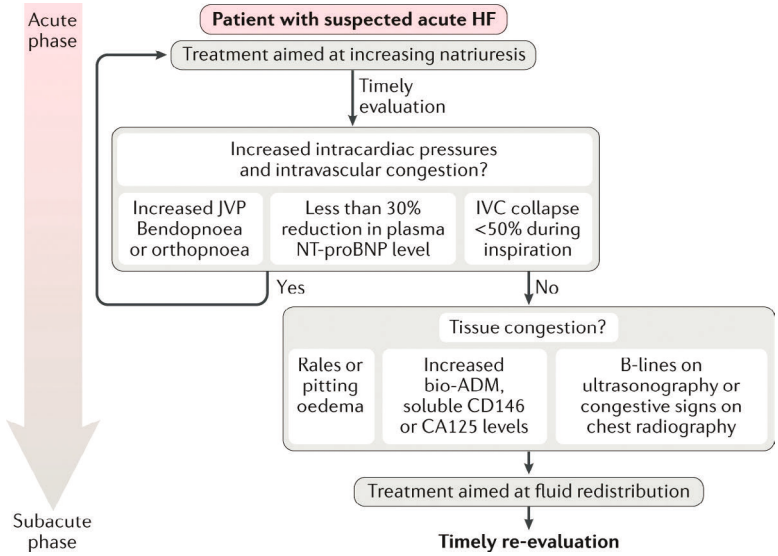


Figure 4 | In patients with acute heart failure (HF), we suggest first treating intravascular congestion with the use of natriuretic drugs. When intravascular congestion is no longer present, but signs and symptoms remain, treatment should be shifted towards fluid translocation, for instance by adding an aquaretic drug. bio-ADM, biologically active adrenomedullin; CA125, carbohydrate antigen 125; IVC, inferior vena cava; JVP, jugular venous pressure; NT-proBNP, N-terminal pro-B-type natriuretic peptide.

Given the different modes of action of aquaretic versus natriuretic therapies, aquaretics might have some advantages over loop diuretics in terms of tissue decongestion and avoiding worsening renal function. In Figure 4, we propose a treatment algorithm on the basis of the presence or absence of tissue and/or intravascular congestion. In brief, we suggest re-evaluating patients soon after the initiation of diuretic treatment. The presence of intravascular congestion is likely to prevent the translocation of fluid from the tissues to the intravascular space; therefore, intravascular congestion should be treated first. However, when intravascular congestion is no longer present, but signs and symptoms remain, treatment should be shifted towards fluid translocation, for instance by adding an aquaretic drug. Potential therapies to reduce residual congestion are discussed below.

Increasing natriuresis

Loop diuretics and thiazides

Heart failure guidelines currently recommend the use of loop diuretics and/or thiazide diuretics to reduce the clinical signs and symptoms of congestion in patients

with chronic or acute heart failure[13,105], including heart failure with preserved ejection fraction. Reduction of (cardiovascular) mortality with loop diuretics and thiazides has not been proven, but one meta-analysis showed a reduction in the risk of death and worsening heart failure with loop and thiazide diuretic therapies, and these drugs seem to improve exercise capacity[106].

Mineralocorticoid-receptor antagonists

Mineralocorticoid-receptor antagonists inhibit the effects of aldosterone, thereby increasing sodium excretion and potassium retention[107]. The beneficial effects of mineralocorticoid-receptor antagonists on clinical outcomes in patients with chronic heart failure with reduced ejection fraction were shown in two large randomized clinical trials[108,109]. In patients with left ventricular dysfunction after myocardial infarction, patients receiving 25–50 mg of eplerenone daily had significantly more weight loss and higher haemoconcentration than patients receiving placebo, indicating a diuretic effect of eplerenone[110]. However, in the RALES trial[111] in patients with severe chronic heart failure, no increase in natriuresis could be identified in patients receiving equivalent doses of spironolactone (12,5–50mg, dose equivalent of 25-100 mg eplerenone[112]). Conversely, doses of ≥100 mg of spironolactone daily have been shown to increase natriuresis[113,114] but did not lead to better decongestion compared with standard of care in patients with acute heart failure[115].

Acetazolamide

Acetazolamide, a carbonic anhydrase inhibitor, blocks the reabsorption of sodium and bicarbonate in the proximal convoluted tubule, resulting in natriuresis and self-limiting metabolic acidosis. Acetazolamide was a popular diuretic in the 1950s but the use of this drug gradually declined after the introduction of loop diuretics. However, several small studies in patients with heart failure have shown that acetazolamide given in addition to loop diuretics might increase natriuresis compared with the use of loop diuretics alone[116-118].

Fluid redistribution

Vasopressin antagonists: increasing aquaresis

Arginine vasopressin is a hormone secreted by the posterior pituitary gland in response to increased osmolality of the blood. Arginine vasopressin acts on three receptors: V1a, V1b and V2. Activation of the V1a receptor induces vasoconstriction, increased platelet aggregation and myocyte hypertrophy, whereas the V1b receptor is located in the anterior pituitary and has a role in the cortisol axis. Blockade of V2 receptors results in decreased expression of aquaporin 2 in the late distal tubules and the collecting

ducts, the transporter responsible for the greatest amount of water reabsorption in the kidneys[8]. Therefore, blocking the effects of vasopressin leads to potent aquaresis. By increasing aquaresis but not natriuresis, intravascular osmotic pressure rises[119], hypothetically allowing fluid to migrate from the interstitium to the vasculature.

The EVEREST trial[120], the first and largest trial on the effects of a V2 receptor antagonist tolvaptan in patients with heart failure, showed no improvement in the dual primary end point of all-cause mortality and cardiovascular death or hospitalization for heart failure with tolvaptan therapy compared with placebo. However, tolvaptan therapy had short-term beneficial effects on dyspnoea relief, clinical congestion scores and change in body mass and/or net fluid loss in the EVEREST and TACTICS-HF trials[120,121]. These effects were most pronounced in patients with hyponatraemia, suggesting an important role for the change in plasma osmolality with tolvaptan therapy. Moreover, these patients had no significant changes in blood pressure, only modest changes in filling pressures, and renal function was unchanged or improved over time compared with patients receiving placebo[122]. These findings again support the hypothesis that interstitial fluid translocates into the blood as a result of changes in plasma osmolality. The beneficial effects of tolvaptan therapy on fluid loss and dyspnoea relief in patients with acute decompensated heart failure have been confirmed in several other studies[102,123,124].

SGLT2 inhibitors: combining natriuresis and increasing osmosis

Sodium/glucose cotransporter 2 (SGLT2) inhibitors cause natriuresis by inhibition of glucose transport, which is driven by concurrent sodium transport in the proximal convoluted tubule in the kidney. Therapy with the SGLT2 inhibitor dapagliflozin has been shown to reduce mortality and hospitalizations for heart failure compared with placebo in patients with heart failure with reduced ejection fraction[125]. Although the mechanism of this benefit remains to be elucidated, natriuretic effects are generally thought to be important[126]. However, one study hypothesized a larger role for aquaresis, suggesting the possibility of using SGLT2 inhibitors in the treatment of tissue congestion, similar to the use of vasopressin antagonists[127]. Outcomes of other trials on the use of SGLT2 inhibitors in patients with heart failure are expected soon (Table 2).

Table 2 | Recent and ongoing trials targeting natriuresis or aquaresis in heart failure

Trial	Year of publication	Study design	Intervention	Study population (*n*)	Primary end point	Secondary end points[a]	Status	Refs
Natriuresis: combination of loop and thiazide diuretics								
Ng et al.	2013	Retrospective analysis	Metolazone in addition to furosemide versus furosemide single therapy	Acute decompensated HF (242)	Increase in mean hourly urine output (P = 0.383) and incidence of worsening renal function (P = 0.819)	Increase in mean net fluid balance (P = 0.048) and total urine output at 24 h (P = 0.505) and at 48 h (P =0.832)	Completed	[143]
CLOROTIC	NA	Randomized, controlled	Thiazide diuretics versus placebo, in addition to loop diuretic treatment	Acute decompensated HF (304)	Changes in body weight and VAS dyspnoea	Length of hospital stay, mortality (all-cause and HF) and rehospitalizations (all-cause and HF)	Recruiting	[144]
Prospective Comparison of Metolazone Versus Chlorothiazide for Acute Decompensated Heart Failure With Diuretic Resistance	NA	Randomized, open-label	Metolazone per os 5 mg versus chlorothiazide 500 mg intravenous	Acute decompensated HF with unresponsive and ineffective diuresis (48)	Net urine output at 24 h	Net urine output at 48 h, net fluid balance over 12 h and 24h, and weight change after 48 h	Recruiting	[145]
Natriuresis: mineralocorticoid-receptor antagonists								
ATHENA-HF	2017	Randomized, controlled	High-dose spironolactone (100 mg) versus placebo or low-dose spironolactone (25 mg)	Acute decompensated HF (360)	Decrease in plasma NTproBNP level from baseline to 96 h (P = NS)	Decrease in Clinical congestion score, dyspnoea, increase in net urine output and net weight change (P = NS for all)	Completed	[115]

Table 2 *(Continued)*

Trial	Year of publication	Study design	Intervention	Study population (*n*)	Primary end point	Secondary end points[a]	Status	Refs
Ferreira et al.	2014	Single-blinded	Spironolactone 50–100 mg versus standard of care	Acute decompensated HF (100)	Proportion of patients free from congestion at day 3 (P = 0.001)	Change in body weight (P = NS), decrease in plasma NTproBNP level (P = 0.05) and proportion of patients receiving oral furosemide at day 3 (P < 0.001)	Completed	[146]
Pilot Study of Natriuretic Versus Standard Doses of Mineralocorticoid Receptor Antagonists in Heart Failure and Loop Diuretic Resistance in Outpatients	NA	Randomized, controlled	Spironolactone 100 mg versus spironolactone 25 mg	Worsening HF (20)	Change in body weight between baseline and day 7	Change in estimated jugular venous pressure on physical examination, change in 6-min walking test, change in VAS dyspnoea and change on Likert scale	Recruitment complete	[147]
Natriuresis: sodium/glucose cotransporter 2 inhibitors[b]								
DAPA-HF	2019	Randomized, controlled	Dapagliflozin 10 mg versus placebo	Chronic HFrEF (4,744)	Combined rate of worsening HF or cardiovascular death (HR 0.74)	HF hospitalization (HR 0.75), change in KCCQ score (HR 1.18), worsening renal function (HR 0.71) and all-cause mortality (HR 0.83)	Completed	[125]

Table 2 *(Continued)*

Trial	Year of publication	Study design	Intervention	Study population (*n*)	Primary end point	Secondary end points[a]	Status	Refs
EMPA-RESPONSE	NA	Randomized, controlled	Empagliflozin 10 mg versus placebo	Acute decompensated HF (80)	Combination of dyspnoea relief, diuretic response, length of hospital stay and change in plasma NT-proBNP level (*P* = NS)	Combination of mortality, HF rehospitalizations within 30 days and worsening HF (*P* = 0.014)	Completed	[139]
EMPEROR Reduced/ Preserved	NA	Randomized, controlled	Empagliflozin 10 mg versus placebo	Chronic HFrEF or HFpEF (3,600 and 5,750)	Composite of time to first HF event, HF hospitalizations or death	Adjudicated HF hospitalization, worsening renal function, time to onset of T2DM, change in KCCQ score from baseline	Recruitment complete	[148, 149]
RECEDE-CHF	NA	Randomized, controlled, cross-over	Empagliflozin 25 mg versus placebo	Stable HF and T2DM (34)	Net urinary output	Glomerular filtration rate, plasma cystatin C levels and urinary sodium excretion	Recruiting	[150]
DAPA-Shuttle 1	NA	Randomized, controlled	Dapagliflozin 10 mg versus placebo	Stable HFrEF and T2DM (40)	Change in urinary osmolyte concentration	Concentration of plasma copeptin, tissue sodium content on ^{23}Na-MRI, and changes in muscle and liver lipid content	Recruiting	[151]

Table 2 *(Continued)*

Trial	Year of publication	Study design	Intervention	Study population (*n*)	Primary end point	Secondary end points[a]	Status	Refs
ELSI	NA	Randomized, controlled	Empagliflozin 10 mg versus placebo	Chronic HFmrEF (84)	Lower-leg skin sodium content (^{23}Na-MRI)	Sodium excretion, skin sodium and water content, plasma NT-proBNP level and vascular stiffness	Recruiting	[152]
ERADICATE-HF	NA	Randomized, controlled	Ertugliflozin 15 mg versus placebo	Chronic HF and T2DM (36)	Fractional excretion of Na after 1 and 12 weeks	Glomerular filtration rate, effective renal plasma flow and plasma RAAS hormone levels	Recruiting	[153]
EMPAG-HF	NA	Randomized, controlled	Empagliflozin 25 mg versus placebo	Acute decompensated HF and T2DM (60)	Total urinary output in 5 days	Worsening renal function, worsening HF, liver function and net fluid output	Recruiting	[154]
EMBRACE-HF	NA	Randomized, controlled	Empagliflozin 10 mg versus placebo	Chronic HF (60)	Change in pulmonary artery diastolic pressure	Changes in other right heart pressures, KCCQ score, 6-min walking test, plasma BNP level and HbA_{1c}	Recruiting	[155]
EMPULSE	NA	Randomized, controlled	Empagliflozin 10 mg versus placebo	Stabilized, acute decompensated HF (500)	Combination of time to death, HF events, time to first HF event and change in KCCQ	Change >10 points on KCCQ, change in plasma NT-proBNP level, days alive out of hospital, rehospitalization within 30 days and diuretic effect	Recruitment starting	[156]

Table 2 *(Continued)*

Trial	Year of publication	Study design	Intervention	Study population (*n*)	Primary end point	Secondary end points[a]	Status	Refs
Natriuresis: carbonic anhydrase inhibitors								
Kataoka et al.	2019	Observational, prospective	Acetazolamide 250–500 mg intravenous	Acute HF (18) or stable, chronic HF with hypochloraemia (12)	Short-term increase in plasma chloride level (P = 0.013) and long-term increase in plasma chloride level (P < 0.0001)	Haemoconcentration and worsening renal function (P = NS for both)	Completed	[157]
Imiela et al.	2017	Randomized, open-label	Acetazolamide dose adjusted to body mass (250–500 mg) intravenous versus standard of care	Acute decompensated HF (20)	Net fluid output and natriuresis over the first 4 days (P = NS for both)	Cumulative fluid balance after 4 days (P = 0.035) and presence of dyspnoea at day 4 (P < 0.001)	Completed	[158]
ADVOR	NA	Randomized, controlled	Acetazolamide 500 mg intravenous versus placebo	Acute decompensated HF (519)	Decongestion achieved at day 3 without need to escalate treatment	All-cause mortality, HF readmission within 3 months, length of hospital stay and changes in EuroQol-5 score	Recruiting	[159]

Table 2 *(Continued)*

Trial	Year of publication	Study design	Intervention	Study population (*n*)	Primary end point	Secondary end points[a]	Status	Refs
ACETA	NA	Randomized, controlled	Acetazolamide 500 mg intravenous versus placebo	Acute decompensated HF (90)	Diuresis and negative fluid balance	Worsening renal function, inotropic or vasopressor need, arrhythmias, death, plasma bicarbonate levels and plasma BNP levels	Recruiting	[160]
Aquaresis: vasopressin antagonists								
EVEREST	2007	Randomized, controlled	Tolvaptan 30 mg versus placebo	Acute decompensated HF (4,133)	All-cause mortality (HR 0.98) and composite of cardiovascular mortality and HF hospitalizations (HR 1.04)	Decrease in body mass at day 1 ($P < 0.001$), increase in plasma sodium level at day 7 ($P < 0.001$), presence of patient-assessed dyspnoea at day 1 ($P < 0.001$) and incidence of clinical worsening of HF ($P = 0.62$)	Completed	[4]
AQUAMARINE	2016	Randomized, controlled, open-label	Tolvaptan 15 mg versus standard of care	Acute decompensated HF with impaired renal function (217)	Increase in cumulative urine output over 48 h (mean difference 1,564 ml; $P < 0.001$)	Patient-reported dyspnoea after 48 h ($P = 0.02$), worsening renal function (P = NS), furosemide dose after 48 h ($P < 0.001$) and change in plasma BNP level ($P = 0.602$)	Completed	[161]

Table 2 *(Continued)*

Trial	Year of publication	Study design	Intervention	Study population (*n*)	Primary end point	Secondary end points[a]	Status	Refs
AVANTI	NA	Randomized, controlled	Pecavaptan 30 mg versus placebo (part A) or versus furosemide (part B)	Acute decompensated HF with incomplete decongestion (414)	Part A: change in body mass and plasma creatinine level. Part B: change in body mass and BUN:creatinine ratio	Incidence of treatment-emergent adverse event (including serious adverse events) and change in augmentation index	Recruiting	[137]

[a]Some trials have additional secondary end points not listed in the table. [b]Given that ≥43 trials to assess sodium–glucose cotransporter 2 inhibitors are ongoing, only selected trials have been included in the table. BNP, B-type natriuretic peptide; BUN, blood urea nitrogen; HF, heart failure; HFmrEF; heart failure with mid-range ejection fraction, HFpEF; heart failure with preserved ejection fraction, HFrEF, heart failure with reduced ejection fraction; KCCQ, Kansas City Cardiomyopathy Questionnaire; NA, not applicable; NS, not significant; NT-proBNP, N-terminal pro-B-type natriuretic peptide; RAAS, renin–angiotensin–aldosterone system; T2DM, type 2 diabetes mellitus; VAS, visual analogue scale.

Hypertonic saline: increasing osmosis

Infusion of a hypertonic saline solution theoretically increases the osmotic pressure of the intravascular compartment, attracting fluid from both the interstitium and cells. Moreover,, renal flow is thought to increase, which might result in improved availability of diuretic drugs at their site of action, the kidney[128]. Taken together, these effects would result in an increased diuretic response to decongestive therapies. Preliminary data from one group showed an increase in weight loss, preserved renal function and reductions in length of hospital stay, mortality and rehospitalization with the use of hypertonic saline infusion in addition to loop diuretic therapy compared with loop diuretics alone in patients with acute heart failure[129,130]. However, high-quality, blinded, randomized clinical trials have not yet been performed.

Compression therapy: increasing hydrostatic pressure

Compression therapy (multi-layered bandaging, manual lymphatic drainage and/or compression stockings) is advised as the main conservative treatment to improve lymphatic function and venous circulation, for instance in venous insufficiency[131]. However, compression therapy for the reduction of oedema in heart failure is controversial. Apprehension about increasing cardiac preload and pulmonary pressures has prohibited conclusive advice on the use of this therapy in heart failure[132,133].

Increasing lymphatic flow

Animal studies indicate that olprinone, a phosphodiesterase type 3 inhibitor, increases lymphatic flow in acute heart failure[134].

Splanchnic nerve block

The abdominal vascular compartment is the largest pool of intravascular blood. In heart failure, as a result of sympathetic nerve overactivity, vasoconstriction in this compartment forces venous blood to the thoracic compartment[19]. Preliminary data on splanchnic nerve blockade to interfere with this process showed a decrease in PCWP and patient-reported symptoms and an increase in cardiac output[20,135].

Extracorporeal blood ultrafiltration

Blood ultrafiltration is performed with a transmembrane pressure gradient in an extracorporeal unit to filter free water directly from the plasma. Several clinical trials have investigated extracorporeal ultrafiltration as an alternative to (loop) diuretic therapy in patients with acute heart failure. All but one trial found extracorporeal ultrafiltration to be superior in terms of reducing both long-term and short-term rehospitalizations and mortality[136]. Moreover, ultrafiltration resulted in greater net fluid loss than standard of care. However, patients treated with extracorporeal ultrafiltration also had significantly

more treatment-related adverse events, such as infection requiring antibiotics and bleeding requiring transfusion[136]. To date, insufficient knowledge about patient selection and adjustment of filtration rate prohibits general usage of extracorporeal blood ultrafiltration. Guidelines advise the use of extracorporeal ultrafiltration as a bail-out option for residual congestion despite treatment with a combination of diuretics[105].

Future perspectives

Several clinical trials are addressing the clinical unmet need to find better and additive decongestive therapies. Clinical trials published in the past 13 and ongoing trials to assess the effect of decongestive therapies on either mortality and hospitalization or end points related to sodium homeostasis or plasma volume measurements are described in TABLE 2. Of particular interest is the AVANTI trial[137] into the use of pecavaptan, a dual V1a–V2 receptor antagonist, in patients with acute heart failure and incomplete decongestion despite standard therapy including loop diuretics.

Conclusions

Residual congestion is frequently found in patients who are hospitalized for heart failure and is associated with a poor prognosis and a high rate of (short-term) rehospitalization. Therefore, better treatments or an improvement of current therapies are needed to treat residual congestion. An increased understanding of the pathophysiology of congestion will lead to better treatments of these patients with severe conditions. Intravascular congestion and tissue congestion have important differences.

Clinical assessments, biomarkers, emerging technologies and imaging tools can help to distinguish between the **presence** of predominant intravascular congestion versus tissue congestion. As summary of the markers identifying either type are depicted in Figure 4. Moreover, we argue that existing and novel therapies have different effects on each type of congestion. Natriuretic drugs can be used in relieving intravascular congestion, whereas residual tissue congestion might be better treated with an aquaretic drug, such as a vasopressin antagonist. Several clinical trials targeting residual congestion are ongoing, which will hopefully improve our understanding and lead to better outcomes and a more personalized approach to the treatment of congestion in heart failure. The main aim of this Review is to lay a foundation for a clinical subdivision of congestion into intravascular congestion and tissue congestion. Further research is needed to confirm the distinction between these two entities and to test our hypothesis of the clinical importance of distinguishing between the types of congestion.

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Chapter 3

Randomized, double-blind, placebo-controlled, multicentre pilot study on the effects of empagliflozin on clinical outcomes in patients with acute decompensated Heart Failure (EMPA-RESPONSE-AHF)

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Abstract

Aims | Inhibition of sodium–glucose co-transporter 2 (SGLT2) reduces the risk of death and heart failure (HF) admissions in patients with chronic HF. However, safety and clinical efficacy of SGLT2 inhibitors in patients with acute decompensated HF are unknown.

Methods and results | In this randomized, placebo-controlled, double-blind, parallel group, multicentre pilot study, we randomized 80 acute HF patients with and without diabetes to either empagliflozin 10 mg/day or placebo for 30 days. The primary outcomes were change in visual analogue scale (VAS) dyspnoea score, diuretic response (weight change per 40mg furosemide), change in N-terminal pro brain natriuretic peptide (NT-proBNP), and length of stay. Secondary outcomes included safety and clinical endpoints. Mean age was 76 years, 33% were female, 47% had *de novo* HF and median NT-proBNP was 5236 pg/mL. No difference was observed in VAS dyspnoea score, diuretic response, length of stay, or change in NT-proBNP between empagliflozin and placebo. Empagliflozin reduced a combined endpoint of in-hospital worsening HF, rehospitalization for HF or death at 60 days compared with placebo [4 (10%) vs. 13 (33%); $P = 0.014$]. Urinary output up until day 4 was significantly greater with empagliflozin vs. placebo [difference 3449 (95% confidence interval 578–6321) mL; $P < 0.01$]. Empagliflozin was safe, well tolerated, and had no adverse effects on blood pressure or renal function.

Conclusions | In patients with acute HF, treatment with empagliflozin had no effect on change in VAS dyspnoea, diuretic response, NT-proBNP, and length of hospital stay, but was safe, increased urinary output and reduced a combined endpoint of worsening HF, rehospitalization for HF or death at 60 days.

Introduction

Multiple randomized clinical trials indicated that sodium–glucose co-transporter 2 (SGLT2) inhibitors reduce the risk for heart failure (HF) hospitalization in patients with type 2 diabetes.[1-4] Although only a minority of patients included in these trials had pre-existing HF, the results showed the potential to also improve outcomes in patients with established HF. [3, 5-8] Beneficial effects in patients with established chronic HF were shown in a recent trial where the SGLT2 inhibitor dapagliflozin reduced the risk of death and HF admissions in patients with established chronic HF with a reduced ejection fraction (HFrEF), either with or without diabetes.[9] These beneficial effects are at least partly explained by the diuretic/natriuretic effects of SGLT2 inhibitors, although other mechanisms such as direct cardiometabolic and renal enhancing effects have been proposed as well. [10-12]

Acute (decompensated) HF is one of the leading causes of hospital admissions worldwide with a post-discharge mortality and rehospitalization risk as high as 20–30% within the first 3 to 6 months. Unlike chronic HF, there is no established therapy available that improves clinical outcome in acute HF.[13] Despite treatment with loop diuretics, many are discharged with residual congestion, which is related to an even higher risk of early rehospitalization and death.[14] Renal failure and worsening renal function in patients with acute HF are common and related to an impaired outcome when diuretic response is poor.[15, 16] Based on both the promising pharmacological profile of the SGLT2 inhibitor empagliflozin and the demonstrated benefits on HF and renal outcomes, we hypothesized that empagliflozin exerts positive effects in patients admitted with acute HF, with or without diabetes mellitus.

Methods

Study design

EMPA-RESPONSE-AHF was an investigator initiated randomized, placebo-controlled, double-blind, parallel group, multicentre pilot study in subjects admitted for acute (decompensated) HF. Patients with and without type 2 diabetes mellitus could participate. A total of 80 eligible subjects were randomized in a 1:1 ratio to receive either empagliflozin 10 mg/day or matched placebo. A blocked randomization was used (size 4), with stratification by study site. Investigators used a web-based randomization system to determine treatment assignment. The trial was executed in five cardiology centres in the Netherlands (online supplementary *Appendix S1*). The trial was approved by the ethics committee at each study centre and the study was conducted in accordance with the Declaration of Helsinki and the International Conference on Harmonisation Guide-

lines for Good Clinical Practice. All participating patients provided written informed consent. This trial was registered with ClinicalTrials.gov, number NCT03200860.

Patient population

Eligible patients were male or female aged >18 years who were hospitalized for acute HF, defined as all of the following: (i) dyspnoea at rest or with minimal exertion, (ii) signs of congestion, such as oedema, rales, and/or congestion on chest radiograph, (iii) brain natriuretic peptide (BNP) ≥350 pg/mL or N-terminal pro BNP (NT-proBNP) ≥1400 pg/mL (for patients with atrial fibrillation: BNP ≥500 pg/mL or NT-proBNP ≥2000 pg/mL), and (iv) treated with loop diuretics at screening. Patients needed to have an estimated glomerular filtration rate (based on the Chronic Kidney Disease Epidemiology Collaboration formula[17]) ≥30 mL/min/1.73m2 between presentation and randomization. Exclusion criteria were: (i) type 1 diabetes mellitus, (ii) dyspnoea primarily due to non-cardiac causes, (iii) cardiogenic shock, (iv) acute coronary syndrome within 30 days prior to randomization, (v) planned or recent percutaneous or surgical coronary intervention within 30 days prior to randomization, (vi) signs of ketoacidosis and/or hyperosmolar hyperglycaemic syndrome (pH>7.30 and glucose >15 mmol/L and HCO3 >18 mmol/L), (vii) pregnant or nursing (lactating) women, (viii) current participation in any interventional study, (ix) inability to follow instructions or comply with follow-up procedures, (x) any other medical conditions that would put the patient at risk or influence study results in the investigator's opinion, or that the investigator deemed unsuitable for the study.

Patients were randomized within 24 h of presentation to the hospital. After informed consent, patients were randomly assigned to one of the treatment groups and received the assigned (blinded) therapy after baseline assessment, which included assessment of HF signs and symptoms, visual analogue scale (VAS) dyspnoea score, vital signs, demographics, and urine and plasma sampling. During 4 days following randomization, patients were evaluated daily per protocol and included evaluation of HF signs and symptoms, vital signs, weight, laboratory assessments (including NT-proBNP at day 4), plasma and urine sampling and assessment of adverse events (AE). Three of four primary outcome measures (VAS dyspnoea score, NT-proBNP, diuretic response) were assessed at day 4. If a patient was discharged before day 4, assessment took place outside the hospital at day 4. Randomized treatment was continued through day 30, when a study visit was carried out and assessments were repeated. Patients were followed until day 60 for AE, and the study was concluded by a telephone call at day 60 to assess AE and vital status.

Study endpoints

The primary endpoints of this study were (i) change in dyspnoea, VAS from baseline to day 4, (ii) diuretic response (defined as Δ weight kg/[(total intravenous dose)/40mg]+[(-total oral dose)/80mg)] furosemide or equivalent loop diuretic dose) through day 4, (iii) length of initial hospital stay, and (iv) percentage change in NT-proBNP from baseline to day 4. [18]

Secondary endpoints of the study included worsening HF (defined as worsening signs and/or symptoms of HF that require an intensification of intravenous therapy for HF or mechanical ventilatory, renal or circulatory support), all-cause death and/or HF readmission through day 30 and through day 60 as part of AE assessment.

Safety endpoints included (i) AE (general), (ii) AE that lead to treatment discontinuation, (iii) serious AE (which could include a secondary endpoint), (iv) AE of special interest (AESI), including hepatic injury, worsening renal function, metabolic acidosis, ketoacidosis and diabetic ketoacidosis (online supplementary *Methods S1*).

Randomized treatment was required to be discontinued per protocol if systolic blood pressure dropped below 90mmHg or decreased below 100mmHg with signs/symptoms of hypotension, or signs of ketoacidosis and/or hyperosmolar hyperglycaemic syndrome, or any increase in serum creatinine >50%. Treating physicians were encouraged to reinitiate randomized treatment after resolution of the above mentioned criteria.

Statistical analysis

Normally distributed continuous variables are presented as mean ± standard deviation (SD), on-normally distributed variables as median and 25th–75th percentile. Categorical variables are presented as numbers (percentage). Power calculation was based on capturing the primary outcome measures in the placebo group with a degree of certainty. With 40 patients in the control group, a given mean continuous response can be estimated within ± 0.2 SD with 80% confidence intervals (CI). We estimated the following mean responses for the primary outcome measures (in the placebo group):

1. Change in VAS dyspnoea score: 1756 ± 2353mm × h at day 4.
2. Diuretic response [of 0.56 ± 0.78 kg/40mg furosemide (or equivalent)] at day 4.
3. Length of stay 9.6 to 10.5 days (± 9.1).
4. Percentage change in NT-proBNP 24 (−1.0–88.7)% (SD 67%) at day 4.

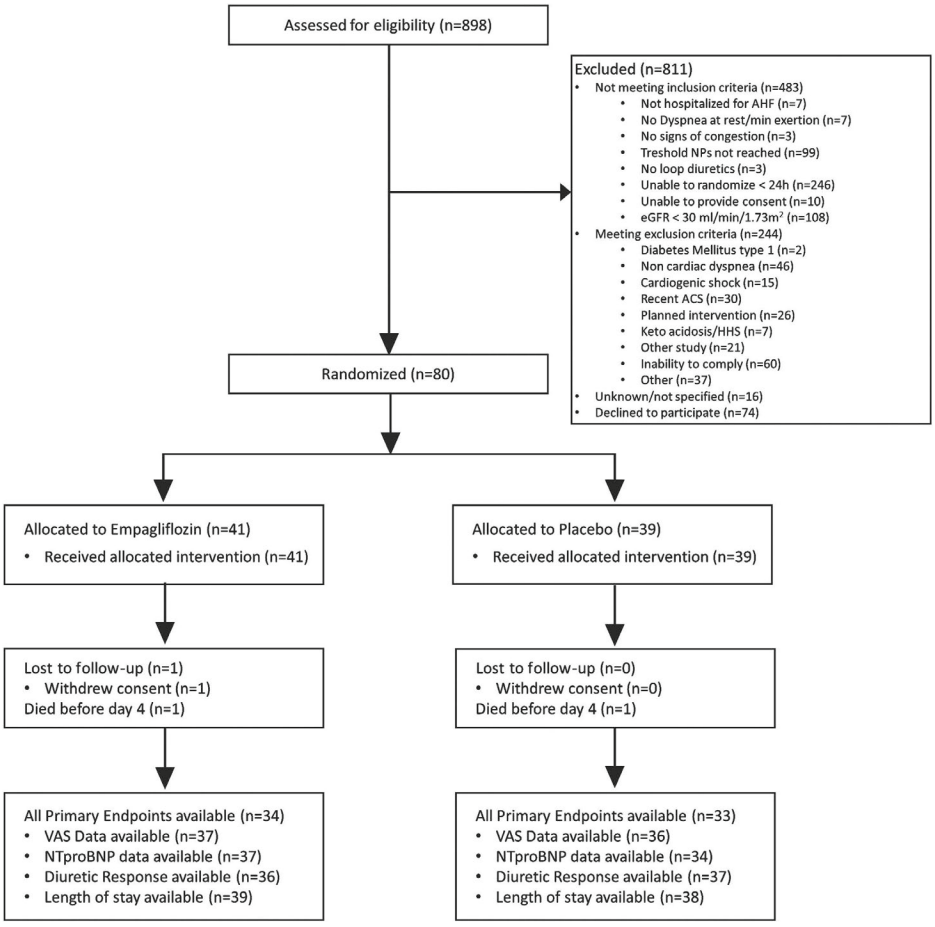

Figure 1 | Consort diagram. ACS, acute coronary syndrome; AHF, acute heart failure; eGFR, estimated glomerular filtration rate; HHS, hyperosmolar hyperglycaemic syndrome; NP, natriuretic peptide; NT-proBNP, N-terminal pro brain natriuretic peptide; VAS, visual analogue scale.

Consequently, 40 patients per group would provide approximately 80% power to detect standardized mean treatment differences of approximately 0.48 SDs at the two-sided 20% significance level for this pilot study. The difference in change in dyspnoea VAS from baseline to day 4 was assessed by comparing the area under the curve (AUC) of change in VAS dyspnoea score by Student's *t*-test. To do so, individual changes in VAS score were visualized (virtually) as a curve where the x-axis shows study day baseline to day 4, and y-axis shows VAS score. Using this approach, AUC for each study day (trapezoids) can be calculated, and added together, resulting in an overall VAS AUC score (mm × h) that can be compared across treatment groups.[19] Difference in diuretic response and percentage change in NT-proBNP at day 4 was assessed by

Student's *t*-test. Difference in length of stay was assessed by Wilcoxon rank-sum test. All analyses were carried out in the full analysis set based on the intention-to-treat principle where all randomized patients were analysed. As this was an exploratory study with limited power, all four individual primary endpoints were tested separately, with no formal correction for multiplicity. Only as a sensitivity analysis, if at least two out of four primary endpoints showed significant difference in the same direction (favouring either investigational drug or placebo), a Bonferroni correction would be applied ($P < 0.05$).

As an exploratory analysis, and for graphical presentation, individual responses to the above-mentioned endpoints were standardized. This was done by dividing the difference from the overall mean (or log transformed mean if non-normally distributed) of each endpoint by the overall SD of that variable, which generates a z-score. The treatment effect can then be measured by the mean difference of standardized z-scores, which was visually presented on a forest plot by mean ± 80% CI (given $P < 0.2$).

Then, all four standardized scores for each individual endpoints were averaged, and mean treatment difference and associated 95% CI for this overall treatment effect visually presented. Statistical analysis for the treatment difference was carried out by Student's *t*-test. Two tailed *P*-values <0.2 were considered statistically significant for the (exploratory) primary endpoints with 80% CI. Statistical analyses were performed using STATA SE 12.0 (Stata Corp., College Station, TX, USA).

Results

Patients

From December 2017 through July 2019 patients were enrolled at five centres in the Netherlands. The CONSORT diagram of this study is presented in *Figure 1*. A total of 80 patients were randomized (41 to empagliflozin and 39 to placebo). One patient randomized to empagliflozin withdrew informed consent, leaving 40 empagliflozin and 39 placebo patients for our analyses. *Table 1* shows the baseline characteristics in the two groups. Mean age of the patients was 76 years, 33% were female, 47% had *de novo* acute HF, mean left ventricular ejection fraction was 36%, and median NT-proBNP was 5236 pg/mL. One third of patients had type 2 diabetes mellitus. Groups were reasonably well-balanced, although patients in the empagliflozin group were older, more often female and had lower NT-proBNP levels.

Table 1 | Baseline characteristics

	Randomized Treatment		
	Empagliflozin	**Placebo**	**P-value**
N	40	39	
Age	79 (73 – 83)	73 (61-83)	0.14
Females (N (%))	16 (40)	10 (26)	0.17
Caucasian race (%)	100	95	0.15
Body weight at baseline (kg)	87 ± 23	83 ± 20	0.42
SBP (mmHg)	127 ± 22	121 ± 25	0.25
DBP (mmHg)	76 ± 15	72 ± 15	0.27
HR (mmHg)	83 ± 19	80 ± 23	0.50
Respiratory rate (breaths/min)	19 ± 4	20 ± 5	0.60
NYHA class (% III/IV)	92	97	0.57
LVEF if known (%) (n =46)	36 ± 17	37 ± 14	0.87
De Novo acute HF (%)	48	46	0.90
Ischemic aetiology (%)	28	29	0.89
Medical History (%)			
Myocardial infarction	30	38	0.43
Hypertension	68	56	0.31
Atrial fibrillation/flutter	78	64	0.19
Diabetes mellitus type II	38	28	0.38
Cerebrovascular accident	5	5	0.98
COPD	28	26	0.85
Cancer	38	13	0.012
Medical Therapy (%)			
ACEi	40	47	0.51
ARB	5	3	0.45
Beta-blocker	70	66	0.69
MRA	48	45	0.81
Loop diuretic	100	100	NA
ICD	8	23	0.054
CRT	15	13	0.78
Laboratory at Baseline			
NT-proBNP (pg/mL)	4406 (2873-6979)	6168 (3180-10489)	0.14
Serum creatinine (µmol/L)	114 ± 34	116 ± 33	0.72
eGFR (mL/min/1.73m^2)	55 ± 18	55 ± 18	0.97
Sodium (mmol/L)	135 ± 17	135 ± 5	0.99

ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; ARNI, angiotensin receptor–neprilysin inhibitor; COPD, chronic obstructive pulmonary disease; CRT, cardiac resynchronization therapy; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; HF, heart failure; HR, heart rate; ICD, implantable cardioverter defibrillator; LVEF, left ventricular ejection fraction; MRA, mineralocorticoid receptor antagonist; NA, not applicable; NT-proBNP, N-terminal pro brain natriuretic peptide; NYHA, New York Heart Association; SBP, systolic blood pressure.

Primary outcome

Results of the primary endpoints are presented in *Figure 2*. The AUC of the change in dyspnoea VAS over the first 4days was 1264 ± 1211mm × h) in the empagliflozin group vs. 1650 ± 1240mm × h in the placebo group (*P* = 0.18). Diuretic response through day 4 was -0.35 ± 0.44) kg/40mg furosemide equivalents in the empagliflozin group vs. -0.12 ± 1.52 kg/40 mg furosemide equivalents in the placebo group (*P* = 0.37). Percentage change in NT-proBNP through day 4 was -46 ± 32% in the empagliflozin group vs. -42 ± 31% in the placebo group (*P* = 0.63). Length of hospital stay was 8 (6–10) days in the empagliflozin group vs. 8 (6–9) days in the placebo group (*P* = 0.58). There were no differences in the primary outcome measures in subgroups of *de novo* vs. decompensated HF.

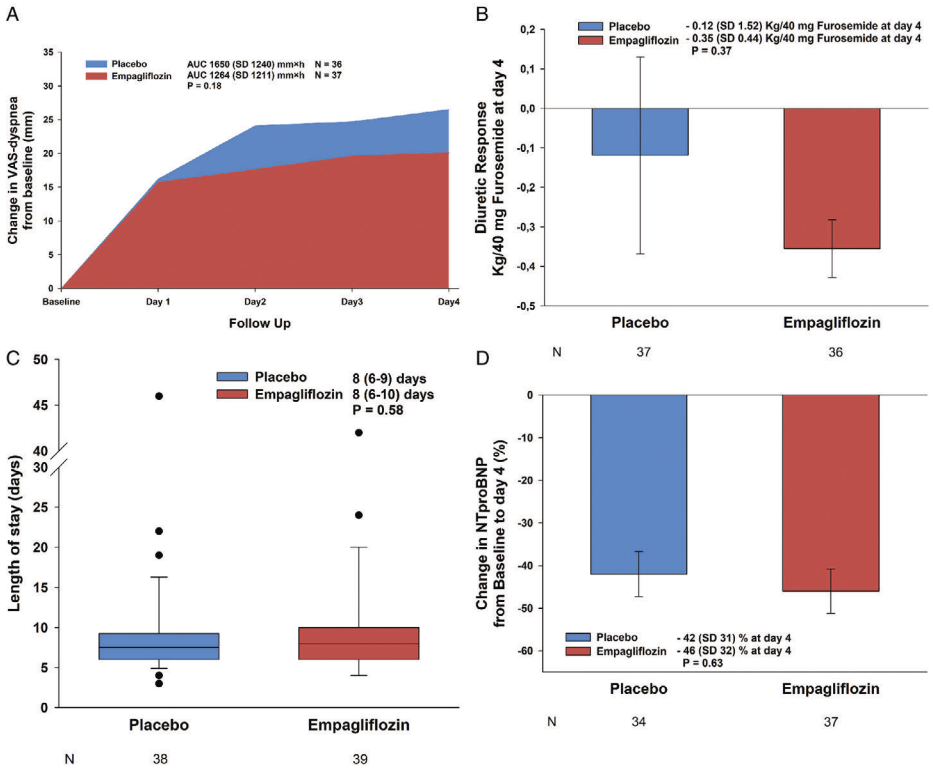

Figure 2 | Primary endpoints

a | Change in VAS Dyspnea, **b** | Diuretic Response, **c** | Percentage change in NT-proBNP, **d** | Length of stay. Error bars represent 80% confidence intervals for B and C. Abbreviations: AUC: Area Under the Curve, VAS: Visual Analogue Scale, NTproBNP: N Terminal Pro Brain Natriuretic Peptide, SD: Standard Deviation

A summary of the four endpoints, standardized by z-scores, are presented in *Figure 3*. The overall combined z-score was not significantly different between empagliflozin and placebo (mean difference −0.019, 95% CI −0.306 to 0.269; *P* = 0.90).

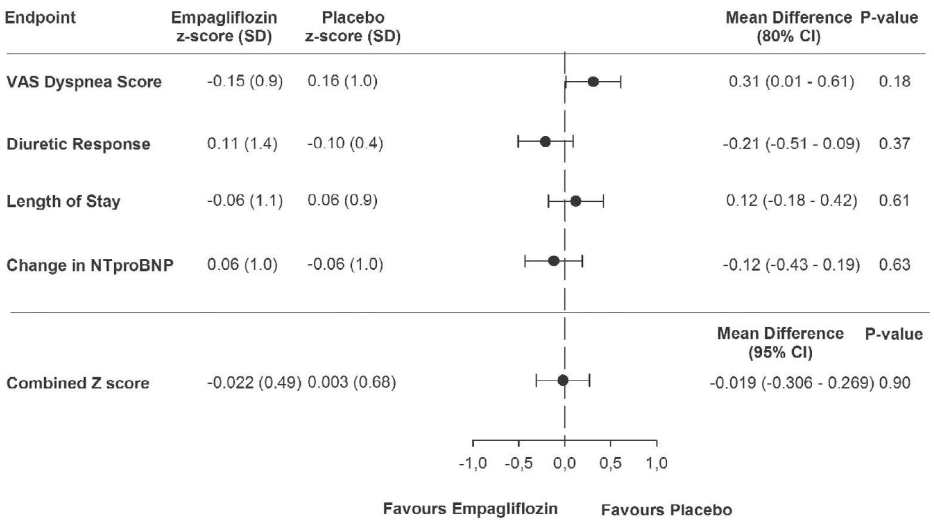

Figure 3 | Z-score presentation of primary endpoints and combined z-score

Abbreviations: CI: Confidence Interval, VAS: Visual Analogue Scale, NTproBNP: N Terminal Pro Brain Natriuretic Peptide, SD: Standard Deviation

Secondary and exploratory analyses

Figure 4 shows the incidence of in-hospital worsening HF, death, and/or HF hospital readmission. A combined endpoint of in-hospital worsening HF, rehospitalization for HF or all-cause death at 60 days occurred in 4 patients (10%) in the empagliflozin group vs. 13 patients (33%) in the placebo group (*P* = 0.014). Online supplementary *Table S1* provides the data for the individual components. There was a greater, but not statistically different, drop in diastolic blood pressure in the empagliflozin treated patients, similar reductions in systolic blood pressure and heart rate, and no differences in renal function were demonstrated up until day 4 (online supplementary *Figure S1*). Patients randomized to empagliflozin more often had a diuretic response better than 0.4 kg decrease/40 mg furosemide equivalent compared with placebo (42% vs. 24%, *P* = 0.14).

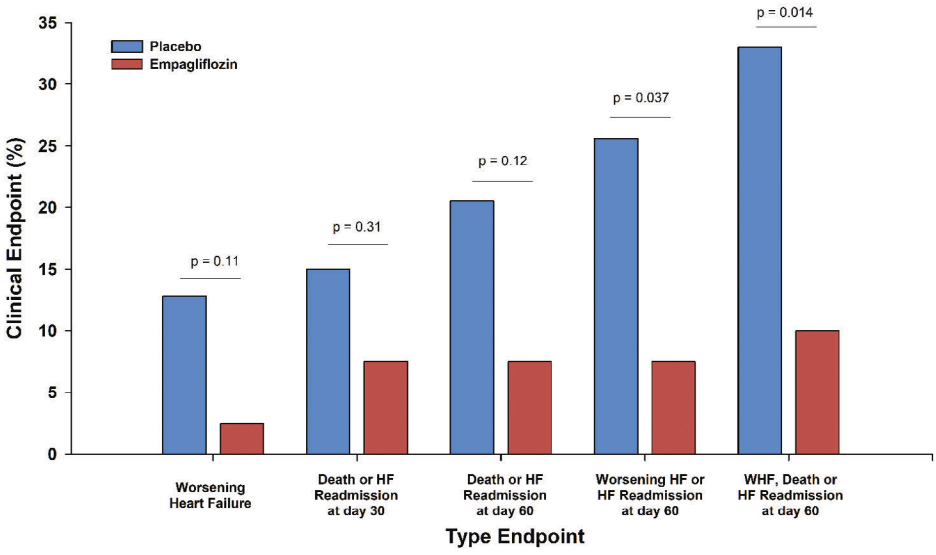

Figure 4 | Clinical events

In a subset of patients, urinary output and net fluid loss were available. The effects of empagliflozin on urinary output and net fluid balance are presented in *Figure 5*. At day 1, there was a significantly greater urine output with empagliflozin (3442 ± 1922 mL) compared with placebo (2400 ± 993 mL) (P = 0.013; n = 58). Net fluid loss at day 1 was also greater with empagliflozin (−2163 ± 1896 mL) compared with placebo (−1007 ± 1049 mL) (P = 0.009; n = 53). After 4 days, the difference in cumulative urine output (3449, 95% CI 578–6321 mL; n = 28) was significantly greater (P = 0.02), whereas net fluid loss with empagliflozin was greater (2701, 95% CI −586 to 8988 mL, n = 25; P = 0.10). Weight change after 4 days was −2.83 ± 3.15 kg in the empagliflozin group vs. −2.30 ± 3.26 kg in the placebo group (P = 0.48). Median loop diuretic dose (re-calculated to furosemide) through day 4 was 320 (194–466) mg furosemide in the empagliflozin group and 300 (200–500) mg furosemide in the placebo group (P = 0.94).

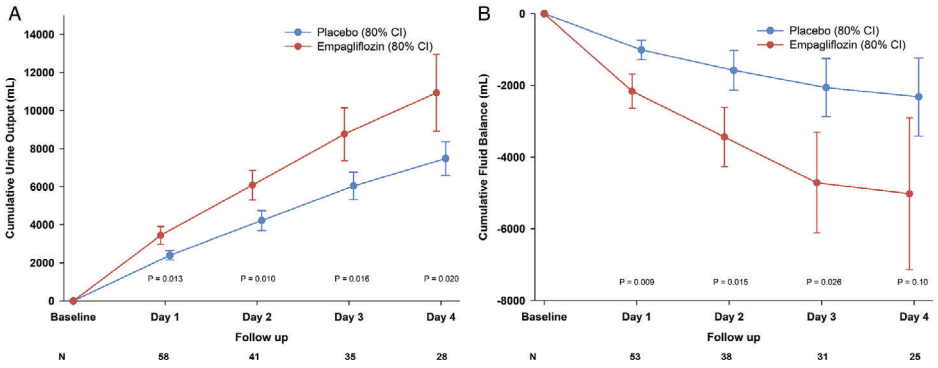

Figure 5 | Urinary output and net fluid balance through day 4

a | Cumulative Urine Output, **b |** Cumulative Net Fluid balance

Abbreviation: CI: Confidence Interval

Safety

Safety data are presented in *Table 2*. The incidence rates of AE were similar in subjects treated with placebo or empagliflozin. Patients randomized to empagliflozin had significantly lower number of cardiovascular AE compared with placebo (9 vs. 17 events, *P* = 0.046). This was mostly due to more frequent worsening HF events in the placebo group. We did not find an excess in urinary tract infections or other adverse effects with the use of empagliflozin. There were 8 serious adverse events in the empagliflozin group vs. 11 in the placebo group (*P* = 0.54). The causes of these events are listed in online supplementary *Table S2*. Overall, seven patients in the empagliflozin group and five patients in the placebo group discontinued study medication due to AE (*P* = 0.36) (online supplementary *Table S3*). There was no difference in the occurrence of AESI. Four patients (10%) in the empagliflozin and three patients (8%) in the placebo group developed a worsening renal function AESI (*P* = 0.74), while one patient with type 2 diabetes in the placebo group experienced diabetic ketoacidosis (online supplementary *Table S4*). There was no sign that empagliflozin was associated with more renal events as the renal/urinary AE rate was similar, and the occurrence of worsening renal function or acute kidney injury was also not different between treatment groups. Online supplementary *Table S5* lists all individual AE.

Table 2. Adverse Events

Adverse event	Empagliflozin (n = 40)	Placebo (n = 39)	P-value
Overall	55	63	
Cardiovascular	9 (23)	17 (44)	0.046
Respiratory	3 (8)	2 (5)	0.67
Gastro Intestinal	6 (15)	9 (23)	0.36
Psychiatric	0 (0)	1 (3)	0.31
Renal/Urinary	15 (38)	13 (33)	0.70
Reproductive	0 (0)	0 (0)	NA
Metabolic	9 (23)	9 (23)	0.95
Musculoskeletal	5 (13)	5 (13)	0.97
Thromboembolic	1 (0)	0 (0)	NA
Infectious	1 (3)	0 (0)	0.32
Other	6 (15)	7 (18)	0.72

NA, not applicable.
First events in a category in an individual patient are shown as n (%).

Discussion

In this randomized, double-blind, placebo-controlled, multicentre pilot study on the safety and efficacy of empagliflozin in patients with acute (decompensated) HF, change in VAS dyspnoea, reduction in NT-proBNP, diuretic response (weight loss per 40 mg furosemide) and length of hospital stay were similar with empagliflozin and placebo. Empagliflozin increased cumulative urinary volume and net fluid balance in a subset of patients. Importantly, empagliflozin appeared to be safe and well tolerated, without major effects on heart rate and blood pressure. Finally, we observed significantly fewer deaths, in-hospital worsening of HF and/or HF readmissions through day 60. As far as we know, this is the first randomized, double-blind, placebo-controlled trial on the effects of a SGLT2 inhibitor in patients with acute HF. SGLT2 inhibitors were originally designed as glucose-lowering agents for glycaemic control. In four large randomized controlled trials in patients with diabetes, SGLT2 inhibitors consistently reduced cardiovascular events, and HF hospitalizations in particular. These data prompted the design of large phase III clinical trials on the effects of SGLT2 inhibitors in patients with established chronic HF, irrespective of whether they had diabetes or not.[20, 21] Recently, main results of the first trial, Dapagliflozin and Prevention of Adverse Outcomes in Heart Failure (DAPA-HF), were presented and published.[9] In 4474 patients with HFrEF with and without diabetes, dapagliflozin reduced the risk of a composite endpoint of worsening HF (hospitalization or an urgent visit resulting in intravenous therapy for HF) or cardiovascular death. These effects were accompanied by an improvement of quality of life. These beneficial effects of dapagliflozin in patients with HFrEF on quality of life

were recently confirmed in a smaller study.[22] Until now, no data on the effects of SGLT2 inhibitors in patients admitted with acute HF, irrespective of left ventricular ejection fraction and diabetes status, have been available. In the present study, we could not show any significant differences for the primary endpoints between empagliflozin and placebo. First, despite both a significantly greater urinary output and a more negative net fluid balance, there was no reduction in dyspnoea, as recorded by a VAS. Second, we did not observe an improvement in diuretic response, defined as weight change per 40mg of furosemide (or equivalent dose of another loop diuretic).[18] This endpoint was chosen to correct for a decreased use of loop diuretic when symptoms had recovered more quickly in the empagliflozin treated patients. However, symptoms in hospital did not recover more quickly and diuretic use during the first 4 days were similar in both groups. Thirdly, although we observed an expected large drop in NT-proBNP in the first days of hospital admission in all patients, we did not demonstrate a greater drop in patients treated with empagliflozin. These findings are similar to a recent study with dapagliflozin in patients with chronic HFrEF. [22]

Finally, length of hospital stay was not shortened by empagliflozin, probably since it may be determined by multiple factors other than those specifically related to improvement in HF, particularly in an elderly, fragile, high-risk patient cohort such as included in this study. We observed a significant reduction of a combined endpoint of in-hospital worsening HF, death and/or hospital readmission through day 60. However, these data should be interpreted with caution for two major reasons. Firstly, this study was not powered and not designed to show an effect on clinical endpoints, and the number of events is very low. Secondly, there was no significant reduction in the pre-defined secondary endpoint of death and/or hospital readmission within 30 days. Nevertheless, the reduction in clinical endpoints is consistent with previous morbidity and mortality benefits for SGLT2 inhibitors in diabetes and in line with results of the DAPA-HF trial in patients with chronic HFrEF.[9] If anything, our results suggest this novel HF treatment can safely be initiated in a high-risk population of acute HF patients and should pave the way for larger studies. Although loop diuretics and nitrates (in selected patients) remain the mainstay of the treatment of acute HF, several drugs have been investigated but failed to improve clinical outcomes in patients with acute HF. Most of these investigational drugs had significant effects on blood pressure and/or renal function. [23-25] However, in our small pilot study, we did not find any clinically relevant effect on blood pressure, heart rate, or renal function. In addition, empagliflozin drug was safe and well tolerated with less AE than in placebo treated patients. Specifically, empagliflozin therapy was not associated with more frequent worsening of renal function or renal AE. Finally, we found a remarkable effect on urinary output and net fluid balance. These findings strongly support an incremental diuretic effect

with empagliflozin treatment, which has not been previously shown in patients with HF. The disconnect between this increase in diuresis, without an effect on symptoms or markers of volume overload is probably related to the limited correlation between these variables in clinical practice. Whether the beneficial effects of SGLT2 inhibitors on clinical outcomes in patients with HFrEF are related to their diuretic effect or whether they are mediated via other pathways where SGLT2 inhibitors exert their actions remains to be established.

Limitations

This study has several limitations. First and foremost, this study is limited by the number of patients and should be considered as a pilot study. The results of this study should therefore be interpreted with caution. Secondly, we screened many more patients than were included in the study due to different reasons. Although for this reason the generalizability of the finding to the average acute HF patient can be questioned, the characteristics of our patients included suggest it represents the phenotype of acute HF patients currently admitted. Thirdly, the number of missing urinary collections and fluid intake limits the interpretability of the data on urinary output and net fluid loss, although an effect was already observed after 24 h. Finally, there was no standardized protocol for in-hospital treatment of acute HF and no protocol for diuretic therapy, which means individual differences in the treatment of these patients may have impacted the results.

Conclusion

In this randomized, double-blind, placebo-controlled pilot study in patients with acute (decompensated) HF, empagliflozin was safe and well tolerated, but did not improve dyspnoea, NT-proBNP, diuretic response and length of hospital stay. However, empagliflozin was associated with greater urinary output and a reduction in a combined endpoint of worsening HF, rehospitalization for HF or death at 60 days. Larger randomized clinical trials with SGLT2 inhibitors are greatly needed to further study the possible beneficial role of SGLT2 inhibitors in patients with acute HF.

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Supplementary material

Appendix S1. EMPA-RESPONSE-AHF site list and investigators

Overall study Principle Investigators:

- Prof dr. A.A. Voors
- Prof dr. H.J. Lambers Heerspink

Sites

1) University Medical Center Groningen
   Investigator: Dr. K. Damman
   Inclusion: 55

2) Antonius Ziekenhuis Sneek
   Investigator: Dr. H.P. Swart
   Inclusion: 10

3) TREANT ziekenhuisgroep
   Investigator: Dr. T.D.J. Smilde
   Inclusion: 7

4) ISALA klinieken Zwolle
   Investigator: Dr. A. Elvan
   Inclusion: 3

5) Jeroen Bosch Ziekenhuis Den Bosch
   Investigator: Dr. J.W.M. van Eck
   Inclusion: 5

Methods S1. AESI Defintions

• Hepatic injury

A hepatic injury is defined by the following alterations of hepatic laboratory parameters after randomisation:

- An elevation of AST and/or ALT $\geq$ 3 fold ULN combined with an elevation of total bilirubin $\geq$ 2 fold ULN measured in the same blood sample
- An isolated elevation of ALT and/or AST $\geq$ 5 fold ULN

These laboratory findings constitute a hepatic injury alert and the patients showing these abnormalities need to be followed up according to medical abnormalities need to be followed up according to medical judgement.

In case of clinical symptoms of hepatic injury (icterus, unexplained encephalopathy, unexplained coagulopathy, right upper quadrant abdominal pain, etc.) without laboratory results (ALT, AST, total bilirubin) available, the investigator should make sure these parameters are analysed, if necessary in an unscheduled blood test.

• Worsening Renal Function
either defined by:

- drop in eGFR below (<) 20 mL/min/1.73m2 in the first 5 days of randomized treatment

or

- A serum creatinine value showing ≥ 2 fold increase from baseline and is above the ULN.

For the AESI "Worsening renal function" the Investigator shall collect an unscheduled laboratory sample for creatinine as soon as judged clinically necessary and initiate follow-up laboratory test of creatinine according to medical judgement. Additionally, the Investigator shall provide information on the medical handling of the AESI, including changes in concomitant treatment.

• Metabolic acidosis, ketoacidosis and diabetic ketoacidosis (DKA)
In case of metabolic acidosis, ketoacidosis and DKA further investigations should be done according to the medical judgement and the clinical course until a diagnosis is made and/or the patient is recovered.

DKA is defined by the diagnostic criteria in the table below, and as defined by the American Diabetes Association (ADA).

Investigators should note that not all criteria in the table below need to apply for the diagnosis of DKA, and clinical judgement should also be taken into consideration. Due to its mechanism of action, empagliflozin may potentially modify the clinical presentation of DKA which may occur at lower plasma glucose levels than stated in the table below.

Table: Diagnostic criteria for DKA

	DKA		
	Mild	Moderate	Severe
Plasma glucose (mg/dL)	>250	>250	>250
Arterial pH	7.25-7.30	7.00-7.24	<7.00
Serum bicarbonate (mEq/L)	15-18	10 to <15	<10
Urine ketones*	Positive	Positive	Positive
Serum ketones*	Positive	Positive	Positive
Effective serum osmolality (mOsm/kg)**	Variable	Variable	Variable
Anion gap***	>10	>12	>12
Alteration in sensoria or mental obtundation	Alert	Alert/drowsy	Stupor/coma

* Nitroprusside reaction method
** Calculation: 2[measured Na (mEq/L) + glucose (mg/dL)]/18
*** Calculation: $(Na^+) - (Cl^- + HCO3^-)$ (mEq/L)

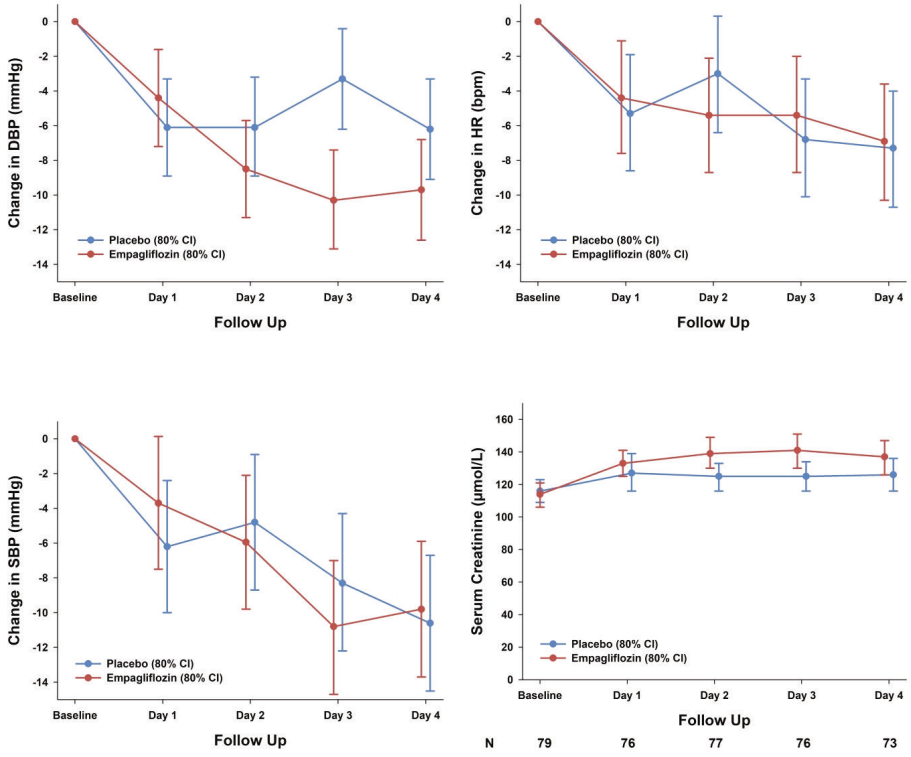

Figure S1. Changes in vitals and serum creatinine

Table S1. Cardiovascular outcome

Cardiovascular Outcome	Empagliflozin	Placebo	P-value
Overall	40	39	
Worsening Heart Failure, n (%)	1 (2.5)	5 (12.8)	0.11
30 day mortality, n (%)	1 (2.5)	1 (2.6)	0.75
60 day mortality, n (%)	1 (2.5)	3 (7.7)	0.36
60 day HF rehospitalization, n (%)	2 (5.0)	5 (12.8)	0.26
60 day mortality or HF rehospitalization, n (%)	3 (7.5)	8 (20.5)	0.12
60 day HF rehospitalization or WHF, n (%)	3 (7.5)	10 (25.6)	0.037
60 day mortality or HF rehospitalization or WHF, n (%)	4 (10.0)	13 (33.3)	0.014

Mortality is all cause mortality. WHF: Worsening heart failure to Day 5 post randomization (or discharge if earlier), defined as worsening signs and/or symptoms of heart failure that require an intensification of intravenous therapy for heart failure or mechanical ventilatory, renal or circulatory support.)

Table S2. Serious adverse events

Adverse Event	Empagliflozin	Placebo	P-value
Overall	8	11	0.54
WHF	3 (8)	4 (10)	
WRF	1 (3)	0 (0)	
AKI	1 (3)	0 (0)	
Angioedema	1 (3)	0 (0)	
Hypovolemic Shock	0 (0)	1 (3)	
Delirium	0 (0)	1 (3)	
Heart Failure	0 (0)	1 (3)	
Atrial Fibrillation	0 (0)	1 (3)	
S. Aureus bacteriemia	1 (3)	0 (0)	
Syncope	1 (3)	1 (3)	
Respiratory Failure	0 (0)	1 (3)	

All serious adverse events counted, multiple similar events in one patient possible
AKI: Acute Kidney Injury, WHF: Worsening Heart Failure, WRF: Worsening Renal Function

Table S3. Adverse events leading to treatment discontinuation

Adverse Event	Empagliflozin	Placebo	P-value
Overall	7	5	0.36
WRF	1 (3)	1 (3)	
AKI	1 (3)	0 (0)	
Angioedema	1 (3)	0 (0)	
HHS	1 (3)	0 (0)	
Itching	1 (3)	0 (0)	
Ketoacidosis	0 (0)	1 (3)	
Nausea	0 (0)	2 (5)	
Noro virus	1 (3)	0 (0)	
Syncope	1 (3)	0 (0)	
Urinary Tract Infection	0 (0)	1 (3)	

AKI: Acute Kidney Injury, HHS: hyperosmolar hyperglycemic syndrome, WRF: Worsening Renal Function

Table S4. Adverse events of special interest

AESI	Empagliflozin	Placebo	P-value
Overall	4	4	0.29
Hepatic	0 (0)	0 (0)	NA
Renal	4 (10)	3 (8)	0.74
DKA	0 (0)	1 (3)	0.30

AESI: Adverse Event of Special Interest, DKA: Diabetic Keto Acidosis

Table S5. List of all adverse events according to treatment

Adverse Event (n (%))	Empagliflozin	Placebo
Acute Kidney Insufficiency	1 (3)	0 (0)
Anemia	1 (3)	2 (5)
Angioedema	1 (3)	0 (0)
Aortic Valve Stenosis	0 (0)	1 (3)
Atrial Fibrillation	0 (0)	2 (5)
Barrett Esophagus	0 (0)	1 (3)
Bursitis	1 (3)	1 (3)
Chest Pain	2 (5)	0 (0)
Cough	1 (3)	0 (0)
Coughing	1 (3)	0 (0)
Cramp In Hands	0 (0)	1 (3)
Cramp In Legs	2 (5)	1 (3)
Creatinine Increase	3 (8)	3 (8)
Cystitis	1 (3)	0 (0)
Dehydration	1 (3)	0 (0)
Delirium	0 (0)	1 (3)
Disorientated	1 (3)	0 (0)
Diarrhoea	0 (0)	3 (8)
Dizziness / Light-headedness	2 (5)	0 (0)
Dry Mouth	1 (3)	0 (0)
Fallen Out Of Bed	1 (3)	0 (0)
Fatigue	0 (0)	1 (3)
Phlebitis	1 (3)	0 (0)
Gout	1 (3)	1 (3)
Haematuria	2 (5)	1 (3)
High Hco3	0 (0)	2 (5)
High Urea	0 (0)	1 (3)
Hyperglycaemia	1 (3)	0 (0)
Hyperkalaemia	0 (0)	1(3)
Hyperosmolar Hyperglycaemic Syndrome	3 (8)	0 (0)
Hypokalaemia	4 (10)	5 (13)
Hyponatremia	1 (3)	0 (0)
Hypotension	2 (5)	3 (8)
Hypovolemic Shock	0 (0)	1 (3)
Incident Tremor	0 (0)	1 (3)
Increased (Right) Cardiac Pressures	0 (0)	1 (3)
Increased CRP	0 (0)	1 (3)
Inflammation Spleen	1 (3)	0 (0)
Irregular Heartbeat	0 (0)	1 (3)
Ischemic Heart Failure	1 (3)	0 (0)

Table S5. *(Continued)*

Adverse Event (n (%))	Empagliflozin	Placebo
Itching	1 (3)	0 (0)
Ketoacidosis	0 (0)	1 (3)
Low Blood Pressure	0 (0)	2 (5)
Lower Back Pain	1 (3)	2 (5)
Mandibular Sensory Loss	0 (0)	1 (3)
Nausea	3 (8)	4 (10)
Nausea/Vomiting	2 (5)	2 (5)
Noro Virus	1 (3)	0 (0)
NT-proBNP Increase	1 (3)	0 (0)
Obstipation	1 (3)	1 (3)
Occasional Hypersensitivity/Swelling Cheek	1 (3)	0 (0)
Pain Due To Catheter	1 (3)	0 (0)
Pain Right Wrist	0 (0)	1 (3)
Painful Hip	1 (3)	0 (0)
THI Procedure	0 (0)	1 (3)
Respiratory Failure	0 (0)	1 (3)
Respiratory Tract Infection	1 (3)	1 (3)
S. Aureus Bacteraemia	1 (3)	0 (0)
Serious Collapse	1 (3)	1 (3)
Sideropenia	0 (0)	1 (3)
Significant Lactate Change	0 (0)	1 (3)
Symptomatic Hypotension	1 (3)	0 (0)
Systemic Amyloidosis	0 (0)	1 (3)
Thrombus After Transurethral Resection Bladder Tumour	1 (3)	0 (0)
Traumatic Supraspinatus Rupture	0 (0)	1 (3)
Two-Vessel Disease	0 (0)	1 (3)
Urea Increase	5 (13)	7 (18)
Urinary Tract Infection	1 (3)	1 (3)
Ventricular Tachycardia	0 (0)	1 (3)
Worsening Heart Failure	3 (8)	10 (26)
Worsening Renal Function	4 (10)	5 (13)

Patients with multiple similar events are counted once, while different events in a single patient are represented in each category.

Chapter 4A

Effects of Empagliflozin on Renal Sodium and Glucose Handling in Patients with Acute Heart Failure

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**Both authors contributed equally to this manuscript*

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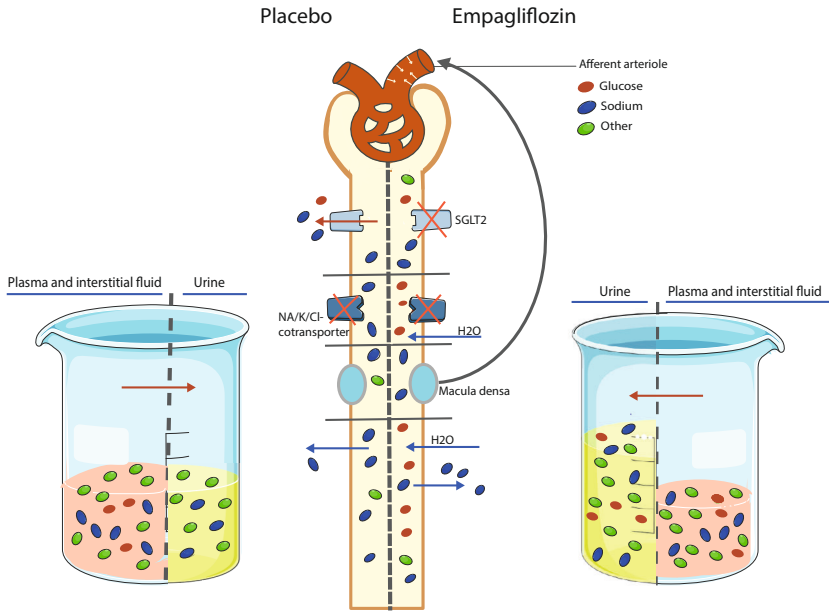
Abstract

Aims | Sodium-glucose cotransporter-2 (SGLT2) inhibitors improve clinical outcome in patients with heart failure (HF), but the mechanisms behind their beneficial effects are not yet fully understood. We examined the effects of empagliflozin on renal sodium and glucose handling in patients with acute HF.

Methods and results | This study was a predefined sub-study of a double-blind, randomized, placebo-controlled, multicenter study (EMPA-RESPONSE-AHF). Patients were allocated within 24 hours of an acute HF admission to either empagliflozin 10 mg/day (n=40) or placebo (n=39) for 30 days. Markers of glucose and sodium handling were measured daily during the first 96 hours and at day 30.

Patients were 76 (range 38-89) years old and 33% had diabetes. The use of loop diuretics during the first 96 hours was similar in both groups. Empagliflozin increased fractional glucose excretion with a peak after 24 hours (21.8 vs 0.1%; P<0.001), without affecting plasma glucose concentration, while fractional sodium and chloride excretion and urinary osmolality remained unchanged (P for all >0.3). However, empagliflozin increased plasma osmolality (delta osmolality at 72 hours: 5±8 versus 2±5 mOsm/kg; P=0.049). Finally, there was an early decline in eGFR with empagliflozin versus placebo (-10±12 vs. -2±12 mL/min/1.73m^2 P=0.009), which recovered within 30 days

Conclusion | In patients with acute HF, empagliflozin increased fractional glucose excretion and plasma osmolality, without affecting fractional sodium excretion or urine osmolality and caused a temporary decline in eGFR. This suggests that empagliflozin stimulates osmotic diuresis through increased glycosuria rather than natriuresis in patients with acute HF.



Graphical abstract | graphical representation of changes in urinary and plasma volume and osmolality. As more glucose is excreted as a result of SGLT2 inhibition, more water is drawn to the urine keeping osmolality constant. As a result of increased electrolyte free water excretion plasma osmolality is moderately increased and total volume of plasma and interstitial fluid is decreased.

Introduction

In patients with diabetes and/or chronic kidney disease, sodium glucose cotransporters-2 (SGLT2) inhibitors consistently showed beneficial effects on cardiovascular outcomes and particularly on HF hospitalizations.[1–4] Recently one larger randomized clinical trial demonstrated that dapagliflozin reduced cardiovascular death and HF hospitalizations in patients with established chronic HF with reduced ejection fraction (HFrEF) with and without diabetes.[5] A post-hoc analysis showed that treatment with dapagliflozin was safe and effective regardless of diuretic use or dose.[6] The beneficial effects of SGLT2-inhibitors on HF outcomes have been attributed to cardiometabolic and renal protective qualities, as well as to its diuretic properties. These were even described in chronic 'stable' euvolemic HF.[6–8] In acute HF, we recently showed that early addition of empagliflozin to standard diuretic treatment increased cumulative diuresis after 4 days with a possible reduction in HF related events.[9] However the mechanisms behind increased diuresis of SGLT2 inhibitors in acute HF are unknown.[10,11] In the present mechanistic study, we investigated the effect of SGLT2 inhibition on renal function,

and urinary sodium, chloride and glucose excretion in acute HF patients randomized to either empagliflozin or placebo.

Methods

Patients

The present study is a predefined analysis of the EMPA-RESPONSE-AHF trial of which the rationale and main results have been published recently.[9] In short, EMPA-RESPONSE-AHF was a double-blind, placebo-controlled multicenter pilot study enrolling 79 patients in 5 centers in the Netherlands, on the safety and efficacy of empagliflozin in patients with acute HF. Within 24 hours of hospital admission, patients were randomized 1:1 to either empagliflozin 10 mg (for 30 days) (n=40) or matching placebo (n=39). The trial was approved by the ethics committee at each study center and the study was conducted in accordance with the Declaration of Helsinki and the International Conference on Harmonization Guidelines for Good Clinical Practice. All patients participating in the trial provided written informed consent.

Biomarkers

Spot urine and plasma samples were collected at baseline, daily during the first 96 hours of hospitalization and after 30 days. Serum sodium, glucose, and creatinine and spot urinary creatinine and sodium were measured as part of safety monitoring and were analyzed according to procedures of the local laboratories of each participating hospital. Urinary glucose, chloride and osmolality were measured at a central laboratory in the University Medical Center Groningen (UMCG) in frozen samples. All samples were stored at -80°C within 2 hours of collection and thawed before analysis. Urinary glucose was measured only after database lock to ensure maintenance of the double-blind nature of the trial. Urinary chloride and potassium were measured using ISE indirect reagents for COBAS C, the measuring range for potassium is 3-100 mmol/L with an analytical variation of <5%, the measuring range for chloride is 20-250 mmol/L, with an analytical variation of <5%. Urinary glucose was measured using the GLUC3 pack for COBAS C, the measuring range for urinary glucose is 0.11-249.6 mmol/L (normal range: 0.06-0.83 mmol/L), with a variation of ~1%. Urinary osmolality, the total number of solute particles (or osmoles) per kilogram of fluid, was both measured and calculated in order to gain insight in the constituents of urine osmolality. Urine osmolality was measured using an automatic freezing point depression osmometer (Osmo Station OM-6050, ARKRAY), with a measuring range of 0-2000 mOsm/kg and an analytical variation of <1%. Between measurements tubes were capped to prevent evaporation.

Aldosterone concentrations were measured using the Aldosterone RIA kit by MT diagnostics. Renin concentrations were measured using the Renin Kit III by Cis Bio International. Aldosterone and renin measurements were performed in the Pharmacology laboratory of the Erasmus Medical Centre Rotterdam.

Calculation of urinary osmolality was done according to the following formula: osmolality = 2 * [Na+] + 2 * [K+] + [Glucose] + [Urea], with all concentrations being concentrations of molecules in urine. The same formula was used to calculate plasma osmolality. All fractional excretion percentages were calculated by a standard formula: 100% * $FE_x = (U_x * P_{creat}) / (P_x * U_{creat})$, in which U_x is the urinary concentration of the analyte and P_x represents the plasma concentration of the analyte. Ucreat and Pcreat represent urinary and plasma concentrations of creatinine, respectively. In sensitivity analysis, estimated 24-hour urinary sodium excretion was calculated using the earlier defined formula by the International Cooperative Study on Salt, Other Factors, and Blood Pressure (INTERSALT) investigators.[12]

Statistical analysis

Baseline characteristics were explored using a t-test for normally distributed variables and a Mann Whitney U-test for non-normally distributed variables. For further analysis variables were normalized by logarithmic transformation where necessary. The effect of empagliflozin use on changes in clinical outcomes (i.e., eGFR, systolic blood pressure, plasma and urinary osmolality, FeNa, FeCl and FeGlu, renin, aldosterone) during 96h and 30 days were analyzed with repeated measures linear mixed-effect (LME) models, which account for individual variations in changes and intercepts by estimating the random effects for per individual. To correct for their highly skewed nature renin and aldosterone were log-transformed before being analyzed in the linear mixed models. For each clinical variable change from baseline was calculated and used as an outcome in the LME model. We performed a nested model adjusted for baseline values and time. Whereas, for each outcome a second model was performed including baseline values, time, treatment arm and the treatment x time interaction term. Further, we compared the two models: without and with the treatment interaction term using analysis of variance (ANOVA), where a P-value <0.05 was considered significant for a treatment effect during the full treatment period (either 96 h or 30 days depending on the variable). The effect of empagliflozin on changes in outcome at specific time point were considered significant for interaction terms P <0.05. LME models were conducted using the lme function in the "nlme" package. All analyses were performed in R studio, version 1.3.959.[13]

Results

Baseline characteristics of the study population have been published elsewhere.[9] In brief, patients were 76 (range 38-89) years old, 33% were female and median NT-proBNP was 5236 (IQR 3482 – 8276) pg/mL. Background medical treatment at baseline was similar between the groups and there were no differences in loop diuretic doses, vasodilator or inotrope use or guideline recommended HF medication. Baseline estimated glomerular filtration rate (eGFR) was 55 ± 18 mL/min/1.73m^2, median plasma glucose was 7.8 (IQR 6.2 – 8.9) mmol/L and 33% had a history of type 2 diabetes mellitus. In urine, glucose concentrations at baseline were 0.1 (IQR 0.1 – 0.2) mmol/L, with low fractional glucose excretion (0.1% (IQR 0.1 – 0.1)). In urine, baseline osmolality was 335 (IQR 322 – 380) mOsm/kg, whereas median plasma osmolality was 305 (IQR 302 – 311) mOsm/kg. Before start of randomized treatment but within 24 hours of admission and after initiation of loop diuretic therapy, spot urinary sodium was 99 (IQR 67 – 111) mmol/L, with a fractional sodium excretion of 2.2 (IQR 0.9 – 4.3) %. No between group differences were observed between patients treated with empagliflozin or placebo for any of these baseline variable (Table 1).

Table 2 shows plasma and spot urinary electrolytes over time, stratified by treatment arm. Empagliflozin significantly decreased spot urinary sodium concentration as compared with placebo. The most pronounced effect was seen after 48 hours (56.2 vs 79.0 mmol/L, P =0.011). In contrast, treatment with empagliflozin did not change fractional excretion of sodium (FeNa) at any time point as compared with placebo (Table 2, Figure 1a, P=0.956 for ANOVA difference between models with and without treatment interaction), indicating that while net urinary sodium concentration decreases, a similar amount of glomerularly filtered sodium is reabsorbed in the renal tubuli compared with placebo. In sensitivity analysis, median calculated 24-hour sodium excretion after 24 hours also did not show differences between patients treated with empagliflozin compared with placebo (P = 0.235). Moreover, no differences in the occurrence of hyponatremia was seen between both treatment arms (p>0.2), delta serum sodium from baseline to 96 hours also did not change between the treatment arms (-0.17 vs -0.18 mmol/L, P=0.99), nor did empagliflozin change serum sodium at any time point (P=0.302 for ANOVA difference between models with and without treatment interaction). Likewise, urinary chloride excretion was lower in patients treated with empagliflozin, but fractional excretion of chloride was unaltered by empagliflozin use (Figure 1b, P=0.922 for ANOVA difference between models with and without treatment interaction).

Table 1 | Baseline characteristics. Categorical variables are depicted as N (%), normally distributed variables are depicted as mean ± SD, non-parametric variables are depicted as median (IQR), abbreviations: eGFR: estimated Glomerular Filtration Rate.

	Empagliflozin (n=40)	Placebo (n=39)	p-value
Age (years)	79 (73 - 83)	73 (61 - 83)	0.141
Female sex (%)	16 (40)	10 (26)	0.263
Systolic blood pressure (mmHg)	128 ± 22	121 ± 25	0.253
eGFR (mL/min/1.73m^2)	53 ± 18	54 ± 16	0.824
Plasma osmolality (mOsm/kg)	305 (302 - 309)	305 (302 - 312)	0.691
Urine osmolality (mOsm/kg)	330 (322 - 379)	341 (324 - 384)	0.530
Serum levels of:			
Creatinine (mg/dL)	1.3 ± 0.4	1.3 ± 0.4	0.723
Urea (mmol/l)	11.0 (7.5 - 12.8)	9.0 (7.3 - 13.1)	0.916
Sodium (mmol/L)	140 (137 - 142)	140 (138 - 142)	0.806
Potassium (mmol/L)	3.9 (3.5 - 4.2)	3.9 (3.5 - 4.4)	0.406
Glucose (mmol/L)	7.9 (6.2 - 9.6)	7.7 (6.3 - 8.8)	0.323
Renin (pg/mL)	12.4 (4.8 – 63.4)	80.1 (14.0 – 179.3)	0.011
Aldosterone (pg/mL)	182.3 (130.5 – 292.0)	192.7 (114.6 – 344.3)	0.653
Urinary levels of:			
Creatinine (mmol/L)	3.5 (1.9 - 5.5)	3.4 (2.0 - 5.1)	0.944
Urea (mmol/L)	111 (60 - 142)	98 (78 - 140)	0.976
Sodium (mmol/L)	100 (68 - 110)	92 (69 - 112)	0.734
Potassium (mmol/L)	27 (21 - 33)	29 (20 - 41)	0.399
Glucose (mmol/L)	0.2 (0.1 - 0.3)	0.1 (0.1 - 0.2)	0.264
Fractional excretion of (in %):			
Sodium	2.1 (0.9 - 4.1)	2.2 (0.9 - 4.4)	0.964
Glucose	0.1 (0.1 - 0.1)	0.1 (0.0 - 0.1)	0.890

Table 2 | Urinary parameters over course of treatment

	Empagliflozin (n=40)	Placebo (n=39)	p-value
Spot urinary sodium, in mmol/L			
Baseline	90 ± 31	87 ± 35	0.706
24 hours	69 ± 28	85 ± 37	0.040
48 hours	56 ± 29	79 ± 44	0.011
72 hours	63 ± 41	70 ± 27	0.400
96 hours	56 ± 29	69 ± 32	0.089
30 days	60 ± 30	56 ± 28	0.683
Fractional excretion of sodium, in %			
Baseline	2.1 (0.9 - 4.1)	2.2 (0.9 - 4.4)	0.964
24 hours	1.4 (0.9 - 2.4)	1.5 (0.6 - 3.2)	0.874
48 hours	1.0 (0.3 - 1.7)	1.4 (0.7 - 2.1)	0.256

Table 2 *(Continued)*

	Empagliflozin (n=40)	Placebo (n=39)	p-value
72 hours	1.4 (0.8 - 1.7)	1.0 (0.6 - 2.3)	0.840
96 hours	0.8 (0.4 - 1.9)	1.0 (0.7 - 1.7)	0.476
30 days	0.7 (0.3 - 1.7)	0.7 (0.4 - 2.0)	0.388
Spot urinary glucose, in mmol/L			
Baseline	0.2 (0.1 - 0.3)	0.1 (0.1 - 0.2)	0.264
24 hours	50.4 (17.1 - 94.8)	0.2 (0.1 - 0.3)	<0.001
48 hours	41.3 (17.2 - 79.1)	0.2 (0.1 - 0.3)	<0.001
72 hours	35.2 (16.2 - 96.2)	0.2 (0.1 - 0.3)	<0.001
96 hours	30.3 (9.9 - 75.2)	0.2 (0.1 - 0.4)	<0.001
30 days	13.1 (1.6 - 58.9)	0.2 (0.1 - 0.3)	<0.001
Fractional excretion of glucose, in %			
Baseline	0.1 (0.1 - 0.1)	0.1 (0.0 - 0.1)	0.890
24 hours	21.8 (10.1 - 29.8)	0.1 (0.1 - 0.1)	<0.001
48 hours	13.6 (5.4 - 24.0)	0.1 (0.0 - 0.1)	<0.001
72 hours	16.0 (4.2 - 24.4)	0.1 (0.0 - 0.1)	<0.001
96 hours	6.0 (2.5 - 21.8)	0.1 (0.0 - 0.1)	<0.001
Spot urinary urea, in mmol/L			
Baseline	108.0 (60.8 – 142.1)	97.5 (77.6 – 139.5)	0.881
24 hours	136.0 (93.3 – 172.7)	124.0 (91.3 – 191.6)	0.935
48 hours	160.2 (116.0 – 194.0)	159.3 (111.3 – 105.7)	0.840
72 hours	154.5 (122.9 – 185.0)	157.5 (128.5 – 207.5)	0.426
96 hours	179.6 (121.0 – 256.7)	172.2 (144.9 – 233.0)	0.427
30 days	214.0 (141.0 – 272.6)	166.6 (85.2 – 294.5)	0.333
Fractional excretion of urea, in %			
Baseline	37.9 (30.3 – 45.0)	37.6 (28.0 – 48.5)	0.984
24 hours	33.1 (28.6 – 41.9)	35.3 (21.4 – 43.0)	0.664
48 hours	29.6 (22.2 – 39.3)	30.5 (25.7 – 39.7)	0.572
72 hours	31.1 (26.3 – 37.1)	32.0 (24.9 – 39.2)	0.952
96 hours	32.4 (24.2 – 38.0)	33.5 (29.5 – 39.0)	0.346

Empagliflozin significantly increased both urinary glucose concentration and fractional excretion of glucose (FeGlu), with a peak in FeGlu after 24 hours (median 21.4% vs 0.1%, P <0.001, Table 2). Even though FeGlu decreased over the course of treatment, it was still significantly higher in patients treated with empagliflozin compared with placebo after 96 hours (median 6.0% vs 0.1%, P <0.001). A similar pattern was seen for urinary glucose concentration up to 30 days of treatment (Table 2). Plasma glucose levels however were not changed by empagliflozin use (P=0.763 for ANOVA difference between models with and without treatment interaction). FeGlu was similar in patients with and without diabetes in the empagliflozin arm (P=0.182 for interaction, Supplementary Figure 2). Plasma urea was additionally increased in patients treated with

empagliflozin, while fractional excretion of urea and urinary urea remained unaffected (P=0.036, 0.99 and 0.782 for ANOVA difference between models with and without treatment respectively).

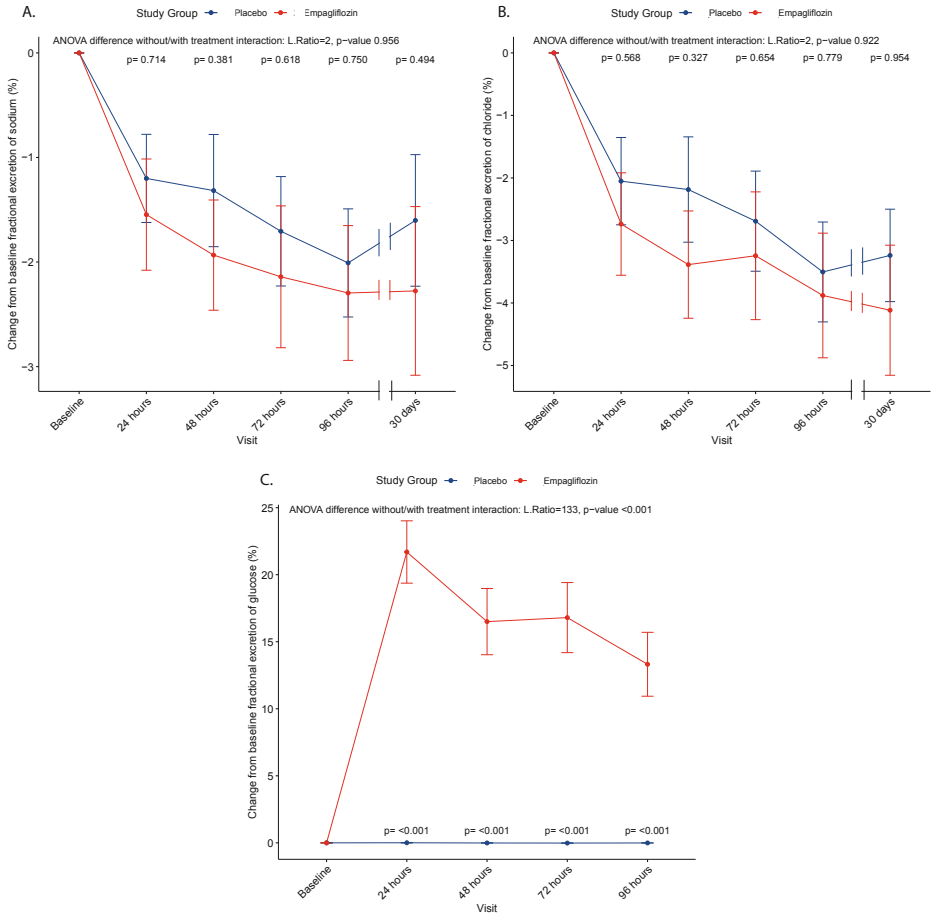


Figure 1 | FeNa, FeGlu, and FeCl progression over the course of treatment

For each clinical variable changes from baseline were calculated and used as outcomes in LME models. Two models were performed, one adjusted for baseline values, the second model adjusted for baseline values and the interaction term between treatment and time. In each panel the results for the ANOVA tests between the two models is depicted, (likelihood ratio and p-value). For placebo (blue) and empagliflozin (red) mean values are shown with dots, the bars represent standard error. A p-value for interaction between each time point and treatment is shown.

Table 3 | eGFR over course of treatment, eGFR= estimated Glomerular filtration rate

	Empagliflozin (n=40)	Placebo (n=39)	p-value
eGFR, in ml/min/1.73m^2			
Baseline	53 ± 18	54 ± 16	0.824
24 hours	44 ± 14	52 ± 19	0.022
48 hours	43 ± 16	53 ± 19	0.013
72 hours	42 ± 16	54 ± 18	0.006
96 hours	45 ± 18	53 ± 20	0.101
30 days	50 ± 21	54 ± 19	0.511
Change in eGFR from baseline, in ml/min/1.73m^2			
24 hours	-9 ± 9	-2 ± 8	0.002
48 hours	-10 ± 12	-2 ± 10	0.004
72 hours	-10 ± 12	-2 ± 12	0.009
96 hours	-7 ± 11	-3 ± 15	0.133
Day 30	-6 ± 15	-4 ± 16	0.681

During the first 72 hours, empagliflozin caused a significant decrease in eGFR (-10 ± 12 vs -2 ± 12 mL/min/1.73m2) compared with placebo (P = 0.009), as shown in Table 3. This significant decline in eGFR was attenuated at 96 hours and 30 days (P = 0.133 and 0.681 respectively). In addition, empagliflozin significantly increased urinary output (cumulative urinary output after 48 hours: 6084 ± 2480 mL vs. 4222 ± 1911 mL p = 0.010, N = 41), which resulted in a greater negative fluid balance (cumulative fluid balance after 48 hours: -3050 (IQR -1280 – -4753) mL vs. -1200mL (IQR -710 – -2425), P = 0.010, *N = 38*). Even though urinary volumes significantly increased after initiation of empagliflozin, no impact on urinary osmolality was seen (Figure 2c). In other words, the number of *particles per kilogram of urine* did not change despite a larger urinary volume. However, a significant shift was seen in the constituents making up urine osmolality, with glucose making up a larger proportion of the total urinary particles compared with placebo (Figure 3). Measured and calculated urine osmolality showed a strong correlation (r of log-transformed variables 0.91 – 0.99) (Supplementary Table 1).

At baseline, plasma osmolality was similar in both groups. During the first 72 hours, empagliflozin modestly increased plasma osmolality (delta plasma osmolality 5 ± 8 mOsm/kg vs 2 ± 5 mOsm/kg; P = 0.049). Moreover, we found a significant interaction for plasma osmolality between time at 72 hours and treatment effect (P = 0.031, Figure 2d).

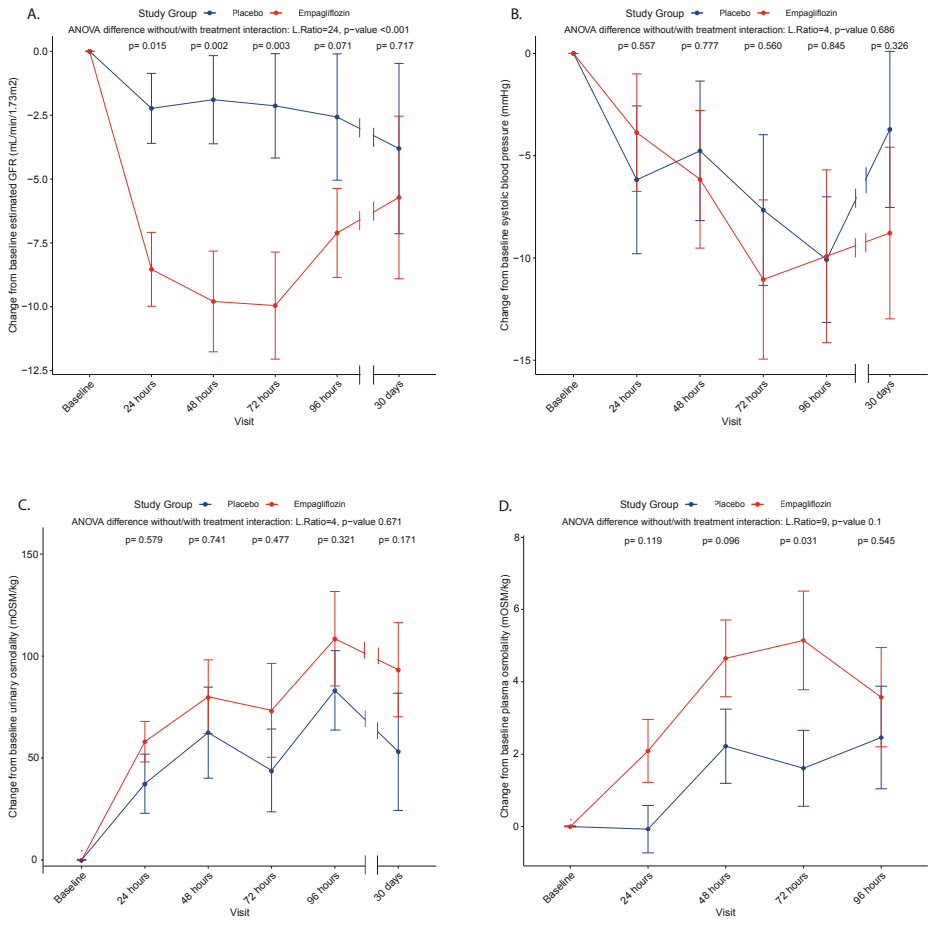


Figure 2 | Delta eGFR (A), delta systolic blood pressure progression (B), delta urine osmolality (C) and delta plasma osmolality (D) over the course of treatment

For each clinical variable changes from baseline were calculated and used as outcomes in LME models. Two models were performed, one adjusted for baseline values, the second model adjusted for baseline values and the interaction term between treatment and time. In each panel the results for the ANOVA tests between the two models is depicted, (likelihood ratio and p-value). For placebo (blue) and empagliflozin (red) mean values are shown with dots, the bars represent standard error. A p-value for interaction between each time point and treatment is shown.

Median baseline renin was 28.8 pg/ml (range 1.0 – 1820 pg/ml), median baseline aldosterone was 185.9 pg/ml (31.0 – 1810 pg/ml). Median plasma renin concentration at baseline was higher in the placebo group than in the empagliflozin group (12.4 pg/ml vs. 80.1 pg/ml, P = 0.011). Empagliflozin significantly increased renin with a peak after 72 hours, after which a plateau was reached and significance was lost (P= 0.016 for

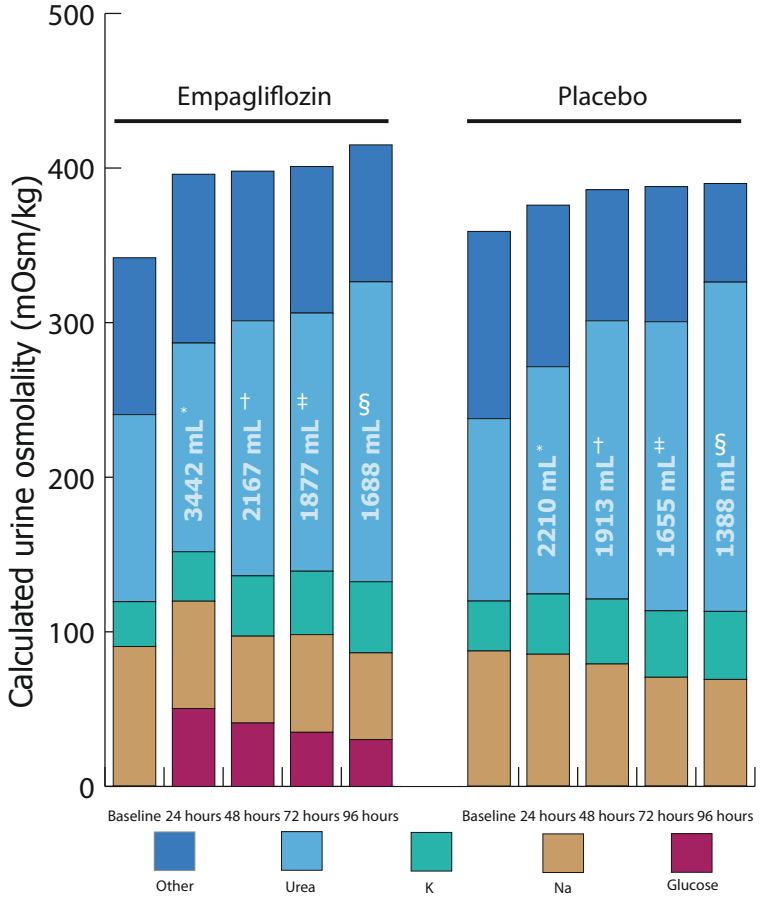


Figure 3 | Composites of urinary molecules making up osmolality

Osmolality per time point for both empagliflozin and placebo. Volumes depicted on the bars represent total urinary volume per 24 hours, y axis represents spot urinary osmolality. * N = 58; † N = 47; ‡ N = 44; § N = 35

ANOVA between models with and without treatment effect) (Figure 4a). Aldosterone was not altered by empagliflozin use (P=0.217 for ANOVA between models with and without treatment effect) (Figure 4b).

No significant correlations, as analyzed with linear regression, could be seen for log FeGlu and log FeNA, for log FeGlu and fluid balance or for log FeNa and fluid balance (Supplementary Figure 1).

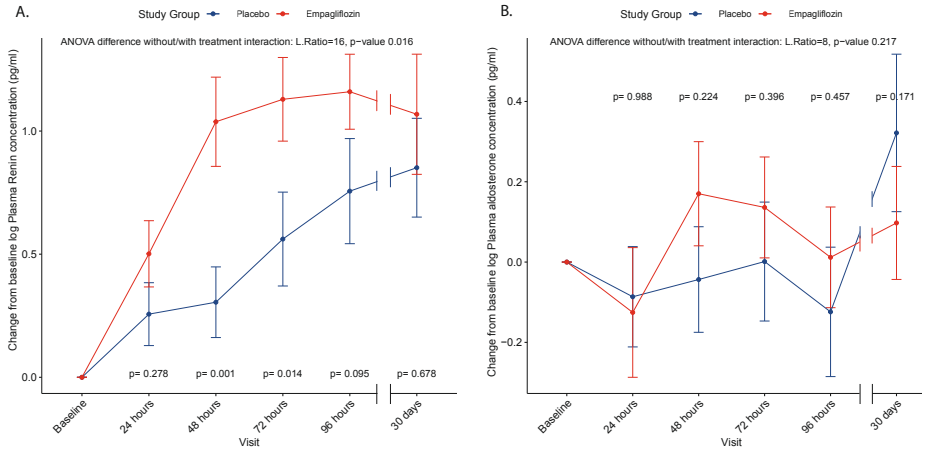


Figure 4: Delta renin and aldosterone over the course of treatment

For renin and aldosterone (both log-transformed) changes from baseline were calculated and used as outcomes in LME models. Two models were performed, one adjusted for baseline values, the second model adjusted for baseline values and the interaction term between treatment and time. In each panel the results for the ANOVA tests between the two models is depicted, (likelihood ratio and p-value). For placebo (blue) and empagliflozin (red) mean values are shown with dots, the bars represent standard error. A p-value for interaction between each time point and treatment is shown.

Discussion

In this predefined post-hoc analysis of the EMPA-RESPONSE-AHF trial, we found that patients with acute HF receiving empagliflozin had a higher urinary output and a more negative fluid balance. Interestingly, fractional sodium excretion did not increase and urinary osmolality remained similar between both groups. Nonetheless, fractional glucose excretion significantly increased after initiation of empagliflozin. Moreover, empagliflozin temporarily reduced renal function in the first days after an acute heart failure hospital admission.

The increase in fractional glucose excretion with SGLT2-inhibition by empagliflozin was expected, as blocking the receptor responsible for tubular reabsorption of glucose would intuitively lead to increased excretion of filtered glucose. Importantly, fractional glucose excretion was similar in patients with and without diabetes and plasma glucose levels remained unchanged, suggesting that pre-existing diabetes and/or plasma glucose supply to the glomerulus do not play an important role. Our finding that fractional sodium excretion was not increased with empagliflozin was somewhat unexpected, since blockage of the SGLT2 receptor in the proximal tubule prevents reabsorption of both glucose and sodium. However, it is well known that in contrast to glucose, sodium

can be reabsorbed throughout the entire tubule. Therefore, our findings suggest that sodium reabsorption might have been blocked by empagliflozin in the proximal tubule, and that this was compensated by an increased reabsorption of sodium in the rest of the tubule and collecting duct.[14]

Similar to a post-hoc analysis of the EMPA-REG OUTCOME Trial, a transient decline in eGFR was seen after initiating treatment with empagliflozin.[15] This course in eGFR over time was seen in the CANVAS program as well.[16] This has been postulated to be related to the juxtaglomerular feedback mechanism and a correction in glomerular hyperfiltration.[17,18] Loss of chloride is sensed by the macula densa in the distal convoluted tubule and leads to release of adenosine, causing afferent vasoconstriction and a decreased renal blood flow, in order to spare salt. If we assume that the established drop in GFR is indeed the result of activation of the macula densa, there should still be high levels of sodium and chloride present in the distal convoluted tubule in order for juxtaglomerular feedback mechanism to be activated.[19] This would mean that although we do not find increased concentrations of sodium in the urine, proximal tubular sodium resorption is indeed diminished. This notion is further supported by the fact that renin levels were increased compared with placebo for the first 72 hours, following a similar pattern compared to drop in eGFR. The increased osmotic diuresis resulting from glycosuria probably leads to an increase in renin levels. Of course, these results need to be interpreted in light of background therapy and one should be wary to draw conclusions based on these data alone. Consequently, sodium is likely reabsorbed distally from the macula densa, e.g. in the collecting duct. In the collecting ducts multiple mediators regulate urine dilution and sodium reabsorption, one of which is vasopressin.[20] Yet, since urinary osmolality remained unchanged and vasopressin only plays a minor role in sodium reabsorption, it is unlikely that an increase in vasopressin is truly responsible for this increase in sodium reabsorption in the collecting duct. We did not find an increase in aldosterone after initiation of empagliflozin, making aldosterone an unlikely cause for distal tubular or collecting duct sodium reabsorption. Other drivers of sodium reabsorption include insulin and insulin like growth factor-1, while endothelin-1 and nitric oxide decrease sodium reabsorption.[21–23] The precise contribution of each of these factors on sodium reabsorption in the collecting duct after blocking the SGLT2 receptor and treatment with loop diuretics have to be assessed in future studies.

Interestingly, our data show a modest increase in plasma osmolality. These effects were even more pronounced in patients with lower serum sodium and lower plasma osmolality. In our data no effect on serum sodium could be found, which might be explained by the fact that only a small proportion of patients presented with hyponatremia and the lowest measured sodium upon admission was 129 mmol/L. Moreover,

this might position SGLT2 inhibitors as a treatment for tissue congestion or residual congestion as increased plasma osmolality attracts fluid from the interstitial space into the blood stream.[24] This notion is supported by earlier findings that the SGLT2 inhibitor dapagliflozin has been calculated to reduce interstitial fluid volume 3 times more than it reduces blood volume, compared to an interstitial fluid reduction of only 66% of the reduction in blood volume by bumetanide.[25] The fact that empagliflozin did not lower blood pressure in these severely diseased patients despite larger negative fluid balance also supports the theory of a more stable refill rate from interstitial fluid to plasma as a result of increased plasma osmolality. However, we have to account for the small study group here and the fact that other larger trials did find a reduction of 5-10 mmHg in systolic blood pressure after initiation of a SGLT2 inhibitors.[26,27] Still, this increase in plasma osmolality might also be expected since distal diluting segments receive higher tubular flow as a result of empagliflozin. The kidneys perceive this as a state of hypervolemia which, through stimulation of the countercurrent system of the vasa recta, leads to more distal sodium reabsorption and less free water clearance.[28]

As described earlier, the majority of HF patients are discharged with residual congestion with consequent impaired prognosis.[29] Our analyses might provide insights into new possibilities to overcome residual congestion due to significantly improved net fluid loss (negative fluid balance) and increased plasma osmolality. Naturally, our data need to be validated in another (larger) clinical cohorts.

Taken together, our findings that empagliflozin increased fractional excretion of glucose, while both fractional excretion of sodium and urinary osmolality remained unchanged, suggest that empagliflozin most prominently stimulates osmotic diuresis as a result of increased fractional excretion of glucose instead of natriuresis. Our results are in line with earlier studies that reported that SGLT2-inhibitors increased urinary volume without an increase in fractional sodium excretion.[8,30] In another study comprising of patients with type 2 diabetes and stable chronic HF (median NT-proBNP: 399 pg/mL) an early increase in urinary sodium excretion was seen. However, in this study fluid administration was high in these patients, sodium intake was not standardized per protocol, and intermittent urinary measurements were only obtained throughout the first six hours. Therefore, the circadian rhythm in sodium excretion could still have affected these findings. Since we included acute HF patients with more signs and symptoms of fluid congestion (median NT-proBNP: 5236 pg/mL), differences might further be explained by our sicker cohort with likely even higher neurohumoral activation potentially leading to increased sodium reabsorption and eventually maintaining fractional excretion of sodium constant. This is in contrast to a study in patients with euvolemic, stable HF, where empagliflozin increased both fractional excretion

of glucose and sodium, and even exhibited a small synergistic effect of concomitant treatment with intravenous bumetanide.[7]

Limitations

Several potential limitations of the present study can be identified. The first limitation of this study is the relatively small sample size. Second, we collected spot urine samples and not 24-hour measurements. Consequently, we do not know the total sodium output over the course of hospitalization. Conceivably, increased volumes of urine, even with a lower concentration of sodium, will eventually lead to an increased absolute total of urinary sodium. As per protocol, treating physicians were blinded to the treatment arm and urinary excretion of glucose. However, urinary levels of sodium excretion were measured in the local labs and could therefore be perceived in the electronic medical records. Third, plasma levels of glucose were not measured at the 30-day follow-up visit, as this was not incorporated in the study protocol. Therefore, no fractional excretion of glucose could be calculated at day 30. Fourth, the timing of loop diuretic administration was not standardized. This was left to the discretion of the treating physician. Therefore, some patients were on continuous loop diuretic infusion, while others were on intravenous bolus loop diuretic treatment. So, the natriuretic effect of a recent loop diuretic bolus, rather than that of empagliflozin cannot be ruled out. Still, considering the randomized nature of this trial, these treatment differences should be equal in both arms. Moreover, patients in both treatment arms were on equal total doses of intravenous and oral loop diuretics, and the number of patients on either oral or intravenous loop (bolus vs. continuous) diuretics were similar as well (Supplementary Table 2). Additionally, calculating fractional excretion levels relies on measured plasma creatinine levels. These values changed throughout follow-up which might have affected calculated values of fractional excretion.

Conclusion

In patients hospitalized with acute HF, empagliflozin caused an increase in urinary output, fractional glucose excretion and plasma osmolality, without affecting fractional sodium excretion or urine osmolality. Additionally, a significant, temporary decline in eGFR was seen. These results suggest that empagliflozin primarily stimulates osmotic diuresis in patients with acute HF through increased glycosuria rather than through natriuresis.

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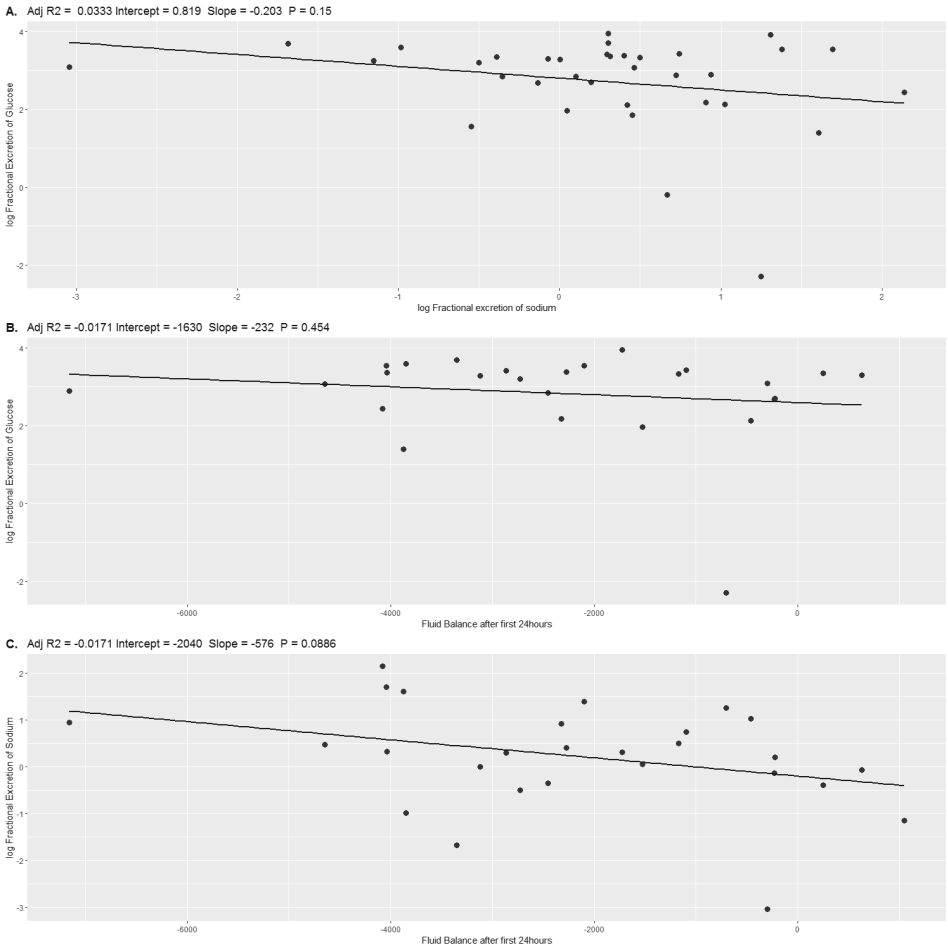
Supplementary material

Supplementary Table 1 | Correlation between measured and calculated urine osmolality per time point

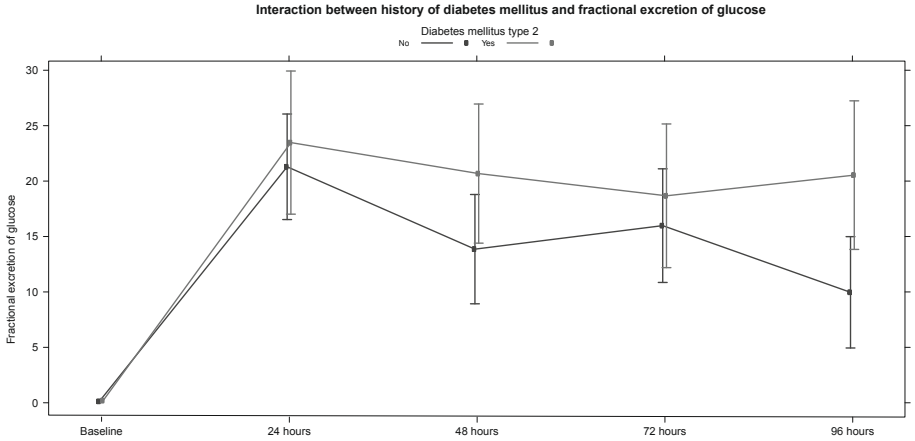
EMPAGLIFLOZIN	2 [Na+] + 2[K+] + [Glucose] + [Urea]			
	Calculated urinary osmol	**Measured urinary osmol**	**Spearman rho**	**Pearson r of log(variables)**
Baseline	342 (320 – 378)	330 (322 – 379)	0.88	0.94
24 hours	396 (354 – 426)	399 (352 – 443)	0.92	0.93
48 hours	398 (367 – 448)	407 (371 – 489)	0.88	0.95
72 hours	401 (371 - 476)	395 (374 – 494)	0.94	0.95
96 hours	415 (358 – 535)	430 (366 - 569)	0.97	0.99
PLACEBO				
	Calculated urinary osmol	**Measured urinary osmol**	**Spearman rho**	**Pearson r of log(variables)**
Baseline	359 (326 – 375)	341 (324 – 384)	0.90	0.93
24 hours	376 (348 – 434)	388 (345 – 410)	0.90	0.91
48 hours	386 (348 – 476)	397 (357 – 450)	0.92	0.96
72 hours	388 (361 – 452)	389 (357 – 435)	0.95	0.97
96 hours	390 (367 – 511)	395 (361 – 496)	0.91	0.97

Supplementary Table 2 | Median cumulative dose of loop diuretics per patient during the first 96 hours of hospitalization

Timepoint	Empagliflozin (n = 40)		Placebo (n = 39)		p-value
Oral dose of loop diuretics received (furosemide dose or equivalent)					
Prior to Baseline	0 [0, 0]	n = 6	0 [0, 10]	n = 10	0.404
Baseline - 24 hours	0 [0, 0]	n = 7	0 [0, 0]	n = 9	0.458
24 hours - 48 hours	0 [0, 80]	n = 18	0 [0, 80]	n = 17	0.974
48 hours - 72 hours	40 [0, 80]	n = 23	0 [0, 80]	n = 19	0.850
72 hours - 96 hours	0 [0, 5]	n = 10	0 [0, 40]	n = 11	0.691
Intravenous dose of loop diuretics received (furosemide dose or equivalent)					
Prior to Baseline	160 [80, 242]	n = 38	148 [80, 200]	n = 37	0.189
Baseline - 24 hours	156 [80, 200]	n = 34	125 [80, 200]	n = 31	0.328
24 hours - 48 hours	20 [0, 160]	n = 20	0 [0, 160]	n = 19	0.899
48 hours - 72 hours	0 [0, 80]	n = 26	0 [0, 113]	n = 24	0.625
72 hours - 96 hours	0 [0, 45]	n = 11	0.00 [0, 0]	n = 9	0.626
Total cumulative diuretic dose of loop diuretics received (oral and intravenous combined)					
Baseline	160 [86, 243]		152 [88, 220]		0.505
24 hours	322 [200, 429]		310 [184, 385]		0.438
48 hours	408 [240, 582]		372 [240, 506]		0.563
72 hours	442 [307, 666]		410 [300, 582]		0.776
96 hours	490 [319, 698]		435 [360, 679]		0.546
Number of patients on continuous vs. bolus loop diuretics					
Continuous	19		15		0.417
Bolus	7		14		0.064
Continuous + bolus	8		5		0.390



Supplementary Figure 1 | correlations between fractional excretion of glucose and fractional exretion of sodium (A), fractional excretion of glucose and fluid balance (B) and fractional excretion of sodium and fluid balance (C)



Supplementary Figure 2 | interaction between history of diabetes and fractional excretion of glucose.

Chapter 4B

Empagliflozin and Renal Sodium Handling: an Intriguing Smart Osmotic Diuretic

supplementary editorial by W. Mullens and P. Martens

The Heart Failure Association (HFA) of the European Society of Cardiology (ESC) recommend sodium–glucose co-transporter 2 inhibitors (SGLT2i) to be used in type 2 diabetes mellitus patients with either established cardiovascular (CV) disease or at high CV risk in order to prevent or delay the onset of and hospitalizations for heart failure (HF).[1] Based upon randomized prospective trials, dapagliflozin and empagliflozin are now also recommended in symptomatic patients with HF and reduced ejection fraction, on guideline-directed medical therapy, regardless of the presence of type 2 diabetes mellitus.[2-4] At present, the mechanisms underlying the beneficial CV and renal effects of SGLT2i are not completely understood and several, not mutually exclusive, mechanisms have been proposed.[1]

In this issue of the Journal, Boorsma *et al*.[5] describe the effects of empagliflozin on renal sodium and glucose handling in a pre-defined sub-analysis of the EMPA-RESPONSE-AHF trial. This was a randomized, double-blind, placebo-controlled, multicentre pilot study which demonstrated that empagliflozin was safe and well tolerated in acute HF on top of classic loop diuretics, while it did not improve dyspnoea, N-terminal pro B-type natriuretic peptide, diuretic response and length of hospital stay.[6] Importantly, the trial demonstrated that early addition of empagliflozin to standard diuretic therapy did increase cumulative diuresis during the decongestive phase of the treatment of acute HF, which was associated with less in hospital worsening of HF and less readmission after discharge.[6]

In the current mechanistic sub-study using spot urine and plasma samples during the first four consecutive days of decongestive therapy, Boorsma *et al*.[5] illustrate that empagliflozin leads to an increased diuresis through an increased glycosuric effect rather than increased natriuresis. While urinary volume was significantly increased, urine osmolality remained similar indicating a significant change in urine content, which was solely related to the presence of urinary glucose.

As such, the additional diuretic effect of empagliflozin given on top of classical diuretic therapy during acute HF was attributed due to increased osmotic diuresis by empagliflozin. Surely, the fact that the investigators only used spot urine samples and did not standardize diuretic therapy are serious limitations to the study. Indeed, several other studies did show an enhanced natriuretic effect of SGLT2i in addition to a glycosuric effect.[7, 8]

Nevertheless, the data are intriguing as they shed light on the potential effects of SGLT2i on diuresis in acute HF. If we consider these drugs to be 'smart osmotic diuretics', we have to realize that their effect on diuresis is strikingly different than our classi-

cal diuretic therapy, which increases natriuresis with concomitant increased diuresis.[9] Indeed, SGLT2i produce a glycosuric effect (60–90 g/day) which is dependent on blood glucose levels. In addition, we need to realize that most of the SGLT2i is bound to proteins, and that the pharmacokinetic effects of SGLT2i differ greatly between different molecules as well as patients and their HF status.[10] They reach their target site in the proximal nephron significantly more by active secretion into the renal proximal tubule than by passive filtration, especially when plasma concentrations are low. As such, these drugs should have less diuretic effect in case of low renal blood flow (RBF) as well as low estimated glomerular filtration rate (eGFR), which is extremely relevant in the case of acute HF. Interestingly, the expression of SGLT2 is also up-regulated in case of poor RBF and renin– angiotensin system activation, which additionally contribute to increased proximal sodium reabsorption in HF.[11] As the glucose–sodium transport ratio of SGLT2 is 1:1, this also implies that a significant amount of filtered sodium will not be reabsorbed by the proximal renal tubules when SGLT2i are administered, especially since there is also cross-reactivity with the sodium/hydrogen exchanger 3 (another proximal sodium transporter).[12] As a result, a significant drop in body weight (water) and arterial blood pressure is observed within the first weeks of

treatment with a SGLT2i. The fact that homozygous SLC5A2 mutation carriers – who have defective SGLT2 function – do have significantly elevated plasma renin activity (PRA) (preventing further volume depletion) supports the concept that SGLT2i contribute to renal sodium loss.[13] However, the current study suggests that treatment with SGLT2i once daily does not significantly increase 24 h urinary sodium excretion, indicating compensatory distal nephron sodium reabsorption.[9] Nevertheless, reduced proximal renal sodium reabsorption remains a good thing for HF patients even though overall renal sodium excretion is not increased.[14] Indeed, SGLT2i, through inhibition of proximal tubular sodium transport and osmotic diuresis with solvent drag, enhance distal tubular flow in the nephron counteracting salt sensitivity, facilitating decongestive treatment and boosting loop diuretic responsiveness.[14]

Importantly, several mechanistic studies highlight that SGLT2i reduce eGFR (also demonstrated by Boorsma *et al.*) while only minimally reducing RBF.[15, 16] This will lead to a reduced filtration fraction, which limits proximal tubular sodium reabsorption through the glomerotubular balance.[16] The precise mechanism of this acute drop in GFR might be divergent in different patient populations, depending on the background glomerular haemodynamics, but relate to tubuloglomerular feedback. Vasoconstriction of the afferent arteriole is proposed in hyperfiltrating type 1 diabetes patients (i.e. high total GFR).[15, 17] In contrast, in type 2 diabetes patients and HF patients who have significantly reduced total GFR (but also single nephron hyperfiltration), an efferent

glomerular arteriolar vasodilatation is proposed despite the fact that SGLT2i increase PRA.[18] One might interpret the more pronounced rise in PRA previously reported after initiation of dapagliflozin, and now corroborated by Boorsma *et al*. during decongestion with empagliflozin, as an indicator of neurohumoral stimulation. However, PRA is depressed during episodes of acute HF with volume overload, and rise due to effective

decongestion.[18] Nevertheless, one has to take into consideration the significant limitations in the interpretation of PRA as there is significant variation in the measurements between patients.[5] Moreover, PRA levels can only be interpreted as an indicator of neurohumoral stimulation if plasma was taken with the patient in the supine position after an adaptation period of 30 min as PRA is very sensitive to changes in body tonus. The information on the collection of the samples was lacking in this study.[5]

In addition, Boorsma *et al*.[5] also indicate an increased plasma osmolality during empagliflozin utilization. Importantly, data from direct blood volume measurement in acute HF indicate that total blood volume in congested patients with mainly volume overload as phenotype, is increased by almost 40%.[19] Assuming an initial blood volume of 5 L, this equates to ±1.5 L additional blood volume, but also more than ±6 L of additional interstitial fluid, as only one quarter of retained sodium and water is located intravascularly.[19] As such, the goal of decongestive therapy in volume overload is to relieve the interstitium of excessive sodium and water while the plasma compartment is merely used as a vehicle to decongest the interstitium (Figure 1). As urine is always hypotonic in case of loop diuretic utilization, this will result in concentration of the plasma compartment, leading to both a small hydrostatic pressure drop and oncotic pressure increase.[9] This will shift the Starling forces between the plasma and interstitium towards net reabsorption. The rate of sodium and fluid entering the plasma is referred to as the plasma refill rate. With adequate decongestion, haemoconcentration will occur if the rate of diuresis exceeds the plasma refill rate, paralleled with restoration of interstitial and plasma volume. One very important observation, however, is that during decongestion the plasma refill rate drops[20] (Figure 1). This drop in plasma refill rate is well studied in the dialysis literature and relates to a decrease in vasodilatory hormones during decongestion.[20]

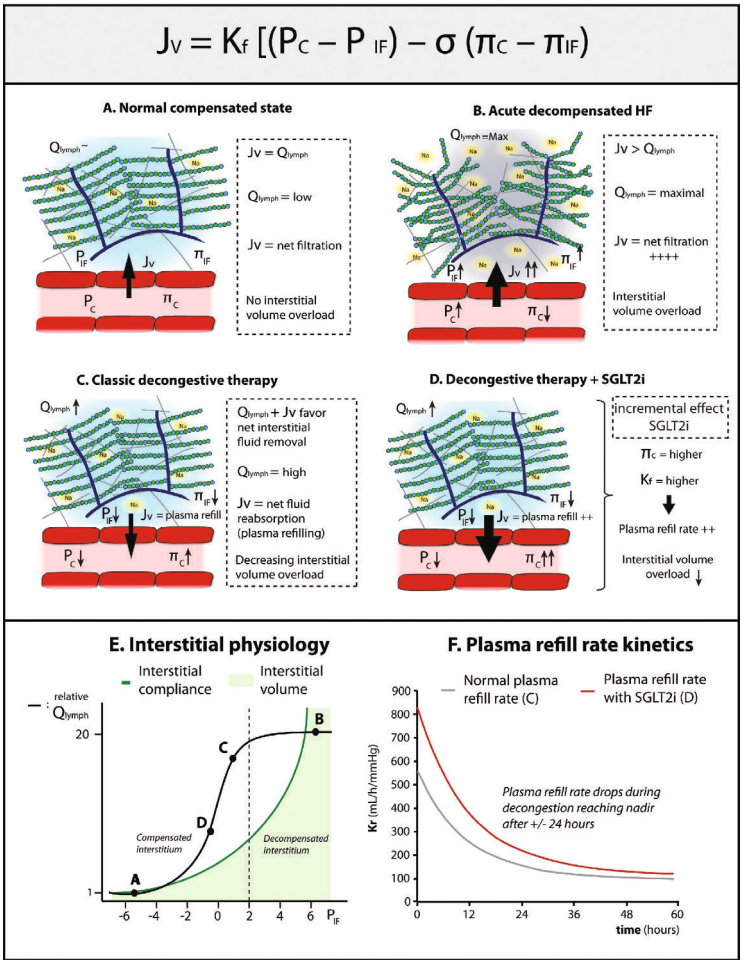


Figure 1 | Sodium–glucose co-transporter 2 inhibitors (SGLT2i) might act as 'smart osmotic diuretics' facilitating removal of interstitial fluid overload. Jv represents the net capillary filtration and is determined by Starling forces. **a |** Normal state. **b |** Acute heart failure (HF) with interstitial volume overload. **c** | Decongestion with classic loop diuretics resulting in plasma refill occurring over the capillary surface. **d** | Incremental effect of SGLT2i favouring enhanced plasma refilling. **e** | Interstitial physiology. Normally interstitial pressure is negative (*A*), interstitial volume will rise due to increased Jv, once lymph flow reaches a plateau (black curve, lymphatic flow can rise up to 20 fold) and Jv > Qlymph (state of decompensated interstitium with low compliance, *B*). Decongestion results in drop in interstitial volume (*C*), which is enhanced by SGLT2i (*D*). (*F*) Plasma refill rate kinetics. Plasma refill rate drops during decongestion reaching a steady state nadir after ±24 h. SGLT2i could potential result in an enhanced plasma refill rate due to higher πC and Kf. Jv, net capillary filtration/reabsorption; Pc, capillary hydrostatic pressure; PIF, interstitial hydrostatic pressure; πC, capillary oncotic pressure; πIF, interstitial oncotic pressure; Qlymph, lymphatic flow.

The reduced vasodilatation will result in a decrease of capillary hydraulic conductivity times capillary area, therefore the area over which refill takes place declines during decongestion. The importance of plasma refill rate is perhaps underappreciated in acute HF. Plasma refill is actually an important event needed to remove sodium and water from the interstitium towards the kidney for effective natriuresis/diuresis. Importantly, SGLT2i act as a smart diuretic through incremental osmotic diuresis, thereby increasing plasma osmolality more compared to classical diuretic therapy, which should improve plasma refill rate considerably (Figure 1). Indeed, the current study nicely corroborates previous findings as the direct increase in plasma osmolality induced by SGLT2i should further facilitate the effect of tissue water mobilization if they are provided on top of standard loop diuretic therapy. In addition, the improved neurohumoral profile with subsequent vasodilatation in response to SGLT2i might further increase the plasma refill rate through a larger capillary area (Figure 1). Indeed, we showed in a small study that dapagliflozin results in lower filling pressures almost immediately upon administration probably through an additional vasodilatory effect.[21]

In conclusion, the reported data clearly indicate a potential role for SGLT2i as 'smart osmotic diuretics' which act through osmotic diuresis, hereby resulting in improved mobilization of interstitial fluid. Additionally, as documented previously, the proximal site of action of SGLT2i improves intrarenal haemodynamics.

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Chapter 5

Dipeptidyl Peptidase-3, a Marker of the Antagonist Pathway of the Renin-angiotensin-aldosterone System in Patients with Heart Failure

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Abstract

Background | Recently, dipeptidyl peptidase 3 (DPP3) has been discovered as the peptidase responsible for cleavage of Angiotensin(1-7) (Ang(1-7)). Ang(1-7) is part of the ACE II – Angiotensin(1-7)- Mas (AAM) pathway which is considered to antagonize the Renin-Angiotensin-Aldosterone system (RAAS). Since DPP3 inhibits the counteracting pathway of the RAAS, we hypothesize that DPP3 might be deleterious in the setting of heart failure. However, no data is available on DPP3 in chronic heart failure. We therefore investigated the clinical characteristics and outcome related to elevated DPP3 concentrations in patients with worsening HF.

Methods | DPP3 was measured in 2156 serum samples of patients with worsening heart failure using luminometric immunoassay (DPP3-LIA) by 4TEEN4 Pharmaceuticals GmbH, Hennigsdorf, Germany. Predictors of DPP3 levels were selected using multiple linear regression with stepwise backward selection.

Results | Median DPP3 concentration was 11.45 ng/mL with a range from 2.8 to 84.9 ng/mL. Patients with higher DPP3 concentrations had higher renin (78.3 (IQR 26.3, 227.7) vs 120.7 UI/mL (IQR 34.74, 338.9), p <0.001, for Q1-3 vs Q4) and aldosterone (88 (IQR 44, 179) vs 116 UI/mL (IQR 46, 241), p <0.001, for Q1-3 vs Q4) concentrations. The strongest independent predictors for higher concentration of DPP3 were log ALT, log-total bilirubin, the absence of diabetes, higher osteopontin FGF-23 and NT pro BNP concentrations (all p<0.001). In univariable survival analysis DPP-3 was associated with mortality and the combined endpoint of death or HF hospitalization (P <0.001 for both). After adjustment for confounders this association was no longer significant.

Conclusion | In patients with worsening heart failure DPP3 is a marker of more severe disease with higher RAAS activity. It may be deleterious in HF by counteracting the Mas-receptor pathway. Procizumab, a specific antibody against DPP3 might be a potential future treatment option for patients with heart failure.

Background

It was recently discovered that dipeptidyl peptidase-3 (DPP3) is responsible for enzymatic cleavage of both angiotensin II (ANGII) and the heptapeptide Angiotensin(1-7) (Ang(1-7)) to Ang(3-7) and Ang(5-7)[1,2](Concept Figure). Ang(1-7) is part of the ACE2 – Angiotensin (1-7) – Mas-receptor (AAM)-axis, which is considered the antagonistic pathway of the RAAS-system[3]. Activation of the AAM-pathway leads to vasodilation, increased renal blood flow and increased natriuresis. The beneficial effects of angiotensin converting enzyme- inhibitors (ACE-i) and angiotensin receptor blockers (ARBs) have been partly attributed to stimulation of the Mas-receptor[4,5]. In contrast, breakdown of Ang(1-7) by DPP3 would inhibit the potentially beneficial effects of the AAM-pathway, and might therefore have deleterious cardiovascular effects[4] (figure 1). This is supported by a study showing that DPP3 infusion in healthy mice caused cardiac depression and these effects were antagonized by Procizumab, a specific antibody directed against circulating DPP3[6]. In patients with cardiogenic shock, higher DPP3 concentrations were associated with a higher short-term mortality and severe organ dysfunction[7].

Aim

Since activation of the RAAS plays a key role in the development and progression of heart failure, the role of DPP3 might be of interest in these patients as well. However, the prevalence, predictors and clinical outcomes of elevated DPP3 concentrations in patients with heart failure (HF) have not yet been established. We therefore investigated the clinical characteristics and outcome related to elevated DPP3 concentrations in patients with worsening HF.

Methods

We measured DPP3 concentrations in 2314 subjects from a multinational, observational cohort on patients with chronic, worsening HF (BIOSTAT-CHF)[8]. DPP3 was measured in serum samples with a DPP3 luminometric immunoassay (DPP3-LIA) by 4TEEN4 Pharmaceuticals GmbH, Hennigsdorf, Germany[9]. The DPP3-LIA has a measuring range between 0.06 - 400 ng/mL, the upper limit of normal, based on a cohort of healthy volunteers, is 40 ng/ml. We excluded 158 samples due to visible hemolysis with normal hemoglobin levels, leaving 2156 samples for the present analysis. Baseline characteristics were evaluated between the lowest 3 quartiles and the highest quartile of DPP3 concentrations, using a t-test or Mann Whitney U for parametric and non-parametric variables, respectively. A multivariable lineair regression analysis was performed to identify predictors of DPP3 concentration. All variables with a $P < 0.1$ in univariable

regression were added to the multivariable regression model. Stepwise backwards selection eliminated the non-significant variables.

Results

Table 1 shows baseline characteristics comparing the highest quartile of DPP3 with the 3 lowest quartiles. Spearman correlations for the continuous variables showing a statistical difference in the baseline table can be found in Supplementary Table 1. Median DPP3 concentration was 11.45 ng/mL with a range from 2.8 to 84.9 ng/mL. Out of 2156 patients in whom DPP3 was measured, only 31 (1.4%) showed DPP3 levels above the median found in cardiogenic shock patients (33.4 ng/mL)[6]. Patients in the highest quartile (median DPP3: 17.95 ng/mL) versus the other three quartiles (median DPP3 respectively 7.84, 10.13, 12.93 ng/mL) were characterized by higher New York Heart Association class (NYHA IV 13.4% vs 11.3%, p = 0.001), more frequent history of valvular surgery (12.1% vs 6.1%, p = <0.001) as well as valvular etiology of HF (11.0% vs. 6.9%, p = 0.003). Atrial fibrillation was more common in the highest quartile (51.6% vs. 43.3%, p = 0.001), but diabetes mellitus was less frequently present (27.5% vs. 33.0%, p = 0.020). Men had higher DPP3 concentration than women (mean 13.0 vs. 12.2 ng/mL for men and women respectively, p = 0.010). Patients in the highest quartile showed more signs and symptoms of congestion, higher liver enzymes, lower cholesterol levels, higher renin and aldosterone, as well as higher biomarkers predictive of more severe disease compared to the lower 3 quartiles. Patients in the highest quartile of DPP3 were less likely to use an ACE-i/ARB at baseline. There was however no significant association between baseline DPP3 levels and target dose of ACE-i/ARB after 9 months of encouraged uptitration. DPP3 levels were not predictive of an inability to uptitrate ACE-i/ARB to guideline recommended target doses.

Table 1 | Baseline characteristics

	Q1-3 combined	Q4	p-value (Q1-3 vs Q4)
n	1617	539	
DPP3 (ng/mL)	10.13 [8.45, 12.18]	17.95 [16.09, 21.70]	
Demographics			
Age (years)	69 ± 12	69 ± 12	0.591
Female sex	447 (27.6)	117 (21.7)	0.008
Systolic Blood Pressure (mmHg)	125 ± 22	123 ± 21	0.006
Diastolic Blood Pressure (mmHg)	75 ± 13	74 ± 12.44	0.011
Heart Rate (beats/min)	79 ± 20	80 ± 19	0.485
Weight (kg)	82 ± 18	83 ± 19	0.400

Table 1 *(Continued)*

	Q1-3 combined	Q4	p-value (Q1-3 vs Q4)
n	1617	539	
NYHA class			0.001
I	44 (2.8)	4 (0.8)	
II	594 (37.8)	166 (31.7)	
III	756 (48.1)	284 (54.2)	
IV	178 (11.3)	70 (13.4)	
Medication use			
ACE-i/ARB			
Percentage of target dose at baseline			0.001
0-49%	989 (61.2)	358 (66.4)	
50-99%	387 (23.9)	135 (25.0)	
100%	241 (14.9)	46 (8.5)	
Percentage of target dose at 9 months			0.068
0-49%	746 (46.1)	273 (50.6)	
50-99 %	496 (30.7)	165 (30.6)	
100%	375 (23.2)	101 (18.7)	
Beta blockers	1382 (85.5)	419 (77.7)	<0.001
Mineralocorticoid Receptor Antagonists	867 (53.6)	279 (51.8)	0.485
Loop Diuretics	1610 (99.6)	539 (100.0)	0.274
Digoxin	289 (17.9)	116 (21.5)	0.070
Medical history			
Myocardial infarction	634 (39.2)	197 (36.5)	0.295
Coronary artery bypass graft	280 (17.3)	98 (18.2)	0.695
Valvular surgery	99 (6.1)	65 (12.1)	<0.001
PCI	369 (22.8)	104 (19.3)	0.098
Atrial fibrillation	700 (43.3)	278 (51.6)	0.001
Stroke	142 (8.8)	59 (10.9)	0.158
Peripheral Vascular Disease	176 (10.9)	57 (10.6)	0.904
Hypertension	1030 (63.7)	311 (57.7)	0.015
Smoking			0.894
None	586 (36.3)	201 (37.3)	
Past	807 (50.0)	267 (49.5)	
Current	222 (13.7)	71 (13.2)	
Diabetes Mellitus	533 (33.0)	148 (27.5)	0.020
COPD	271 (16.8)	103 (19.1)	0.237
Renal Disease	424 (26.2)	171 (31.7)	0.016

Table 1 *(Continued)*

	Q1-3 combined	Q4	p-value (Q1-3 vs Q4)
n	1617	539	
Treated thyroid disease			0.033
No	1467 (90.7)	474 (87.9)	
Hypothyroidism	124 (7.7)	47 (8.7)	
Hyperthyroidism	26 (1.6)	18 (3.3)	
Current Malignancy	60 (3.7)	20 (3.7)	0.999
Heart Failure etiology			
Hypertension	171 (10.8)	48 (9.0)	0.266
Cardiomyopathy	401 (25.3)	132 (24.7)	0.817
Valvular Disease	110 (6.9)	59 (11.0)	0.003
Clinical profile			
Bibasilar rales/crackles	600 (38.2)	226 (43.0)	0.058
Peripheral edema			<0.001
Not Present	574 (43.5)	153 (32.2)	
Ankle	404 (30.6)	125 (26.3)	
Below Knee	273 (20.7)	136 (28.6)	
Above Knee	70 (5.3)	61 (12.8)	
Elevated JVP	339 (31.3)	129 (37.7)	0.033
Hepatomegaly	202 (12.5)	104 (19.4)	<0.001
Third heart tone	156 (9.7)	55 (10.3)	0.754
Orthopnea	532 (33.0)	215 (40.0)	0.004
Dyspnoe VAS score	50 ± 22	44 ± 23	0.012
Laboratory values			
Angiotensin converting enzyme-2*	5.19 (0.70)	5.65 (0.76)	<0.001
Hemoglobin (g/dL)	13.3 [11.9, 14.5]	13.4 [12.0, 14.6]	0.299
Hematocrit (%)	40 [36, 43]	41 [37, 44]	0.014
Serum Creatinine (umol/L)	101 [83, 127]	106 [86, 141]	0.004
Urea (mmol/L)	11.0 [7.4, 17.4]	12.1 [8.0, 19.9]	0.006
Sodium (mmol/L)	140 [137, 142]	139 [136, 141]	<0.001
Potassium (mmol/L)	4.2 [3.9, 4.6]	4.2 [3.9, 4.6]	0.125
NT pro-BNP (ng/L)	2469 [1098, 5340]	3333 [1520, 6513]	<0.001
AST (U/L)	24 [18, 32]	32 [25, 46]	<0.001
ALT (U/L)	24 [16, 35]	28 [20, 47]	<0.001
Alkaline Phophatase (ug/L)	81 [63, 112]	89 [70, 128]	<0.001
Gamma-GT (U/L)	47 [26, 91]	81 [42, 133]	<0.001
Total bilirubin (umol/L)	13 [9, 20]	18 [12, 27]	<0.001
TSH (mU/L)	1.85 [1.16, 3.00]	2.00 [1.32, 3.20]	0.085
Glucose (mmol/L)	6.3 [5.4, 8.0]	6.1 [5.2, 7.6]	0.011
Triglycerides (mmol/L)	1.2 [0.9, 1.7]	1.1 [0.9, 1.5]	0.002

Table 1 *(Continued)*

	Q1-3 combined	Q4	p-value (Q1-3 vs Q4)
n	1617	539	
Total Cholesterol (mmol/L)	4.1 [3.4, 5.0]	3.9 [3.2, 4.8]	<0.001
HDL cholesterol (mmol/L)	1.1 [0.9, 1.3]	1.0 [0.8, 1.3]	0.005
LDL cholesterol (mmol/L)	2.5 [1.8, 3.2]	2.4 [1.7, 3.0]	0.111
bio-ADM (pg/mL)	33.7 [22.5, 54.0]	40.9 [26.9, 74.8]	<0.001
Troponin T (ug/L)	0.03 [0.02, 0.06]	0.03 [0.02, 0.06]	0.535
Aldosterone (pg/mL)	88 [44, 179]	116 [46, 241]	<0.001
Renin (UI/mL)	78.3 [26.3, 227.7]	120.7 [34.74, 338.9]	<0.001
FGF-23 (RU/ml)	192.0 [110.0, 453.4]	377.2 [155.1, 1308.8]	<0.001
Osteopontin (ng/mL)	211 [175, 252]	237 [190, 285]	<0.001
GDF-15 (pg/mL)	2535. [1616, 4038]	3647 [2041, 6289]	<0.001
CA-125 (U/ml)	36.1 [15.6, 108.4]	69.9 [21.6, 201.2]	<0.001

Variables with a normal distribution are displayed with a mean ± SD, for variables with a non-normal distribution median [interquartile range] is depicted, categorical variables are depicted as n (%).
**ACE-2 values are reported in relative quantification, meaning the value depicted is not an absolute concentration, but a concentration relative to the rest of the BIOSTAT index cohort.*
Abbreviations: ACE-i/ARB: angiotensin converting enzyme-inhibitor or angiotensin receptor blocker: AST: aspartate aminotransferase; ALT; alanine aminotransferase; bio-ADM; biologically active adrenomedullin; CA-125: Cancer antigen-125; COPD: chronic obstructive pulmonary disease; DPP-3: dipeptidyl peptidase 3; GDF-15; Growth/differentiation factor-15; FGF-23: fibroblast growth factor 23; HDL: high density lipoprotein; JVP: jugular venous pressure; LDL: low density lipoprotein; mmHg: millimeter mercury; NT pro-BNP: N-terminal pro-brain natriuretic peptide; NYHA: New York Heart Association; PCI: percutaneous coronary intervention; TSH: thyroid stimulating hormone; VAS: visual analogue scale;

Table 2 | Multivariable linear regression analysis for log DPP3 levels For continuous variables standardized beta-coefficient is depicted per standard deviation of the variable.

Variable	Standardized Beta	95% Confidence interval	T-value	p-value
log ALT	0.096	0.07-0.12	7.473	<0.001
Diabetes mellitus	-0.128	-0.18 - -0.07	-4.497	<0.001
log Total Bilirubin	0.062	0.03 - 0.09	4.485	<0.001
Osteopontin	0.062	0.03 - 0.09	4.344	<0.001
log FGF-23	0.065	0.03-0.10	3.650	<0.001
NT pro-BNP^-2	0.048	0.02 - 0.07	3.534	<0.001
GDF-15^0.5	0.052	0.02 - 0.08	3.167	0.002
Aldosterone	0.037	0.01 - 0.06	3.045	0.002
Female sex	-0.085	-0.15 - -0.03	-2.799	0.005
History of valvular surgery	0.126	0.03 - 0.22	2.63	0.009
Alkaline Phosphatase^0.5	0.029	0 – 0.05	2.24	0.025

N= 739, adjusted R2 = 0.327, **Abbreviations:** ALT: alanine aminotransferase; FGF-23: fibroblast growth factor-23; NT pro BNP: N-terminal brain natriuretic peptide;

Table 3 | Cox regression analysis for mortality and the combined endpoint of HF hospitalization or mortality.

	Mortality		
	Hazard ratio (per log increase of DPP3)	**95% confidence interval**	**p-value**
Univariate	1.848	1.513 – 2.256	<0.001
Corrected for OPN and FGF-23	1.174	0.945 - 1.459	0.147
Biostat risk model	1.173	0.965 - 1.425	0.109
	Hospitalization or mortality		
	Hazard ratio (per log increase of DPP3)	**95% confidence interval**	**p-value**
Univariate	1.558	1.319 - 1.839	<0.001
Corrected for OPN and FGF-23	1.128	0.948 - 1.343	0.172
Biostat risk model	1.096	0.937 – 1.281	0.254

Abbreviations: OPN: osteopontin, FGF-23: fibroblast growth factor 23

From a multivariable linear regression analysis with stepwise backwards selection, we identified that the six strongest predictors for higher concentration of DPP3 were the absence of diabetes, higher log-ALT, log-total bilirubin, higher osteopontin, FGF-23 and NT-pro BNP concentrations (all p<0.001) (table 2). Other independent predictors of higher DPP3 concentrations were GDF-15 (beta 0.052, p=0.002), , aldosterone

concentrations (beta 0.037, p=0.002), male sex (beta 0.085, p=0.005), valvular surgery (beta 0.126, p=0.009), and higher alkaline phosphatase (beta 0.029, p=0.025). Due to missing values across several variables, the multivariable regression is based on 739 subjects.

During a median follow-up of 21 months, 561 (26%) patients died and 870 (40%) either died or had a hospital admission for HF. Mortality ranged from 20.4% in the lowest quartile to 36.0% in the highest quartile. Similarly, death or HF hospitalization occurred in 34.7% in the lowest quartile to 50.3% in the highest quartile. In a univariable survival analysis, higher DPP3 concentrations were significantly associated with an increased risk of mortality (p <0.001) and with the combined endpoint of death or HF hospitalization. Although higher DPP3 concentrations were associated with worse clinical outcomes, this association was no longer significant after adjustment for potential confounders, in particular osteopontin and FGF-23, as well as after adjustment for the previously published BIOSTAT risk prediction model[10] (table 3).

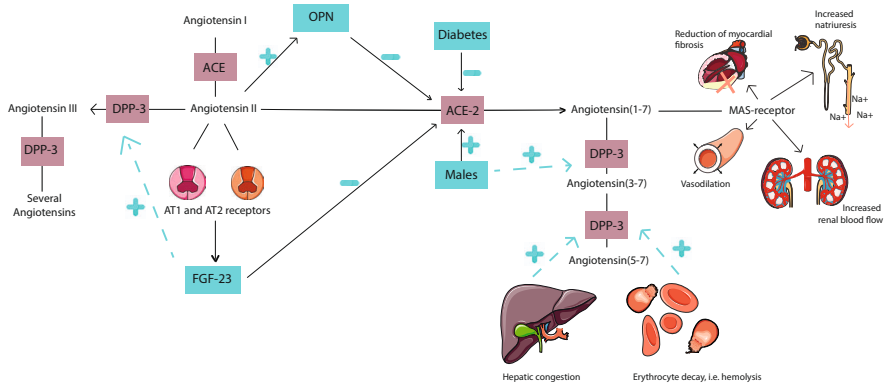

Figure 1 | Concept Figure. Simplified depiction of the RAAS-system.

Angiotensin I is converted to Angiotensin II by ACE (angiotensin converting enzyme). Angiotensin II is degraded to ANGIII and several other angiotensins. Angiotensin II directly stimulates OPN (osteopontin) synthesis and activates the AT1 and AT2 receptors, eventually leading to increased FGF-23 concentrations. FGF-23 has a proposed direct stimulation effect on DPP3, as indicated by the dotted arrow. Both OPN and FGF-23 negatively influence ACE-2 (angiotensin converting enzyme 2) concentrations. ACE-2 converts Angiotensin II to Angiotensin(1-7). Angiotensin(1-7) activates the MAS-receptor, leading to reduction of myocardial fibrosis, increased natriuresis, vasodilation and increased renal blood flow. DPP3 converts Angiotensin(1-7) to Angiotensin(3-7) and Angiotensin(5-7). Diabetes has a negative effect on ACE-2 concentrations. Male sex has a positive effect on ACE-2 levels and a proposed direct positive effect on DPP3 levels, as indicated by the dotted arrow. Both decay of liver cells and erythrocytes will release DPP3 into the plasma, depicted by the dotted arrows. Abbreviations: ACE(-2): Angiotensin converting enzyme (-2); AT1/AT2; angiotensin receptor 1/2; FGF-23: fibroblast growth factor-23; DPP3: dipeptidyl peptidase-3; OPN; osteopontin

Discussion

In this study the main predictors of higher DPP3 concentrations in patients with HF were higher bilirubin, osteopontin and FGF-23 concentrations, while diabetes and female sex were associated with lower DPP3 concentrations. The hypothetical explanations why these variables are associated with DPP3 concentrations are depicted in the Concept Figure, and will be discussed below The Concept Figure is meant to be hypothesis generating on the role of DPP3 in HF and should be regarded as such. As is always the case with correlations, we cannot assume causation.

Moreover, we showed that patients with higher DPP3 concentration also had higher renin and aldosterone levels, higher NYHA class, more valvular disease, more signs and symptoms of congestion and higher liver enzymes. We also observed an increased risk of adverse outcome in patients with higher DPP3 levels, significance was however lost after adjustment for the BIOSTAT risk model.

Under normal physiological circumstances DPP3 resides inside the cytoplasm. Our finding that bilirubin andALT were associated with DPP3 levels as well as a history of valvular surgery, might suggest that a low degree of continuous hemolysis, possibly originating from an artificial heart valve, contributed to increased DPP3 concentrations. Alternatively these biomarkers, combined with an increase in alkaline phosphatase, could originate from liver cell decay, from right sided congestion. Moreover, increased liver enzymes in HF, in particular total bilirubin, are independent predictors of worse outcome, thereby, along with higher NT-pro BNP and GDF-15, reflecting a more diseased patient[11,12].

Fibroblast growth factor-23 (FGF-23) is elevated in HF and CKD patients and correlates with disease severity. Activation of the AT1/AT2 receptors leads to decreased expression of the Klotho receptor, the receptor for FGF-23, leading to increased concentrations of FGF-23[13]. ANGII infusion in mice resulted in a 1.5-1.7 time increase in FGF-23 serum concentrations[14]. Moreover, FGF-23 attenuates the beneficial effect of ARBs on the AAM-axis[15].

Osteopontin (OPN) is a protein associated with inflammation, angiogenesis and bone resorption, and is activated by ANGII[16]. Several studies demonstrated that ARBs nearly normalized OPN serum concentrations and intramyocardial expression both in patients with hypertension and rats with either dilated cardiomyopathy or oxalate deposited kidney disease[16-18]. Activation of the AAM-axis is suggested to be responsible for this normalization of OPN levels.

We recently showed that men with heart failure have higher circulating ACE2 concentrations than women[19]. ACE2 is also expressed in testis tissue, potentially explaining higher serum concentrations in men[19]. In a healthy cohort, no sex differences regarding DPP3 could be found[9]. Men with heart failure might express higher levels of DPP3 similar to ACE2 expression. However, this sex differences theory is still debatable. In contrast, patients with diabetes mellitus exhibit lower levels of ACE2, likely due to glycosylation of the enzyme[20,21]. Furthermore, in patients with diabetes mellitus the functionality of both ACE2 and ANG1-7 is altered[22].

Recently, a monoclonal antibody specifically directed at DPP3 was designed. This antibody, Procizumab, rapidly improved cardiac function in an acute heart failure mouse model[6]. Procizumab might therefore serve as a potential AAM activating therapy that could amplify the beneficial effects of RAAS-blockers.

This study has several strengths and limitations. To our knowledge, this is the first study on DPP3 levels in HF. The large number of subjects as well as number of biomarkers available allows for adequate positioning of DPP3 in HF. One limitation is that we could not correct for low grade hemolysis, even though macroscopic hemolytic samples (n = 158, mean DPP3 30.6 ng/ml) were excluded. While we expect hemolysis to increase DPP3 concentrations, we could not verify this as reticulocytes, LDH and haptoglobin were not available in our cohort. Another important limitation is the fact that concentrations of circulating DPP3 were very low in general, meaning that the hypothesized effects might be modest in patients with chronic heart failure. In addition, several studies have shown effects of DPP3 to be related to degradation of ANGII. We cannot say with certainty whether in chronic HF DPP3 (antagonism) will affect ANGII or ANG(1-7), however considering the relationship with outcome in our study we consider an effect on the AAM-axis to be more likely.

Conclusion

DPP3 is an enzyme that counteracts the antagonistic AAM-pathway of the RAAS-system. Predictors of DPP3 concentrations were related to either cell decay or to the RAAS-system. Higher osteopontin, FGF-23 and aldosterone levels indicate more disease severity and suggest a deleterious role for DPP3 by counteracting the Mas-receptor pathway. DPP3 was univariately associated with worse outcomes, significance was lost after correction for confounders. Future research is warranted to further investigate the potential of DPP3 as an actionable biomarker in HF, alongside classic RAAS-inhibitors. Procizumab, a specific antibody against DPP3 might be a potential future treatment option for patients with heart failure.

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Chapter 6

Albuminuria as a Marker for Systemic Congestion in Heart Failure

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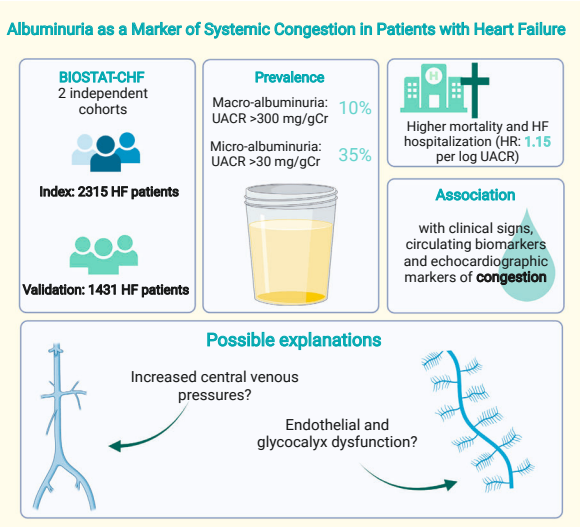
Abstract

Aims | Albuminuria is common in patients with heart failure and associated with worse outcomes. The underlying pathophysiological mechanism of albuminuria in heart failure is still incompletely understood. The association of clinical characteristics and biomarker profile with albuminuria in patients with heart failure with both reduced and preserved ejection fraction were evaluated.

Methods and results | 2,315 patients included in the index cohort of BIOSTAT-CHF were evaluated and findings were validated in the independent BIOSTAT-CHF validation cohort (1,431 patients). Micro-albuminuria and macro-albuminuria were defined as urinary albumin-creatinine ratio (UACR) >30 mg/gCr and >300 mg/gCr in spot urines, respectively.

The prevalence of micro- and macro-albuminuria was 35.4% and 10.0%, respectively. Patients with albuminuria had more severe heart failure, as indicated by inclusion during admission, higher New York Heart Association functional class, more clinical signs and symptoms of congestion, and higher concentrations of biomarkers related to congestion, such as bio-ADM, CA-125, and NT pro-BNP (all P <0.001). Presence of albuminuria was associated with increased risk of mortality and heart failure (re) hospitalisation in both cohorts. The strongest independent association with log UACR was found for log NT pro-BNP (standardised regression coefficient 0.41, 95% CI 0.32 - 0.50, P <0.001). Hierarchical clustering analysis demonstrated that UACR clusters with markers of congestion and less with indices of renal function. The validation cohort yielded similar findings.

Conclusion | In patients with new-onset or worsening heart failure, albuminuria is consistently associated with clinical, echocardiographic, and circulating biomarkers of congestion.



Introduction

Under normal circumstances, only 0.008% of plasma albumin is filtered by the glomeruli[1]. Leakage through the glomerular membrane, caused by damage to (one of the layers of) the glomerular endothelial membrane will cause more albumin to pass through the glomeruli, leading to albuminuria.[1] This is a disease mechanism which is well described in hypertensive and diabetic kidney disease and is usually related to intraglomerular hypertension.[2, 3] In patients with chronic heart failure, the presence of albuminuria is a strong prognostic indicator of adverse events, such as mortality and heart failure hospitalisation, even after correction for diabetes, hypertension and concomitant renal disease[4, 5]. While its prognostic value is well recognised, the underlying mechanisms of albuminuria in heart failure are incompletely understood. First, albuminuria could be the result of renin-angiotensin-aldosterone (RAAS)- system activation, as angiotensin may directly cause podocyte injury.[6] Secondly, albuminuria might be the result of endothelial dysfunction, manifesting in both the peripheral vessels and the glomeruli. Third, albuminuria could be the result of increased renal venous pressure. Two studies from the same group indicated that when renal venous pressure was increased this led to albuminuria, while external pressure on the kidney parenchyma did not lead to albuminuria.[7, 8] Lastly, albuminuria could be an indicator of comorbidities which frequently occur alongside heart failure, such as diabetes and hypertension[9-11]. In addition, its differential relevance in heart failure with reduced and preserved ejection fraction has not been described. We therefore aimed to study the clinical characteristics and biomarker profile associated with albuminuria, in addition to previously described clinical outcomes, in patients with heart failure with both reduced and preserved ejection fraction.

Methods

Patient population

For the present study, we used the index and validation cohort of BIOSTAT-CHF (A Systems Biology Study to Tailored Treatment in Chronic Heart Failure). The trial design has been published before.[12] In brief, BIOSTAT-CHF was a European, multicentre clinical study executed in 11 different countries, consisting of 2,516 patients in the index cohort. Patients were included after presentation with either new onset or worsening heart failure, which was defined as left ventricular ejection fraction (EF) ≤40% or BNP (brain natriuretic peptide) >400 pg/mL or NT pro-BNP (N-terminal pro-B-type natriuretic peptide) >2000 pg/mL. Patients were encouraged to be uptitrated to recommended treatment doses of betablockers and angiotensin converting enzyme (ACE) inhibitors or angiotensin receptor blockers (ARB).

Data were validated in the independent BIOSTAT-CHF validation cohort consisting of 1,738 patients with heart failure from Scotland, United Kingdom.

All patients enrolled in BIOSTAT-CHF provided written informed consent to participate in the study and BIOSTAT-CHF was conducted in concordance with the declaration of Helsinki, national ethics and legal requirements, as well as relevant EU legislation.

Biomarkers

Spot urinary samples were collected at the baseline visit. Samples were frozen in -80°C and thawed prior to analyses. Standard urinary chemistry measurements for albumin, creatinine, sodium, potassium, urea, and uric acid were performed in the laboratory of the University Medical Center Groningen, using routine clinical chemistry measurement on a Roche Cobas® analyser.

At baseline, a total of 2,315 patients in the index cohort and 1,431 patients in the validation cohort had urinary samples available. Micro-albuminuria was defined as an urinary albumin-creatinine ratio (UACR) >30 mg/gCr, while macro-albuminuria was defined as an UACR >300 mg/gCr[3].

Statistical analysis

Patients were divided in groups based on normo-, micro- or macro-albuminuria respectively. Normally distributed continuous data are presented as means and standard deviation, not normally distributed data as medians and 25th until 75th percentile, and categorical variables as percentages and frequencies. Intergroup differences were tested using one-way ANOVA for normally distributed continuous data, whereas skewed data

was analysed using Chi-squared test or Kruskal-Wallis test depending on whether the data was nominal or continuous. Whether variables were parametric or non-parametric was assessed visually using QQ-plots. When QQ-plots were inconclusive, a histogram was created. When the histogram was also inconclusive, the variable was deemed to be non-parametric. In a subgroup analysis, HFpEF was defined as an LVEF ≥50% and HFrEF was defined as an LVEF <40%, per the most recent ESC HF guidelines.[13]

Associations of UACR were analysed using univariable and multivariable regression analyses, in which all variables with P<0.10 in univariable analysis were included in multivariable analysis and subjected to the backward elimination method. Assumption of normality were checked with the use of QQ-plots, the assumption of linearity was checked using scatter-plots and independence of residuals was analysed using residual time series plots. Moreover, we have checked the assumption of homoscedasticity with the use of fitted value vs residual plots. If the assumption was not met, log-transformation was performed to ensure homoscedasticity. The models obtained using stepwise backward selection were validated by repeating the variable selection using least absolute shrinkage and selection operator (LASSO) regression. First, variables with more than 15% missingness were excluded from the analysis, second LASSO-regression was performed using the R package glmnet.[14] Alpha was set to 1 and the optimal penalization parameter (lambda) was obtained using 10-fold cross-validation. To derive the most parsimonious model, the lambda within 1 standard error of the minimum lambda was used. Variables selected with LASSO-regression were then used in a linear regression model to obtain their coefficients. The coefficients of determination (R^2) has been calculated for all regression models.

A Cox regression analysis was used to investigate the association of UACR with clinical endpoints of mortality and heart failure hospitalisation, first in a univariable model, secondly corrected for known causes of albuminuria (i.e. renal disease, diabetes mellitus, and hypertension), and lastly corrected for the BIOSTAT-CHF risk model, which consists of the strongest predictors of each clinical outcome[15]. Proportional hazards assumption of the Cox regression models were checked by the use of Schoenfeld residuals in R, with the use of the Survival and Survminer package to test and plot the Schoenfeld residuals.

The dendrograms were built using hierarchical clustering based on Euclidean distance, which has the benefit of using real-valued vectors, as compared to Spearman's correlation, which uses ranked variables. The optimal number of clusters was decided in a supervised manner and based on clinical experience. The current number of clusters is 9. Any number of clusters of 7 or higher puts UACR in between the congestion cluster.

A similar approach was used in the validation cohort. All variables were standardised prior to analysis. Aside from urinary and plasma biomarkers, a clinical congestion score was added to the dendrogram. This congestion score was modified from the validated Ambrosy score[16] and constructed by assigning points based on clinical findings: 1 point for orthopnoea, 1 point for jugular vein distention and respectively 1/3, 2/3, or 1 point for peripheral oedema until the ankle, below the knee and above the knee, for a maximum of 3 points[17]. A two-sided p-value of <0.05 was considered to be significant.

Results

Baseline Characteristics

The prevalence of macro-albuminuria and micro-albuminuria was 242 (10.0%) and 819 (35.4%) respectively, in the index cohort. Baseline characteristics based on these groups are depicted in *Table 1*. In summary, patients with micro- or macro albuminuria had more severe heart failure indicated by inclusion during admission, more frequent New York Heart Association functional class of III or IV, higher blood pressures and heart rate, more clinical signs of congestion, and higher plasma concentrations of congestion-related biomarkers, such as NT pro-BNP, biologically active adrenomedullin (bio-ADM) and cancer antigen 125 (CA-125) (all P < 0.001).

Patients with any albuminuria in the index cohort were less likely to be on ACE-inhibitor or angiotensin receptor blocker (63.2% (macro-) and 69.0% (micro-) vs. 75.8% (normo-albuminuria), P <0.001), while they were on higher doses of loop diuretics (114 ± 141 (macro-) and 101 ± 120 (micro-) vs 85 ± 116 mg of furosemide or equivalent daily (normo-albuminuria), P <0.001). No differences in medical treatment between those with and without albuminuria were seen in the validation cohort, with the exception of loop diuretic doses.

Echo parameters that provide assessment of right sided pressures and volume (only available in the validation cohort) suggested that pulmonary pressures were higher in patients with albuminuria (Peak tricuspid regurgitation gradient 40.7 mmHg, 36.4 mmHg and 33.2 mmHg for macro-, micro and normo-albuminuria, respectively, P<0.001) and inferior caval vein diameter >2.1 cm (22.5%, 25.9% and 15.6% for macro-, micro and normo-albuminuria respectively, P<0.001).

Table 1 | Baseline Characteristics according to normo-, micro- and macro-albuminuria

	Index Cohort				Validation Cohort			
	Macro (UACR >300 mg/gCr)	**Micro (UACR 30 – 300 mg/gCr)**	**Normal (UACR <30 mg/gCr)**	**P-value**	**Macro (UACR >300 mg/gCr)**	**Micro (UACR 30 – 300 mg/gCr)**	**Normal (UACR <30 mg/gCr)**	**P-value**
N	242 (10.0%)	819 (35.4%)	1254 (54.2%)		112 (7.0%)	446 (31.2%)	873 (61.0%)	
Demographics								
Age	69.75 (11.26)	70.44 (12.17)	67.69 (11.79)**	<0.001	74.5 (10.8)	76.1 (9.5)	72.2 (10.7)**	<0.001
Female sex (%)	56 (23.1)	240 (29.3)	323 (25.8)	0.083	30 (26.8)	136 (30.5)	286 (32.8)	0.368
Duration of heart failure (years)	2.12 (3.38)	4.16 (6.37)	2.38 (4.69)*	0.088	2.95 (4.82)	3.25 (4.26)	3.56 (4.73)	0.280
Inpatient hospitalisation (%)	168 (69.4)	596 (72.8)	786 (62.7)**	<0.001	69 (61.6)	278 (62.3)	374 (42.8)**	<0.001
NYHA-class (%)			**	<0.001				<0.001
Class I	4 (1.7)	9 (1.1)	39 (3.2)		0 (0.0)	2 (0.4)	13 (1.5)	
Class II	57 (23.9)	242 (30.6)	508 (41.7)		33 (29.5)	152 (34.1)	420 (48.2)	
Class III	140 (58.8)	411 (51.9)	568 (46.7)		55 (49.1)	200 (44.8)	361 (41.4)	
Class IV	37 (15.5)	130 (16.4)	102 (8.4)		24 (21.4)	92 (20.6)	78 (8.9)	
Systolic Blood Pressure (mmHg)	131.32 (22.60)**	126.50 (23.40)	122.51 (20.80)**	<0.001	141.09 (25.68)**	127.44 (21.99)	123.42 (20.77)**	<0.001
Diastolic Blood Pressure (mmHg)	76.16 (13.70)	75.71 (14.66)	74.17 (12.50)*	0.013	74.96 (14.55)**	69.81 (13.45)	68.19 (11.83)*	<0.001
Heart Rate (beats/min)	80.42 (18.40)	82.82 (20.70)	77.82 (18.59)**	<0.001	76.43 (17.25)	75.57 (17.95)	72.10 (15.27)**	<0.001
Weight (kg)	83.16 (17.38)	81.25 (18.58)	81.72 (18.11)	0.361	83.13 (20.26)	81.79 (19.71)	82.79 (20.11)	0.651
BMI (kg/m2)	28.5 (5.0)	27.8 (5.6)	28.8 (5.3)	0.191	29.0 (6.2)	28.8 (6.4)	29.2 (6.3)	0.357
Waist Hip Ratio					1.01 (0.10)*	0.98 (0.09)	0.97 (0.09)	0.002
Medication								
ACE-inhibitors or ARB (%)	153 (63.2)	565 (69.0)	950 (75.8)**	<0.001	78 (69.6)	311 (69.7)	634 (72.6)	0.493
Beta blockers (%)	201 (83.1)	667 (81.4)	1056 (84.2)	0.258	73 (65.2)	334 (74.9)	631 (72.3)	0.116
Mineralocorticoid Receptor Antagonists (%)	107 (44.2)	407 (49.7)	727 (58.0)**	<0.001	38 (33.9)	142 (31.8)	295 (33.8)	0.764
Loop diuretic dose (mg furosemide or equivalent)	114 (141)	101 (120)	85 (116)**	<0.001	86 (67)**	78 (53)	62 (43)**	<0.001
Medical History								
Myocardial infarction (%)	109 (45.0)**	288 (35.2)	487 (38.8)	0.016	52 (46.4)	223 (50.0)	442 (50.7)	0.696
Atrial fibrillation (%)	107 (44.2)*	419 (51.2)	519 (41.4)**	<0.001	59 (52.7)	241 (54.5)	343 (39.6)**	<0.001
Stroke (%)	28 (11.6)	82 (10.0)	102 (8.1)	0.136	21 (18.8)	98 (22.2)	147 (17.0)*	0.076
Peripheral Arterial Disease (%)	42 (17.4)	110 (13.4)	107 (8.5)**	<0.001	37 (33.3)	108 (24.9)	182 (21.4)	0.014
Hypertension (%)	192 (79.3)**	534 (65.2)	729 (58.1)**	<0.001	82 (73.2)	285 (64.3)	458 (52.7)**	<0.001

Table 1 *(Continued)*

	Index Cohort				Validation Cohort			
	Macro (UACR >300 mg/gCr)	**Micro (UACR 30 – 300 mg/gCr)**	**Normal (UACR <30 mg/gCr)**	**P-value**	**Macro (UACR >300 mg/gCr)**	**Micro (UACR 30 – 300 mg/gCr)**	**Normal (UACR <30 mg/gCr)**	**P-value**
N	242 (10.0%)	819 (35.4%)	1254 (54.2%)		112 (7.0%)	446 (31.2%)	873 (61.0%)	
Current Smoking (%)	35 (14.5)	118 (14.4)	178 (14.2)	0.751	17 (15.3)	48 (10.9)	121 (13.9)	0.424
Diabetes Mellitus (%)	137 (56.6)**	280 (34.2)	347 (27.7)**	<0.001	55 (49.5)	175 (39.5)	236 (27.2)**	<0.001
COPD (%)	44 (18.2)	150 (18.3)	208 (16.6)	0.561	22 (19.6)	82 (18.6)	143 (16.6)	0.533
Renal Disease (%)	114 (47.1)**	247 (30.2)	283 (22.6)**	<0.001	70 (62.5)	237 (53.6)	336 (39.3)**	<0.001
Clinical Profile								
Bibasilar rales/crackles	117 (49.4)	372 (46.2)	418 (34.5)**	<0.001	51 (46.8)	209 (48.3)	250 (29.9)**	<0.001
Peripheral Oedema (%)			**	<0.001	*		**	<0.001
Not Present	66 (30.8)	218 (31.5)	493 (48.3)		26 (24.1)	133 (32.1)	351 (45.3)	
Ankle	69 (32.2)	201 (29.0)	304 (29.8)		35 (32.4)	136 (32.9)	230 (29.7)	
Below Knee	53 (24.8)	208 (30.0)	176 (17.3)		30 (27.8)	116 (28.0)	163 (21.1)	
Above Knee	26 (12.1)	66 (9.5)	47 (4.6)		17 (15.7)	29 (7.0)	30 (3.9)	
Jugular Venous Distention (%)	67 (38.5)	213 (37.6)	230 (26.3)**	<0.001	43 (41.3)	136 (34.3)	195 (25.8)**	<0.001
Haepatomegaly (%)	46 (19.1)	132 (16.2)	150 (12.0)**	0.002	8 (8.1)	21 (5.0)	23 (2.9)*	0.017
Orthopnoea (%)	93 (38.4)	330 (40.4)	382 (30.5)**	<0.001				
VAS Dyspnoea score	40.71 (21.30)*	47.89 (22.87)	48.49 (22.30)	0.077	2.36 (0.80)	2.32 (0.85)	2.02 (0.83)**	<0.001
Chest X-ray								
Congestion present (%)	48 (30.6)	200 (35.1)	194 (24.5)**	<0.001				
Cardio Thorax Ratio >0.5 (%)	114 (72.6)	406 (71.5)	501 (63.3)**	0.002				
Echo parameters								
LVEF (%)	32.32 (10.45)	31.26 (11.72)	30.66 (9.71)	0.083	43.20 (15.61)	40.47 (13.39)	39.66 (13.21)	0.076
LVEDD (mm)	60.75 (10.24)	60.32 (10.25)	61.59 (9.55)**	0.028	53.97 (8.40)	55.19 (9.67)	55.28 (9.10)	0.436
Intraventricular septal thickness (mm)	11.30 (2.66)**	10.69 (2.62)	10.47 (2.36)	<0.001	13.39 (2.90)*	12.51 (3.07)	12.17 (4.37)	0.041
Posterior wall thickness (mm)	11 [9, 12]**	10 [9, 12.00]	10 [9, 11]	0.001	12 [10,14]*	11 [10, 13]	11 [9, 13]	0.002
Left atrial diameter (mm)	48.55 (7.29)	47.85 (8.60)	46.90 (7.77)*	0.010	46.53 (6.20)	46.96 (7.85)	44.40 (6.94)**	<0.001
Mitral Regurgitation (%)	114 (49.1)	377 (49.1)	556 (46.3)	0.433	38 (34.5)	160 (37.4)	243 (29.3)**	0.012
E/A Ratio	1.50 [0.99, 2.48]	1.50 [0.80, 2.40]	1.10 [0.72, 2.07]**	0.005	1.10 [0.76, 2.10]	1.10 [0.80, 1.87]	0.90 [0.70, 1.20]**	<0.001
Inferior Caval Vein diameter >2.1 cm					25 (22.5)	113 (25.9)	133 (15.6)**	<0.001
Tricuspid Regurgitation Gradient (mmHg)					40.70 (16.52)	36.83 (14.48)	33.17 (11.57)**	<0.001

Table 1 *(Continued)*

	Index Cohort				Validation Cohort			
	Macro (UACR >300 mg/gCr)	Micro (UACR 30 – 300 mg/gCr)	Normal (UACR <30 mg/gCr)	P-value	Macro (UACR >300 mg/gCr)	Micro (UACR 30 – 300 mg/gCr)	Normal (UACR <30 mg/gCr)	P-value
N	242 (10.0%)	819 (35.4%)	1254 (54.2%)		112 (7.0%)	446 (31.2%)	873 (61.0%)	
Laboratory values								
Haemoglobin (g/dL)	12.90 [11.40, 14.20]	13.20 [11.76, 14.50]	13.40 [12.10, 14.60]**	<0.001	12.50 [11.07, 13.93]*	13.00 [11.50, 14.40]	13.40 [12.20, 14.70]**	<0.001
Serum Creatinine (µmol/L)	123.76 [97.24, 169.00]**	106.08 [87.00, 133.00]	97.24 [80.33, 121.16]**	<0.001	121.00 [89.75, 169.00]**	103.00 [83.00, 135.00]	94.00 [79.00, 117.00]**	<0.001
eGFR (ml/min/m²)	39.1 [28.0, 52.8]**	48.1 [35.9, 66.5]	55.3 [40.0, 72.5]**	<0.001	39.8 [28.2, 56.1]**	48.8 [34.1, 65.6]	55.8 [41.2, 74.1]**	<0.001
Serum Urea (mmol/L)	14.90 [9.30, 24.15]**	11.85 [8.10, 18.65]	10.35 [7.10, 16.42]**	<0.001	10.10 [7.77, 15.05]	9.40 [7.10, 13.50]	8.00 [6.30, 10.40]**	<0.001
Fractional excretion of Urea (%)	25.2 [16.2, 36.9]	26.6 [16.8, 36.2]	25.4 [16.5, 36.4]	0.721	31.2 [26.5, 37.0]	30.4 [24.7, 36.9]	30.5 [25.1, 36.4]	0.434
Serum Albumin (g/L)	30.40 (9.37)	31.34 (8.78)	33.23 (8.55)**	<0.001	36.21 (6.39)	37.32 (6.08)	39.02 (5.68)**	<0.001
ALAT (U/L)	22.00 [15.00, 31.00]*	24.35 [16.00, 37.76]	26.00 [18.00, 38.75]	0.001	21.00 [16.00, 31.75]	22.00 [16.00, 33.00]	22.00 [17.00, 32.00]	0.569
Alkaline Phophatase (µg/L)	111.75 (66.28)	106.96 (70.28)	96.40 (55.22)**	0.006	123.56 (64.43)	111.54 (71.87)	95.68 (44.45)**	<0.001
Gamma-GT (U/L)	63.00 [29.00, 125.00]	63.00 [31.88, 120.00]	46.00 [25.29, 94.00]**	0.001	81.00 [41.75, 146.75]**	51.00 [31.00, 105.50]	40.00 [24.00, 76.00]**	<0.001
Total bilirubin (pmol/L)	29.47 (156.81)	20.32 (21.89)	17.15 (13.49)**	0.049	14.03 (9.99)	14.28 (9.62)	11.35 (6.78)**	<0.001
NT pro-BNP (ng/L)	5045 [2358, 11823]**	3891 [1721, 7516]	1936 [872, 3885]**	<0.001	3957 [1487, 8885]**	2369 [994, 5230]	855 [336, 2137]**	<0.001
Bio-ADM (pg/mL)	45.2 [27.6, 69.6]	40.1 [26.5, 67.5]	28.5 [20.6, 43.0]**	<0.001	41.7 [26.3, 66]**	31.80 [21.0, 50.3]	23.9 [16.8, 35.2]**	<0.001
Glucose (mmol/L)	7.33 [5.66, 10.20]**	6.49 [5.50, 7.99]	6.05 [5.27, 7.40]**	<0.001	7.40 [5.80, 9.60]	6.60 [5.40, 9.40]	6.00 [5.20, 8.00]**	<0.001
Aldosterone (pg/mL)	82.00 [38.00, 177.25]	84.50 [41.00, 183.05]	103.00 [48.00, 211.13]**	0.001				
Renin (UI/mL)	65.43 [22.09, 173.34]	83.52 [25.24, 230.04]	101.61 [32.22, 305.14]**	<0.001				
GDF-15 (pg/mL)	3923 [2373, 6328]*	3623 [2149, 5942]	2189 [1441, 3475]**	<0.001	4826 [2992, 7358]**	3486 [2332, 5598]	2344 [1611, 3764]**	<0.001
FGF-23	460.30 [180.13, 1301.75]	331.89 [149.59, 847.95]	162.25 [100.99, 324.44]	<0.001				
CA-125	69 [22, 205]	58 [21, 149]	28 [14, 105]**	<0.001	56 [25, 159]**	37 [17, 92]	20 [12, 41]**	<0.001

*For differences between groups: * p <0.05 compared to microalbuminuria, ** p <0.01 compared to microalbuminuria.*

Abbreviations: UACR: urinary albumin-to-creatinine ratio; NYHA: New York Heart Association; BMI: body mass index; ACE: angiotensin converter enzyme; ARB: angiotensin receptor blocker; COPD: chronic obstructive pulmonary disease; VAS: visual analogue scale; LVEF: left ventricular ejection fraction; LVEDD: left ventricular end-diastolic diameter; ALAT: alanine transferase; gamma-GT: gamma-glutamyltransferase; NT pro-BNP: N-terminal pro-B type natriuretic peptide; bio-ADM: biologically active adrenomedullin; GDF-15: Growth differentiation factor 15; CA-125: cancer antigen 125

Linear and logistic regression analysis

From multivariable linear regression, the strongest associations with log UACR were found for log NT pro-BNP (standardised regression coefficient 0.438), log urinary KIM-1 (standardised regression coefficient 0.327), log plasma urea, log fractional excretion of urea, a history of diabetes mellitus, systolic blood pressure, log bio-ADM, and log renin (p for all <0.001) (Table 2). Multivariable linear regression in the validation cohort yielded similar results regarding NT pro-BNP, history of diabetes mellitus, systolic blood pressure and bio-ADM. Yet, several differences were also observed, in part driven by the inclusion of different variables in the index in validation cohorts due to availability (Table 2). The adjusted R^2 of the multivariable regression models for the index and validation cohorts were 0.284 and 0.288, respectively. To account for the possibility that a higher NT pro-BNP was the result of poorer renal clearance, the presence of an interaction was tested between log NT pro-BNP and eGFR for the association with log UACR, which was not significant (p for interaction = 0.260). In order to differentiate between congestion and heart failure severity, linear regression was performed in subsets of NYHA-functional class (class I/II, III, and IV). In all NYHA-classes the strongest association with log UACR was found for log NT pro-BNP (Supplementary tables 2a and 2b). The independent associations of log UACR in multivariable linear regression analysis in HFrEF and HFpEF were similar (Table 3a and 3b).

To further validate our findings from the linear regression analyses, variable selection was repeated using LASSO, which gave very similar variables to include in the final model for the complete cohort and the HFrEF subgroups (Supplementary Tables 3a and 3b). However, for the HFpEF subgroup, slightly different variables were selected (Supplementary Table 3c). The regression models build with the variables selected using LASSO can be found in Supplementary Tables 4a-4d. In all these models log NT pro-BNP showed the strongest independent association with log UACR. As a history of diabetes was a major factor associated with log UACR in all subgroups, linear regression analyses were repeated in those with and without a history of diabetes mellitus (Supplementary Table 5). Again, associations found were similar in both subgroups, and NT pro-BNP and systolic blood pressure remained the strongest associations.

Sensitivity analyses using multivariable logistic regression for associations of any vs. no albuminuria showed similar results (Supplementary Table 6). The independent predictors of log UACR in multivariable linear regression analysis in HFrEF and HFpEF were similar (Table 3a and 3b).

Sensitivity analyses using multivariable logistic regression for predictors of any vs. no albuminuria showed similar results (Supplementary Table 3).

Table 2 | linear regression, standardised Beta per log increase UACR.

	Index Cohort			Validation Cohort		
Variable	**Standardised ß (95% CI)**	**T value**	**p-value**	**Standardised ß (95% CI)**	**T value**	**p-value**
log NT pro-BNP	0.438 (0.35-0.53)	9.676	<0.001	0.486 (0.39-0.58)	9.689	<0.001
log Urinary KIM-1	0.327 (0.23 – 0.42)	6.915	<0.001			
Log Plasma Urea	0.373 (0.27 – 0.48)	6.9	<0.001			
Log Fractional Excretion of Urea	0.346 (0.24 – 0.45)	6.68	<0.001			
Hx of Diabetes Mellitus	0.536 (0.37 - 0.7)	6.351	<0.001	0.474 (0.30-0.65)	5.218	<0.001
Systolic Blood Pressure	0.252 (0.17-0.33)	6.136	<0.001	0.410 (0.33-0.49)	9.940	<0.001
log bio-ADM	0.265 (0.18 - 0.35)	6.197	<0.001	0.150 (0.05-0.25)	2.892	0.004
log Ɣ-GT				0.201 (0.12-0.29)	4.654	<0.001
log Renin	-0.228 (-0.31 to -0.14)	-5.228	<0.001			
VAS Dyspnea score				0.146 (0.06-0.23)	3.281	0.001
Body Mass Index				-0.110 (-0.2 to -0.02)	-2.38	0.017
Heart rate				0.10 (0.02-0.18)	2.323	0.019
Log Urinary NGAL	0.139 (0.05 - 0.23)	3.066	0.002			
Log Serum Creatinine				0.094 (0-0.19)	1.997	0.046
Peripheral Edema above knees				0.389 (0.01-0.77)	2.011	0.045
Adjusted R-squared: 0.2844				*Adjusted R-squared: 0.2882*		
N = 1645				*N= 1471*		

Abbreviations: NT pro-BNP: N-terminal pro-B-type natriuretic peptide; KIM-1: kidney injury marker-1; Hx: medical history; bio-ADM: biologically active adrenomedullin; Ɣ-GT: gamma-glutamyltransferase; VAS: visual analogue scale; NGAL: Neutrophil gelatinase-associated lipocalin

Table 3a | Linear regression analysis for log UACR in HFrEF vs HFpEF in the index cohort

Variable	HFrEF (LVEF <40%)			HFpEF (LVEF ≥50%)		
	Standardised regression coefficient (95% CI)	T value	p-value	Standardised regression coefficient (95% CI)	T value	p-value
Log NT pro-BNP	0.414 (0.3-0.52))	7.355	<0.001	0.568 (0.2-0.93)	3.092	0.003
Log plasma Urea	0.42 (0.28-0.56)	6.052	<0.001			
Log urinary KIM-1	0.371 (0.25-0.49)	5.949	<0.001	0.393 (0.09 – 0.69)	2.594	0.011
Systolic blood pressure	0.285 (0.18-0.39)	5.296	<0.001			
Log fractional excretion of urea	0.334 (0.21-0.46)	5.097	<0.001			
Log bio-ADM	0.242 (0.13-0.35)	4.301	<0.001			
Serum Creatinine				0.46 (0.22-0.7)	3.793	<0.001
Log Renin	-0.186 (-0.29--0.08)	-3.362	0.001	-0.49 (-0.81--0.17)	-3.053	0.003
Log plasma CA-125				0.588 (0.19–0.99)	2.92	0.004
History of Diabetes Mellitus	0.379 (0.15-0.6)	3.303	0.001	0.96 (0.37-1.55)	3.253	0.002
Log Urinary Uromodulin	-0.163 (-0.27--0.06)	-3.083	0.002	-0.348 (-0.63- -0.06)	-2.414	0.018
Log Urinary NGAL	0.147 (0.03-0.26)	2.523	0.012			
Plasma Glucose	0.112 (0.01–0.24)	2.211	0.027			
Beta blocker use				0.707 (0.13 – 1.28)	2.428	0.017
	Adjusted R-squared: 0.2771			*Adjusted R-squared: 0.432*		
	N = 1,1031			*N = 113*		

Abbreviations: NT pro-BNP: N-terminal pro-B-type natriuretic peptide; KIM-1: kidney injury marker-1; bio-ADM: biologically active adrenomedullin; NGAL: Neutrophil gelatinase-associated lipocalin

Table 3b | Linear regression analysis for log UACR in HFrEF vs HFpEF in the validation cohort

Variable	HFrEF (LVEF <40%)			HFpEF (LVEF ≥50%)		
	Standardised regression coefficient (95% CI)	T value	p-value	Standardised regression coefficient (95% CI)	T value	p-value
Log NT-proBNP	0.557 (0.42 – 0.69)	8.168	<0.001	0.639 (0.51-0.77)	9.576	<0.001
Systolic blood pressure	0.036 (0.23 – 0.49)	5.522	<0.001	0.326 (0.2 – 0.45)	5.025	<0.001
Log Bilirubine	0.237 (0.1-0.37)	3.512	<0.001			
History of Diabetes Mellitus	0.43 (0.16 – 0.7)	3.101	0.002	0.468 (0.19-0.75)	3.28	0.001
Log bio-ADM				0.178 (0.04-0.31)	2.584	0.01
VAS dyspnea score	0.17 (0.03-0.31)	2.422	0.016			
Log Gamma-GT	0.141 (0.01-0.27)	2.089	0.037			
	Adjusted R-squared: 0.275			*Adjusted R-squared: 0.286*		
	N = 444			*N= 296*		

Hierarchical cluster analysis of UACR and other biomarkers

Figure 1a shows a dendrogram based on Euclidean distance positioning. UACR clearly clusters with biomarkers of congestion (the red cluster), as well as the clinical congestion score. Other clusters that can be appreciated are a 'glomerular cluster' of serum creatinine and serum urea (dark blue), a renal injury cluster of urinary KIM-1 and urinary NGAL (orange), a hepatic cluster of alkaline phosphatase, gamma GT and total bilirubin (light blue) and a RAAS-cluster of aldosterone and renin (purple). Very similar clusters were found in the validation cohort (Figure 1b).

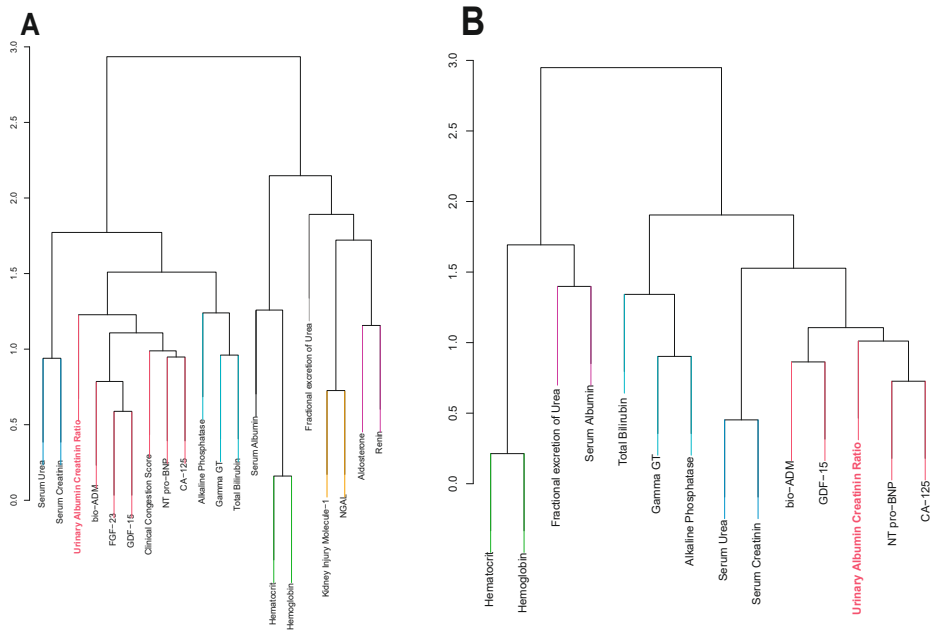


Figure 1 | Dendrograms displaying hierarchical clustering based on Euclidean distance for the index cohort (A) and the validation cohort (B)

Cox regression analysis

Kaplan Meier survival curves showed that the presence of any albuminuria was associated with a higher risk of mortality and heart failure (re)hospitalisation (index cohort: Figure 2a and validation cohort Figure 2b, log-rank P for both <0.001). Multivariable Cox regression analyses showed that log UACR was independently associated with mortality in both the index and validation cohort and was independently associated with the combined endpoint of death or HF hospitalisation in the validation cohort (Table 4).

Table 4 | Cox regression analysis

	Index cohort			Validation cohort		
2-year mortality						
	Hazard ratio	**(95% confidence interval)**	**p-value**	**Hazard ratio**	**(95% confidence interval)**	**p-value**
Univariate	1.148	1.104 – 1.193	<0.001	1.330	1.257 - 1.407	<0.001
Serum Creatinine, Age, History of DM and NT pro-BNP	1.061	1.009 - 1.116	0.02	1.214	1.140 - 1.293	<0.001
BIOSTAT-CHF risk model**	1.059	1.012 - 1.107	0.013	1.134	1.066 – 1.206	<0.001
Combined endpoint of HF hospitalisation or mortality						
	Hazard ratio	**(95% confidence interval)**	**p-value**	**Hazard ratio**	**(95% confidence interval)**	**p-value**
Univariate	1.118	1.084 - 1.153	<0.001	1.296	1.237 - 1.359	<0.001
Serum Creatinine, Age, History of DM and NT pro-BNP	1.037	0.999 - 1.079	0.066	1.198	1.138 - 1.262	<0.001
BIOSTAT-CHF risk model**	1.031	0.995 – 1.067	0.091	1.201	1.143 - 1.263	<0.001

*Variables included in the BIOSTAT risk model for 2-year mortality: Age, blood urea nitrogen, NT pro-BNP, haemoglobin, and failure to prescribe a beta-blocker.
**Variables included in the BIOSTAT risk model for the combined endpoint of HF hospitalisation or mortality: age, previous HF hospitalisation, peripheral oedema, systolic blood pressure, NT pro-BNP, haemoglobin, high-density lipoprotein, sodium, and use of beta-blockers
Abbreviations: DM: diabetes mellitus; NT pro-BNP: N-terminal pro–B-type natriuretic peptide

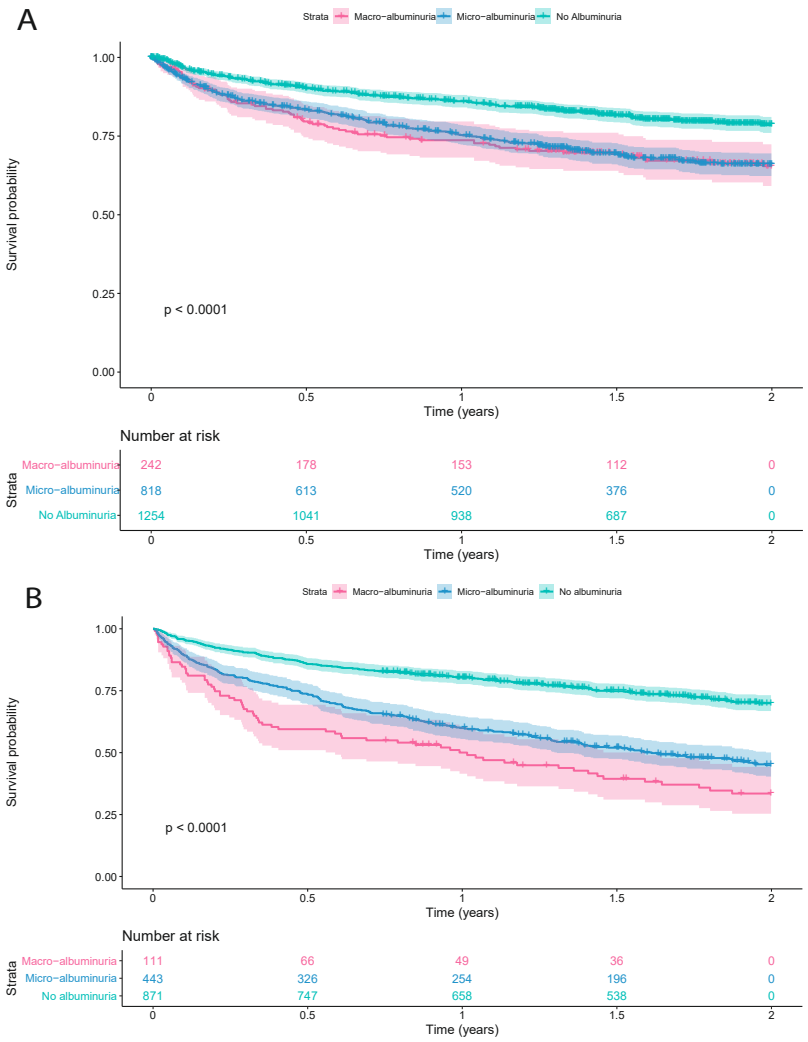


Figure 2 | Survival analyses, proportion free from mortality or heart failure hospitalisation, for the index cohort (A) and the validation cohort (B)

To account for potential differences in biology between patients with HFpEF or HFrEF an interaction term was added to the cox regression model. There however was no significant interaction on outcome (index: likelihood ratio rest P-value = 0.452, P-value = 0.2316 for mortality and the combined endpoint respectively; validation: likelihood ratio rest P-value = 0.978, P-value = 0.5455 for mortality and the combined endpoint respectively).

Discussion

The present study shows that patients with heart failure who had albuminuria showed more signs and symptoms of (systemic) congestion at baseline, compared with those who did not have albuminuria. Even after adjustment for several kidney markers, such as urinary neutrophil gelatinase-associated lipocalin (NGAL) and kidney injury marker-1 (KIM-1), the strongest association with log UACR was found for plasma NT pro-BNP, in multivariable regression analysis. In addition, the correlation between NT pro-BNP and UACR was independent of glomerular filtration and remained present across all NYHA functional classes. Other markers and clinical parameters reflective of congestion, such as bio-ADM and peripheral oedema, were also associated with a higher UACR. Lastly, in hierarchical cluster analysis UACR clustered with established and novel markers of congestion, as well as with the clinical congestion score, rather than with glomerular and tubular markers such as creatinine, NGAL, and KIM-1. Taken together, these findings suggest that in patients with heart failure the extent of albuminuria is more related to severity of congestion than to markers of intrinsic renal disease.

Congestion

Although albuminuria is not yet an established marker of congestion in heart failure, there are several lines of evidence that support a connection between congestion and albuminuria. Firstly, ligation of the renal vein in healthy dogs resulted in albuminuria in the congested kidney after pressures of 18 mmHg were attained, while the non-congested control kidneys did not show albuminuria.[18] Central venous pressures (and with it renal venous pressure) of more than 18 mmHg are not uncommon in acute heart failure.[19, 20] Importantly, after the ligation was lifted, proteinuria quickly recovered.[18] Secondly, in patients admitted for acute decompensated heart failure, the incidence of albuminuria significantly decreased after seven days of diuretic treatment, indicating an effect of congestion relief (or reduction of central venous pressure) on albumin excretion.[21] Thirdly, in patients with the so-called 'nutcracker' syndrome, the left renal vein gets squeezed between the aorta and the superior mesenteric artery. This syndrome is typically associated with albuminuria, among other urological symptoms.[22] Similar to the nutcracker syndrome, in patients with renal vein thrombosis giving rise to occlusion of the renal vein, albuminuria is common, can be severe and is reversible after the occlusion is lifted.[23] Finally, in adult patients with congenital heart disease associated with higher (central and pulmonary) venous pressure (single ventricle Fontan, systemic right ventricle, and Eisenmenger syndrome) a higher prevalence of albuminuria is found as well. In contrast, no increase in albuminuria is found in congenital abnormalities without increased right-sided pressures, such as an aortic coarctation.[24] In addition, pulmonary hypertension is a common complication of sickle cell disease. In this patient category the presence of albuminuria has a positive predictive value of 60% for

concomitant pulmonary hypertension, further solidifying the association between increased right sides pressures and albuminuria.[25]

The association between albuminuria and (central) venous pressure is further supported by available echocardiographic data. In the validation cohort echo parameters related to right-sided pressure, namely vena cava inferior diameters and tricuspid regurgitation gradient, were significantly increased in patients with albuminuria, indicating increased pulmonary and central venous pressures. In logistic regression, the gradient over the tricuspid valve remained independently associated with the presence of albuminuria.

After NT pro-BNP, the second strongest association with log UACR in multivariable linear regression was found for urinary KIM-1. Several animal studies have shown that clipping of the renal vein leads to increased renal interstitial pressures, with one renal congestion model in mice showing increased tubular expression of KIM-1 in the affected kidney.[18, 26, 27] Moreover, clipping of the vein leads to albuminuria and microscopic destruction of podocytes in these mice[27], indicating congestion-induced damage to the glomerular membrane as well as the tubules. Importantly, our data only show correlations between congestion markers, KIM-1 and albuminuria in heart failure and therefore causality cannot be assessed.

One surprising finding is the fact that renin concentration was negatively associated with log UACR. A possible explanation for this finding could be that those with micro- or macro-albuminuria were less likely to be on ACE-i/ARB and MRA, giving rise to lower renin concentrations. This finding is further supported by the fact that ACE-i use was associated with a lower OR for UACR in logistic regression. Our findings are especially interesting in light of the fact that sacubitril/valsartan is associated with a higher incidence of albuminuria, despite a reduction in NT pro-BNP and a slower decline in eGFR, in both HFrEF and HFpEF.[28, 29] Importantly however, no patients in BIOSTAT-CHF were using sacubitril/valsartan, so an influence of this drug on albuminuria could not be tested in our data.

Endothelial dysfunction

Another possible underlying mechanism explaining the relation between albuminuria and congestion could be related to endothelial dysfunction. Dysfunctional endothelial cells are associated with many cardiovascular, metabolic and renal diseases.[30] The main symptoms of endothelial dysfunction are impaired nitric oxide production and increased vascular permeability. Impaired endothelial function in the kidneys might therefore result in more albuminuria.[31] Endothelial dysfunction would also explain the

correlations found with congestion, as the threshold for congestion is lowered in the case of endothelial dysfunction.[32] Of particular interest is the function of the glycocalyx, which is situated on the outer border of the endothelium in both the peripheral vasculature and in the glomerulus. The glycocalyx transduces stress from plasma flow, which releases mediators that in the glomerulus control podocyte function and in the peripheral vasculature regulate permeability.[1, 30] It is postulated that high levels of sodium, resulting from sodium reabsorption in heart failure, structurally alter the glycocalyx, disrupting interstitial stability, which in turn diminishes interstitial protection from overt fluid overload.[33] A similar pathophysiological mechanism seems plausible for the glomerular glycocalyx.[10] In patients with sickle cell disease and pulmonary hypertension the vascular endothelial growth factor soluble fms-like tyrosine kinase-1 has been suggested to be the missing link responsible for endothelial dysfunction and thus albuminuria.[34] Endothelial dysfunction might therefore be an important link between congestion and albuminuria in heart failure. It is however important to acknowledge that the endothelium shows remarkable heterogeneity throughout the body. Endothelial phenomena observed in one part of the body may therefore not be easily transferable to other parts of the body.

Limitations

Limitations of this study include the post-hoc design. Our findings are based on associations; a direct effect of central venous pressure or intrarenal congestion could not be proven. Moreover, an association with low cardiac output, the other main hallmark of heart failure aside from congestion, could not be proven as we did no measure this. However, as albuminuria was associated with a higher systolic blood pressure, rather than a lower systolic blood pressure, this association seems less likely. Similarly, it is technically possible that the inverse is true, meaning albuminuria leads to congestion, through serum hypalbuminaemia. However, hypalbuminaemia in the index cohort was very mild and not present at all in the validation cohort. Moreover, serum albumin was not associated with albuminuria in multivariable regression analyses, making it an unlikely contributor.

Additionally, no data on timing of loop diuretic administration is present. We can therefore not exclude an effect of recent administration of loop diuretics, leading to RAAS activation as a driver of albuminuria, although the total daily dose of loop diuretics was not independently associated with log UACR. Urinary albumin was only available at baseline, so no information on treatment effect or course over time could be provided.

Another important limitation is the fact that our findings cannot easily be extrapolated to the current ESC HF guideline recommendations of optimal medical treatment [13], as no patients in BIOSTAT-CHF used sacubitril/valsartan or an sodium-glucose-cotransporter2 inhibitor and less than half used an MRA. The current findings should therefore be confirmed in contemporary databases.

While we did not find differences in independent assocations of albuminuria in HFpEF and HFrEF, it is important to note that the subgroup of patients with HFpEF in both BIOSTAT-CHF cohorts is limited in number. The overall findings in the entire cohorts are therefore likely driven by patients with HFrEF.

Lastly, as BIOSTAT-CHF did not exclude patients based on comorbidities, our results could still be driven mainly by concomitant comorbidities, such as diabetes and hypertension.

Conclusion

In patients with new-onset or worsening heart failure, albuminuria was consistently related to clinical, echocardiographic, and circulating biomarkers of congestion.

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Supplementary Material

Supplementary table 1: Univariable linear regression for log(UACR) from the index cohort

Variable	Adjusted R^2	P for R^2
Demographics		
Type of visit	0.007	<0.001
Hospitalisation		
Outpatient Visit		
Age (years)	0.014	<0.001
Sex (% Male)	0	0.146
Race	-0.001	0.735
Weight (kg)	0	0.57
BMI (kg/m2)	0	0.484
LVEF (%)	0.003	0.004
Systolic Blood Pressure (mmHg)	0.018	<0.001
Diastolic Blood Pressure (mmHg)	0.002	0.01
Heart Rate (beats/min)	0.009	<0.001
Duration of heart failure (years)	-0.002	0.419
NYHA class	0.025	<0.001
I		
II		
III		
IV		
Medication use		
ACE-inhibitors or ARB (%)	0.007	<0.001
Beta blockers (%)	0.002	0.021
Mineralocorticoid Receptor Antagonists (%)	0.009	<0.001
Digoxin (%)	0	0.488
Loop diuretic dose (furosemide or equivalent)	0.009	<0.001
Medical History		
Myocardial infarction (%)	0	0.847
Coronary artery bypass graft (%)	0	0.268
Valvular disease (%)	0	0.852
PCI (%)	0	0.667
Atrial fibrillation (%)	0.004	0.002
Stroke (%)	0.002	0.017
Peripheral Vascular Disease (%)	0.009	<0.001
Hypertension (%)	0.017	<0.001
Diabetes Mellitus (%)	0.031	<0.001
COPD (%)	0	0.587
Renal Disease (%)	0.024	<0.001
Treated thyroid disease (%)	-0.001	0.781
No		
Hypothyroidism		

Supplementary table 1: *(Continued)*

Variable	Adjusted R^2	P for R^2
Hyperthyroidism		
Current Malignancy (%)	0.001	0.044
Heart failure aetiology	0.014	<0.001
Cardiomyopathy		
Valvular Disease		
Hypertensive		
Other		
Unknown		
Clinical Profile		
Rales/crackles	0.014	<0.001
Pulmonary congestion > 1/3 up lung fields (%)	0.005	0.008
Peripheral oedema (%)	0.04	<0.001
Elevated JVP (%)	0.016	<0.001
Haepatomegaly (%)	0.006	<0.001
Third heart tone (%)	0	0.526
Orthopnoea (%)	0.008	<0.001
Dyspnoea VAS score	0.007	0.023
Chest X-ray		
Pulmonary oedema (%)	0.004	0.005
Upper lobe venous congestion (%)	0.01	<0.001
Cardiomegaly (defined as CTR > 0.5) (%)	0.012	<0.001
Laboratory values		
Haemoglobin (g/dL)**	0.013	<0.001
Log Haematocrit (%)	0.005	0.001
Serum Creatinine (µmol/L)	0.048	<0.001
eGFR (ml/min)**	0.031	<0.001
Log Urea (mmol/L)	0.021	<0.001
Log Fractional excretion of Urea	0.001	0.0845
Sodium (mmol/L)	0	0.353
Potassium (mmol/L)	0.002	0.015
Log NT pro-BNP (ng/L)	0.113	<0.001
Serum Albumin (mmol/L)	0.023	<0.001
ALAT (U/L)*	-0.001	0.68
Alkaline Phophatase (µg/L)*	0.01	<0.001
Gamma-GT (U/L)*	0.006	0.009
Total bilirubin*	0.002	0.084
Transferrin (g/L)	-0.002	0.419
Ferritin (pmol/L)	0.001	0.273
TSH (mU/L)	-0.001	0.971
Triglycerides (mmol/L)*	0.003	0.045

Supplementary table 1: *(Continued)*

Variable	Adjusted R^2	P for R^2
Total Cholesterol (mmol/L)*	0.01	<0.001
HDL (mmol/L)*	0.012	<0.001
LDL (mmol/L)*	0.008	0.003
Log bio-ADM (pg/mL)	0.072	<0.001
Troponin T (ug/L)	-0.002	0.861
Glucose (mmol/L)	0.031	<0.001
Log Aldosterone (pg/mL)	0.007	0.001
Log Renin (UI/mL)	0.011	<0.001
Log GDF-15 (pg/mL)	0.127	<0.001
Log FGF-23 (RU/ml)	0.099	<0.001
Log CA-125	0.037	<0.001
Log Urinary KIM-1 (µg/mL)	0.031	<0.001
Log Urinary NGAL (µg/mL)	0.011	<0.001
Log Plasma NGAL (ng/mL)	0.026	<0.001
Log Urinary osteopontin (µg/mL)	0	0.838
Log Urinary uromodulin (µg/mL)	0.018	<0.001

* Omitted from multivariable model due to >1/3 missingness, **omitted from multivariable model due to collinearity with another variable.

Abbreviations: BMI: body mass index; LVEF: left ventricular ejection fraction; NYHA: New York Heart Association; ACE: angiotensin converter enzyme; ARB: angiotensin receptor blocker; PCI: percutaneous coronary intervention; COPD: chronic obstructive pulmonary disease; VAS: visual analogue scale; CTR: cor-to-thorax ratio; eGFR; estimated glomerular filtration rate; NT pro-BNP: N-terminal pro-B type natriuretic peptide; ALAT: alanine transferase; gamma-GT: gamma-glutamyltransferase; TSH: thyroid stimulating hormone; HDL: high density lipoprotein; LDL; low density lipoprotein; bio-ADM: biologically active adrenomedullin; GDF-15: Growth differentiation factor 15; FGF-23: Fibroblast growth factor 23; CA-125: cancer antigen 125; KIM-1: kidney injury marker-1; NGAL: Neutrophil gelatinase-associated lipocalin.

Supplementary table 2a |

NYHA I/II index				
Variable	**Standardised Beta**	**95% CI**	**T-value**	**P-value**
log NT pro-BNP	0.406	(0.27-0.54)	5.87	<0.001
History of diabetes	0.748	(0.47-1.03)	5.202	<0.001
log Renin	-0.291	(-0.42--0.16)	-4.356	<0.001
log Bio-ADM	0.331	(0.16-0.5)	3.872	<0.001
log Urinary NGAL	0.162	(0.04-0.29)	2.562	0.011
log Creatinine	0.129	(0-0.26)	1.964	0.05
N = 710				
Adj R2 = 0,1779				
NYHA III index				
Variable	**Standardised Beta**	**95% CI**	**T-value**	**P-value**
log NT pro-BNP	0.489	(0.35-0.63)	6.707	<0.001
log Urinary KIM1	0.396	(0.27-0.53)	6.025	<0.001
log UREA	0.365	(0.21-0.52)	4.526	<0.001
Systolic Blood Pressure	0.272	(0.14-0.4)	4.122	<0.001
History of diabetes	0.508	(0.26-0.76)	3.991	<0.001
log Fractional Excretion of Urea	0.317	(0.15-0.48)	3.818	<0.001
Peripheral edema above knees	0.692	(0.25-1.14)	3.066	0.002
log Renin	-0.257	(-0.39--0.13)	-3.876	<0.001
log Urinary Uromodulin	-0.191	(-0.32--0.07)	-3.004	0.003
log Bio-ADM	0.182	(0.05-0.31)	2.795	0.005
N = 661				
Adj R2 = 0,3009				
NYHA IV index				
Variable	**Standardised Beta**	**95% CI**	**T-value**	**P-value**
log Glucose	0.428	(0.19-0.67)	3.518	0.001
log NT pro-BNP	0.483	(0.21-0.76)	3.482	0.001
History of hypertension	0.713	(0.24-1.18)	3	0.003
log Urinary KIM-1	0.397	(0.13-0.66)	2.977	0.003
Jugular venous distention	0.628	(0.15-1.1)	2.609	0.01
log Creatinine	-0.372	(-0.66--0.08)	-2.55	0.012
History of renal disease	0.615	(0.03-1.2)	2.091	0.038
ACE-inhibitor use	-0.512	(-0.99--0.03)	-2.115	0.036
N = 153				
Adj R2 = 0,269				

Supplementary table 2b |

NYHA I/II Validation				
Variable	**Standardised Beta**	**95% CI**	**T-value**	**P-value**
log NT pro-BNP	0.633	(0.5-0.76)	9.701	<0.001
History of diabetes	0.514	(0.27-0.76)	4.182	<0.001
log bilirubin	0.214	(0.09-0.34)	3.365	0.001
History of hypertension	0.361	(0.14-0.58)	3.208	0.001
History of peripheral artery disease	0.367	(0.09-0.65)	2.557	0.011
N = 595				
R^2 = 0.213				
NYHA III validation				
Variable	**Standardised Beta**	**95% CI**	**T-value**	**P-value**
log NT pro-BNP	0.7	(0.57-0.83)	10.669	<0.001
Systolic brood pressure	0.457	(0.33-0.59)	6.994	<0.001
log bio-ADM	0.286	(0.15-0.42)	4.219	<0.001
Age	-0.148	(-0.28--0.02)	-2.219	0.027
Jugular venous distention	-0.271	(-0.54-0)	-1.99	0.047
N = 531				
R^2 = 0.254				
NYHA IV validation				
Variable	**Standardised Beta**	**95% CI**	**T-value**	**P-value**
log NT pro-BNP	0.518	(0.27-0.76)	4.133	<0.001
Systolic Blood Pressure	0.371	(0.18-0.57)	3.741	<0.001
History of diabetes	0.493	(0.04-0.94)	2.167	0.032
log Serum creatinine	0.221	(0-0.44)	1.996	0.047
Heart rate	0.215	(0-0.43)	1.975	0.05
N = 186				
R^2 = 0.228				

Supplementary table 3a | comparison of variable selection using LASSO and stepwise backwards selection for the entire cohort:

Variable	Index Cohort		Validation Cohort	
	Backward stepwise	LASSO >15% miss excluded (lambda 1se)	Backward stepwise	LASSO >15% miss excluded (lambda 1se)
log NT pro-BNP	x	x	x	x
log Urinary KIM-1	x	x		
Log Plasma Urea	x			
FE of Urea	x			
Hx of Diabetes Mellitus	x	x	x	x
Systolic Blood Pressure	x	x	x	x
log bio-ADM	x	x	x	x
log Renin	x	x		
Log Urinary NGAL	x	x		
Creatinine		x	x	
Log GDF-15		x		x
Hypertension		x		
Log UroM		x		
FGF-23		x		
y-GT			x	x
VAS			x	X
BMI			x	
Heart Rate			x	
Oedema			x	x
NYHA class IV				x
CA-125				x

Supplementary table 3b | comparison of variable selection using LASSO and stepwise backwards selection for the HFrEF (LVEF <40%) subgroup:

Variable	Index HFrEF		Validation HFrEF	
	Stepwise backwards	LASSO >15% miss excluded (lambda 1se)	Stepwise backwards	LASSO >15% miss excluded (lambda 1se)
Log NT pro-BNP	x	x	x	x
Log plasma Urea	x			x
Log urinary KIM-1	x	x		
Log bio-ADM	x	x	x	
Systolic blood pressure	x	x	x	
History of Diabetes Mellitus	x	x	x	x
Log Renin	x	x		
Log Urinary NGAL	x	x		
Log fractional excretion of urea	x			
History of hypertension		x		x
Plasma Glucose	x			
FGF-23		x		
Log GDF-15		x		
Log UROM	x	x		
y-GT			x	
VAS dyspnoea score			x	
Peripheral oedema above knee				x
NYHA IV				x
Sodium				x
CA-125				x
Bilirubin			x	x

Supplementary table 3c | comparison of variable selection using LASSO and stepwise backwards selection for the HFpEF (LVEF ≥50%) subgroup:

Variable	Index		Validation	
	Stepwise backwards selection	Index Lasso >15% miss excluded (lambda 1se)	Stepwise backwards selection	Validation Lasso >15% miss excluded (lambda 1se)
log NT pro-BNP	x	x	x	x
log KIM-1	x			
Hx of Diabetes Mellitus	x		x	
log Renin	x			
Creatinine	x			
log Uromodulin	x	x		
Periperal edema	x			
Betablocker use	x			
Renal disease		x		
Systolic Blood Pressure			x	x
Heart Rate				x
VAS				x
HDL				x
Bio-ADM			x	x
Glucose				x
CA-125	X			x

Supplementary Table 4a | Multivariable regression analysis using LASSO variable selection, index cohort:

Variable	Standardised regression coefficient (95% CI)	T-value	P-value
log NT pro BNP	0.34 (0.25-0.43)	7.274	<0.001
log GDF-15	0.325 (0.22-0.43)	6.186	<0.001
Systolic Blood Pressure	0.242 (0.16-0.32)	5.97	<0.001
log Urinary KIM-1	0.246 (0.16-0.33)	5.673	<0.001
History of Diabetes Mellitus	0.457 (0.29-0.62)	5.528	<0.001
log plasma Renin	-0.203 (-0.28--0.12)	-4.925	<0.001
log Bio-ADM	0.207 (0.12-0.29)	4.627	<0.001
log Serum Creatinine	0.087 (0.01-0.17)	2.116	0.034
history of hypertension	0.166 (0.01-0.32)	2.053	0.04
Log urinary NGAL	0.081 (0-0.16)	1.919	0.055
FGF-23	0.004 (-0.08-0.08)	0.088	0.93

Supplementary Table 4b | Multivariable regression analysis using LASSO variable selection, validation cohort:

Variable	Standardised regression coefficient (95% CI)	T-value	P-value
Systolic blood pressure	0.405 (0.32-0.49)	9.606	<0.001
log NT pro-BNP	0.473 (0.37-0.58)	8.864	<0.001
History of Diabetes Mellitus	0.428 (0.25-0.61)	4.645	<0.001
log Gamma-GT	0.156 (0.07-0.24)	3.494	<0.001
VAS Dyspnoea score	0.125 (0.03-0.22)	2.669	0.008
log GDF-15	0.118 (0.01-0.22)	2.189	0.029
log bio-ADM	0.108 (0.01-0.21)	2.065	0.039
log CA-125	0.108 (0-0.21)	2.047	0.041
Peripheral oedema above knees	0.328 (-0.06-0.72)	1.641	0.101
NYHA class IV	-0.057 (-0.31-0.2)	-0.439	0.661

Supplementary Table 4c | Multivariable regression analysis using LASSO variable selection, subgroup of patients with HFrEF (LVEF <40%):

Variable	Index Cohort			Validation Cohort		
	Standardised regression coefficient (95% CI)	T-value	P-value	Standardised regression coefficient (95% CI)	T-value	P-value
log NT pro-BNP	0.361 (0.25-0.47)	6.535	<0.001	0.487 (0.32-0.66)	5.59	<0.001
log GDF-15	0.346 (0.23-0.47)	5.686	<0.001			
Systolic Blood Pressure	0.256 (0.16-0.35)	5.145	<0.001			
log Urinary KIM-1	0.254 (0.15-0.36)	4.801	<0.001			
log bio-ADM	0.216 (0.11-0.32)	3.964	<0.001			
History of Diabetes Mellitus	0.36 (0.17-0.55)	3.678	<0.001	0.525 (0.22-0.83)	3.369	0.001
log Urinary Uromodulin	-0.17 (-0.26--0.08)	-3.594	<0.001			
log Renin	-0.138 (-0.23--0.04)	-2.837	0.005			
History of Hypertension	0.2 (0.02-0.38)	2.145	0.032	0.451 (0.18-0.73)	3.217	0.001
log Urinary NGAL	0.077 (-0.02-0.17)	1.539	0.124			
FGF-23	-0.02 (-0.11-0.06)	-0.471	0.637			
log Biliurubin				0.16 (0.01-0.31)	2.148	0.032
Serum Sodium				0.144 (0.01-0.28)	2.076	0.039
log CA125				0.14 (-0.02-0.3)	1.719	0.086
log Urea				0.102 (-0.04-0.25)	1.405	0.161
NYHA IV				-0.177 (-0.61-0.25)	-0.815	0.416
Peripheral oedema above knees				0.011 (-0.69-0.71)	0.03	0.976

Supplementary Table 4d | Multivariable regression analysis using LASSO variable selection, subgroup of patients with HFpEF (LVEF ≥50%):

Variable	Index Cohort			Validation Cohort		
	Standardised regression coefficient (95% CI)	T-value	P-value	Standardised regression coefficient (95% CI)	T-value	P-value
Log NT pro-BNP	0.755 (0.36-1.15)	3.799	<0.001	0.622 (0.39-0.86)	5.247	<0.001
History of renal disease	0.967 (0.3-1.63)	2.876	0.005			
log Urinary Uromodulin	-0.278 (-0.58-0.02)	-1.843	0.068			
log Glucose				0.363 (0.2-0.52)	4.514	<0.001
Systolic Blood Pressure				0.333 (0.15-0.51)	3.626	<0.001
HDL-cholesterol				0.323 (0.15-0.5)	3.612	<0.001
VAS Dyspnoea score				0.192 (0.01-0.38)	2.048	0.042
Heart Rate				0.148 (-0.05-0.35)	1.462	0.145
log bio-ADM				0.14 (-0.06-0.34)	1.409	0.16
log CA-125				0.141 (-0.09-0.37)	1.219	0.224

Supplementary Table 5 |

Index (N = 614)				Validation (N = 417)		
History of diabetes = YES						
Variable	**Standardised regression coefficient (95% CI)**	**T value**	**p-value**	**Standardised regression coefficient 95% CI**	**T value**	**p-value**
log NT pro-BNP	0.451 (0.28-0.62)	5.285	<0.001	0.548 (0.39-0.71)	6.68	<0.001
Systolic Blood Pressure	0.434 (0.27-0.6)	5.187	<0.001	0.55 (0.41-0.69)	7.508	<0.001
log Renin	-0.328 (-0.49--0.17)	-3.991	<0.001			
Bibasalar Rales				0.545 (0.23-0.85)	3.453	0.001
History of Atrial Fibrillation				0.483 (0.19-0.77)	3.28	0.001
log Serum Creatinin				0.253 (0.1-0.41)	3.18	0.002
VAS dyspnoea scale				0.236 (0.08-0.39)	3.058	0.002
Age				-0.25 (-0.43--0.07)	-2.727	0.007
log Urinary NGAL	0.205 (0.06-0.35)	2.843	0.005			
log Plasma FGF-23	0.256 (0.07-0.44)	2.704	0.007			
log ASAT				-0.281 (-0.51--0.06)	-2.452	0.015
log bio-ADM	0.196 (0.02-0.37)	2.157	0.031			
	Adjusted R^2 = 0.204			*Adjusted R^2 = 0.323*		
History of diabetes = NO						
Index (N = 924)				Validation (N = 826)		
Variable	**Standardised regression coefficient (95% CI)**	**T value**	**p-value**	**Standardised regression coefficient 95% CI**	**T value**	**p-value**
log NT pro-BNP	0.463 (0.35-0.58)	8.121	<0.001	0.513 (0.4-0.62)	9.24	<0.001
Systolic Blood Pressure				0.336 (0.24-0.43)	6.968	<0.001
log bio-ADM	0.283 (0.17-0.39)	5.012	<0.001			
History of hypertension	0.421 (0.22-0.62)	4.098	<0.001			

Supplementary Table 5 *(Continued)*

Index (N = 614)				Validation (N = 417)		
log Bilirubin				0.179 (0.08-0.28)	3.566	<0.001
Serum Albumin	-0.163 (-0.27--0.06)	-3.1	0.002			
VAS dyspnoea scale				0.152 (0.06-0.25)	3.087	0.002
Heart Rate				0.144 (0.05-0.24)	2.924	0.004
Peripheral oedema above knees				0.554 (0.14-0.97)	2.616	0.009
History of malignancy				0.582 (0.13-1.03)	2.537	0.011
Body Mass Index				-0.134 (-0.24--0.03)	-2.481	0.013
Jugular venous distention	0.249 (0.02-0.48)	2.153	0.032			
	Adjusted R^2 = 0.181			*Adjusted R^2 = 0.248*		

Supplementary table 6 | Multivariable logistic regression for any albuminuria

	Index			Validation		
	OR	95% CI	P-value	OR	95% CI	P-value
NT pro BNP *(per 1000 units increase)*	1.091	1.066 - 1.117	<0.001	1.103	1.055 - 1.160	<0.001
History of diabetes	1.575	1.266 - 1.961	<0.001	1.874	1.274 - 2.767	0.002
History of hypertension				1.898	1.301 - 2.787	<0.001
Bio-ADM *(per 10 units increase)*	1.035	1.013 - 1.060	0.003			
GDF-15 *(per 1000 units increase)*	1.009	1.005 - 1.013	<0.001			
Systolic blood pressure *(per 10 units increase)*	1.146	1.093 - 1.204	<0.001			
ACE- inhibitor use	0.783	0.623 - 0.986	0.037			
Aldosterone *(per 10 units increase)*	0.990	0.985 - 0.996	<0.001			
History of atrial fibrillation	1.257	1.024 - 1.544	0.029			
NYHA class IV	1.596	1.166 - 2.193	0.004			
Bilirubin *(per 5 units increase)*				1.183	1.063 - 1.323	0.003
Peripheral edema above knee				2.944	1.339 - 6.781	0.009
Body mass index				0.965	0.936 - 0.995	0.026
Tricuspid regurgitation rate *(per 5 units increase)*				1.079	1.009 - 1.158	0.029
Age *(per 10 units increase)*				1.226	1.015 - 1.489	0.037
Heart rate				1.011	1.000 - 1.021	0.043

Abbreviations: NT pro-BNP: N-terminal pro-B-type natriuretic peptide; bio-ADM: biologically active adrenomedullin; GDF-15: growth differentiation factor-15; ACE: angiotensin converter enzyme; NYHA: New York Heart Association;

Part 2 Renal Compression Syndrome

Chapter 7

Renal Compression in Heart Failure – the Renal Tamponade Hypothesis

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Abstract

Renal dysfunction is one of the strongest predictors of outcome in heart failure. Several studies have revealed that both reduced perfusion and increased congestion (and central venous pressure) contribute to worsening renal function in heart failure. The current *Viewpoint* proposes a novel factor in the link between cardiac and renal dysfunction: "renal tamponade", or compression of renal structures due to limited space for expansion. This space can either be limited by the rigid renal capsule that encloses the renal interstitial tissue, or by the layer of fat around the kidneys or by the peritoneal space exerting pressure on the retroperitoneal kidneys. Renal decapsulation in animal models of heart failure and acute renal ischaemia has been shown effective in alleviating pressure related injury within the kidney itself, thus supporting this concept, and making it a potentially interesting novel treatment in heart failure.

Key points

- Renal dysfunction in heart failure remains highly prevalent and is associated with worse outcomes
- The rigidness of the renal capsule is central in congestion-induced damage to the renal structures
- Renal decapsulation has been shown beneficial in animals, making it an interesting, novel treatment to investigate in heart failure

Introduction

The kidney plays a central role in heart failure (HF), and several large studies have shown that renal dysfunction is one of the most powerful predictors of outcome in HF.[1, 2] Already in 1990 it was demonstrated that the kidney is very sensitive to changes in perfusion. When cardiac index decreases by 25%, renal blood flow decreases by as much as 50%. [3] Later, several groups showed that reduced renal perfusion is among the strongest determinants of glomerular filtration rate in HF. [4-6] Further studies in this field revealed that not only reduced renal perfusion, but also –importantly- increased central venous pressure contribute to a decrease in renal function. [7, 8] Ever since, several studies have investigated associations between clinical factors and renal congestion. Yet, the area of cardiorenal interplay remains complex.

In the present *Viewpoint*, we discuss the three mechanisms that individually or combined, may lead to renal congestion as a result of intrarenal or extrarenal compression. Another (classic) example of renal injury as a result of compression can be found in the Page kidney; a clinical syndrome of diminished renal function and/or hypertension as a result of external compression on the kidney, most commonly subcapsular hematoma.[9] The fact that the kidney is surrounded by a rigid and non-expandable capsule plays a crucial role in the Page kidney, as well as in renal compression in heart failure. [10, 11] We will discuss these mechanisms, and will coin a novel term for this: "renal tamponade" hypothesis.

Anatomy of the kidney

The kidneys are located in the retroperitoneal space. The area surrounding the kidney is called the perirenal space (*Figure 1, no 5*). This space is enclosed by the renal fascia (*Fig. 1, no. 7*). The perirenal space consists mainly of adipose tissue, but also the renal vasculature, lymphatic system and adrenal gland can be found within the perirenal space. The kidney itself is captured by a thick fibrous capsule that serves as protection to the soft renal tissue (*Fig. 1, no. 6*). Of note, the capsule does not cover the renal sinus; leaving an opening for the renal artery, renal vein, renal nerve and renal pelvis to either enter or exit. [12] Within the kidney, the glomeruli are located in the cortex, while the tubules descend into the medulla.

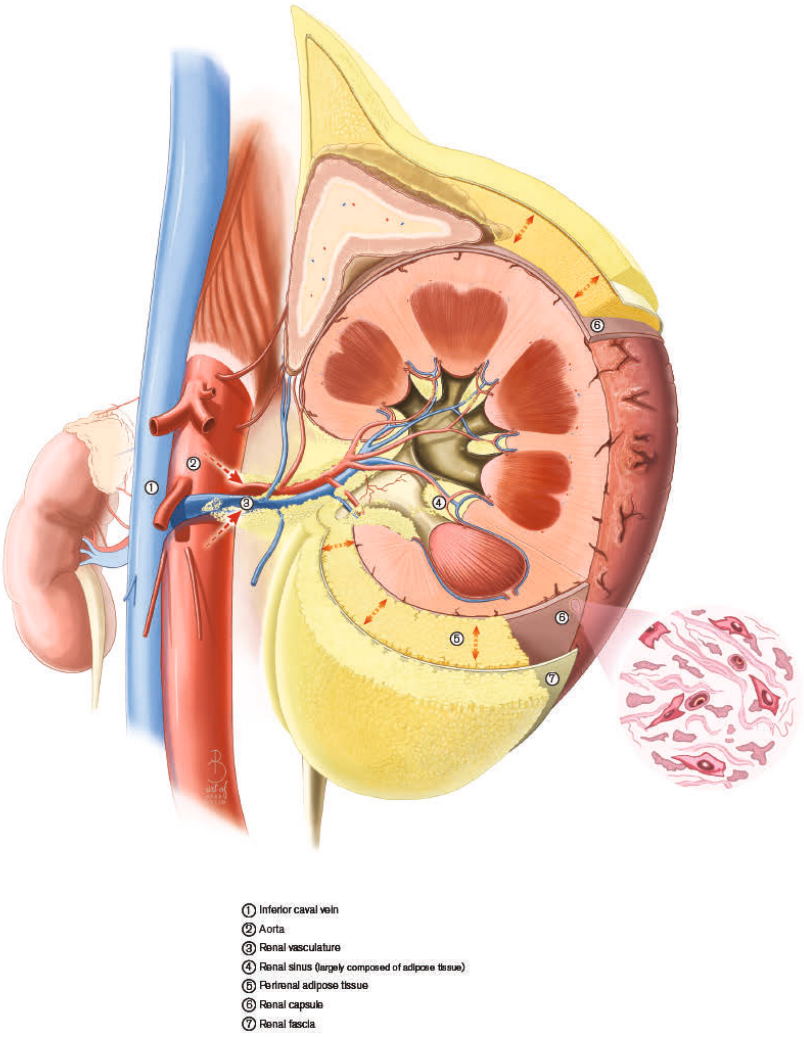

Figure 1: | Anatomy of the kidney and the perirenal space. The kidney is surrounded by the renal capsule. Histologically this capsule is made of dense irregular collagen tissue, making it particularly rigid. Around the renal capsule the perirenal space is bordered by the renal fascia and is made up of mainly adipose tissue. The renal sinus (4) is not covered by renal capsule and is therefore sensitive for local fat infiltration and compression of vasculature.

Compression of the kidney and renal vasculature can therefore theoretically arise from increased pressures in 3 different compartments, an overview of the current data on increased pressure in the different compartments can be found in Table 1.

Table 1 | summary of historic findings regarding the renal tamponade

Increased intracapsular pressure Preclinical studies*	Type of animal (N)	Type of intervention	Outcome(s)
Wegria (1955)	Mongrel dogs	Clamping of the renal vein	• Proteinuria after renal venous pressure >250 mmH2O
Stone (1977)	Monkeys (12)	Renal ischemia induced by clamping of the suprarenal aorta, unilateral renal decapsulation	• Preservation of creatinine and urea clearance in the decapsulated kidney
Burnett (1980)	Mongrel dogs (10)	Clamping of the renal vein	• Increased renal interstitial pressure • Decreased FeNa during volume expansion
Khraibi (1977)	Wister rats (19)	Renal ischemia induced by clamping of the suprarenal aorta, unilateral renal decapsulation, acute volume expansion	• Attenuation of renal interstitial pressures after volume expansion in decapsulated kidney
García-Estañ (1990)	Sprague-Dawley rats (27)	Renal decapsulation and infusion of atrial natriuretic peptide	• Increase in interstitial medullary pressure after ANP infusion • Normalization of cortical interstitial pressure after ANP infusion and decapsulation
Komuro (2017)	Wister rats (9)	Saline administration until CVP of 10 and 15 mmHg were attained	• Correlation between CVP and renal interstitial pressure (r=0.95) • Reduced medullary perfusion, as assessed with contrast enhanced ultrasonography
Shimada (2018)	Sprague-Dawley rats (10)	Clamping of the renal vein Renal decapsulation	• Increased renal interstitial pressure • Proteinuria • Increased expression of markers of tubular d amage • Decreased GFR and urine production • Attenuation of the above after decapsulation

Table 1 *(Continued)*

Increased intracapsular pressure			
Preclinical studies*	**Type of animal (N)**	**Type of intervention**	**Outcome(s)**
Cruces (2018)	Piglets (18)	Ischemia-reperfusion with or without renal decapsulation vs. sham procedure	• Lower intrarenal pressure after renal decapsulation, as compared to ischemia without decapsulation and/or sham procedure • Lower renal lactate release after renal decapsulation, as compared to ischemia without decapsulation and/or sham procedure
Clinical studies	**Study population (N)**	**Type of intervention**	**Outcome(s)**
Stone (1977)	Hemorrhagic shock patients with acute tubular necrosis (21)	Unilateral renal capsular incision	• Greater renal plasma flow on the decapsulated side • Greater urine flow on the decapsulated side • Reduced incidence of anuria/oliguria as compared to anticipated incidence (7 vs 75%)
Nijst (2017)	• Euvolemic HFrEF (40) • Euvolemic HFpEF (40)	• Infusion of 1L hydroxyethyl starch 6% • Intravenous bolus of loop diuretic	• Blunting of intrarenal venous flow patterns after volume expansion • Return to baseline after loop diuretic administration
De la Espriella-Juan (2018)	Acute HF (1)	Aggressive decongestive treatment	• Normalization of renal venous flow patterns
Ter Maaten (2021)	Acute HF (15)	Standard of care decongestive treatment	• Normalization of renal venous flow patterns
Increased perirenal pressure			
Preclinical studies	**Type of animal (N)**	**Type of intervention**	**Outcome(s)**
Hou (2014)	Wistar rats (10)	High fat vs. normal diet	• PRAT was associated with infrarenal aortic endothelial dysfunction • PRAT was associated with albuminuria in obese rats

Table 1 *(Continued)*

Increased intracapsular pressure			
Preclinical studies*	**Type of animal (N)**	**Type of intervention**	**Outcome(s)**
Ma (2016)	Pigs (14)	High fat/high fructose vs standard diet	• PRAT of obese pigs vasodilation of the renal artery was impaored. This was restored after TNF-a blockage.
Cops (2020)	Sprague-Dawley rats (16)	• Surgical wire constriction on IVC • Randomization to sedentary vs. moderately intense endurance exercise	• Endurance exercise lowered perirenal fat pad/ tibia length ratio, while body weight remained similar to sedentary rats • Abdominal pressure was lowered in the endurance group • Cystatine C was lower in the endurance group • No differences between other indices of kidney function could be found
Clinical studies	**Study population**	**Type of intervention/observation**	**Outcome(s)**
Lamacchia (2011)	Type 2 diabetes (151)	Quantification of peri-and pararenal fat thickness on ultrasound	• PRAT was independently associated with eGFR (β = – 0.327), after correction for WC and BMI.
Sun (2013)	• Obese, healthy volunteers (67) • Age –sex matched healthy lean volunteers (34)	Quantification of peri-and pararenal fat thickness on ultrasound	• PRAT was higher in obese patients with albuminuria than in obese patients without albuminuria
Geraci (2018)	Hypertension (269)	Quantification of peri-and pararenal fat thickness on ultrasound	• Correlation between PRAT and eGFR (r = -0.284) • In multivariable regression PRAT remained an independent predictor of eGFR, after correction for BMI and WC

Table 1 *(Continued)*

Increased intracapsular pressure			
Preclinical studies*	**Type of animal (N)**	**Type of intervention**	**Outcome(s)**
Ricci (2018)	Morbid obesity (284)	Sleeve gastrectomy	• PRAT was an independent predictor of systolic blood pressure in obese subjects (β = 0.160), after rigorous correction for known risk factors • PRAT significantly reduced after sleeve gastrectomy
D'Marco (2019)	Chronic kidney disease (class I-V) (103)	Perirenal fat between renal cortex and hepatic/ splenic border measurement on ultrasound	• PRAT was larger in patients with prediabetes, CKD stage 4 or 5 and higher triglyceride levels, but not in those with a history of hypertension
Koo (2020)	Community based cohort (3919)	Estimation of PRAT on CT	• PRAT was associated with renal (OR 2.05) and aortic (OR 1.11) atherosclerosis
Notohamiprodjo (2020)	• Healthy normoglycemic (230) • Pre-diabetes (87) • Type 2 diabetes (49)	Quantification of renal sinus fat volume on MRI	• Diabetes (β = 7.34) and pre-diabetes (β = 7.13) were significantly associated with more renal sinus fat, compared to normoglycemic controls.
Spit (2020)	Type 2 diabetes (51)	Quantification of renal sinus fat volume on MRI	• Correlation between renal sinus fat and GFR (r = -0.38) • Correlation between renal sinus fat and effective renal plasma flow (r = -0.38)
Fang (2020)	Type 2 diabetes (171)	Ultrasound assessment of PRAT diameter	• Correlation between PRAT and eGFR diameter (r = -0.181)
Increased intra-abdominal pressure*			
Preclinical studies	**Type of animal**	**Type of intervention**	**Outcome(s)**

Table 1 *(Continued)*

Increased intracapsular pressure			
Preclinical studies*	**Type of animal (N)**	**Type of intervention**	**Outcome(s)**
Harman (1982)	Mongrel dogs (7)	Insertion of inflatable bags in the peritoneum	• At 20 mmHg GFR decreased to <25% of baseline value • At 40 mmHg dogs became anuric and <7% of baseline GFR remained • CO also diminished to 37%, an effect which could be attenuated by infusion of fluids
Bishara (2011)	• Sprague-Dawley rats, aortocaval fistula, compensated (86) • Sprague-Dawley rats, aortocaval fistula, decompensated (6) • Sprague-Dawley rats, aortocaval fistula, dcompensated and pretreated with NOS inhibitor (6)	Pneumoperitoneum induced by injection of air through a needle	• After IAP >14mmHg GFR, urinary flow and sodium excretion decreased. • This effect was most pronounced in decompensated rats • Pre-treatment with NOS inhibitor exacerbated the decline in renal function

Table 1 *(Continued)*

Increased intracapsular pressure			
Preclinical studies*	**Type of animal (N)**	**Type of intervention**	**Outcome(s)**
Abu-Saleh (2019)	• Sprague-Dawley rats, sham operated (23) • Sprague-Dawley rats, aortocaval fistula, compensated (8) • Sprague-Dawley rats, aortocaval fistula, decompensated (9), and pretreated with tadalafil (6) • Sprague-Dawley rats, acute myocardial infarction (7), and pretreated with tadalafil (11) • Sprague-Dawley rats, sham treated with tadalafil (6)	Pneumoperitoneum induced by injection of air through a needle	• After IAP of 10 mmHg RPF, GFR, urinary flow and sodium excretion all diminish • This effect is most pronounced in decompensated ACF rats • This effect is ameliorated by pre-treatment with tadalafil • GFR recovered after IAP normalizes in all groups • In sham and MI rats natriuresis *increases* to above baseline level after IAP is normalized, in decompensated rats natriuresis remains low
Clinical studies	**Study population**	**Type of intervention/observation**	**Outcome(s)**
Sugerman (1988)	Morbidly obese (15)	• Measurement of abdominal pressure using urinary catheter manometer • Roux-en-Y gastric bypass	• Significant reduction of IAP 1 year after GBS
Cuda (2005)	Morbidly obese with proteinuria pre-operatively (1)	Roux-en-Y gastric bypass	• Normalization of proteinuria 1 year after GBS
Lambert (2005)	Morbidly obese (45)	• Measurement of abdominal pressure using urinary catheter manometer • Roux-en-Y gastric bypass	• Obese patients have a higher IAP compared to general population • IAP correlates to number of comorbidities • IAP is not reduced by incising the peritoneum • The first 2 days post-operatively IAP increases • No long-term data on IAP after GBS

Table 1 *(Continued)*

Increased intracapsular pressure			
Preclinical studies*	**Type of animal (N)**	**Type of intervention**	**Outcome(s)**
Mullens (2008)	Acute decompensated HF refractory to treatment (9)	Paracentesis (5) or ultrafiltration (4)	• After mechanical fluid removal IAP was significantly reduced (mean reduction 5 mmHg) • After mechanical fluid removal renal function improved (mean serum creatinine from 3.4 +/- 1.4 mg/dL to 2.4 +/- 1.1 mg/dL)
McIsaac (2019)	Morbidly obese (471)	Gastric bypass (sleeve gastrectomy or Roux-en-Y)	• In patients with microalbuminuria GFR increased from 109 ± 10 mL/min to 120 ± 36 mL/min 24 months after GBS • In patients with microalbuminuria albumin-creatinine-ratio normalized 24 months after GBS
Rubio-Gracia (2020)	Acute decompensated HF (43)	• Measurement of abdominal pressure using urinary catheter manometer • Standard of care decongestive treatment	• Higher baseline IAP was associated with poorer diuretic and natriuretic response • Higher baseline IAP was associated with higher serum creatinine • IAP >12 mmHg after 72 hours of decongestive treatment was associated with higher mortality and rehospitalization rates

*Studies published in any language other than English, or with no available abstract were omitted from inclusion in this table.
Abbreviations: mmH20: millimeters water pressure, FeNa: fractional excretion of sodium, (e)GFR: (estimated) glomerular filtration rate; ANP: atrial natriuretic peptide; PRAT: perirenal adipose tissue; TNF-a: tumor necrosis factor-alpha; WC: waist circumference; BMI: body mass index; CT: computed tomography; GBS: gastric bypass surgery

1- Increased intracapsular pressure (Figure 2, first panel.): Increased pressure within the renal parenchyma as a result of increased volume in the kidney caused by increased interstitial fluid in HF, in the context of an organ (the kidney) that cannot expand in volume.

Histologically, the fibrous renal capsule consists of many collagen fibers in a dense, irregular structure, making it decidedly rigid.[10] Pressures up to 10.000 mmHg are required to stretch the capsule to twice its size or even rupture it (*Fig. 1, no 6*). [13] Pressures of such a magnitude are generally only reached during traumatic events or polycystic kidney disease. [14, 15] In other instances, such as congestion from heart failure, pressures are likely deflected inwards by the rigid capsule. To illustrate, in heart failure (both with reduced and preserved ejection fraction), as a result of maladaptive water and sodium homeostasis, intravascular pressures rise. As intravascular pressures reach a tipping point, fluid will exit the blood stream into the interstitium. [16] In the skin this gives pitting edema, in the lungs it leads to alveolar edema. Interstitial edema is similarly present in the kidneys, albeit less visible. The kidneys however do not have the ability to expand similar to the skin and subcutaneous tissue. [11] The reason for this lack of expandability is the presence of the very rigid renal capsule (*Figure 1, no. 6*). [13]

Several kidney congestion models in rats and dogs have demonstrated that when central or renal *vascular* pressures are increased, usually from clipping of the respective vein, renal *interstitial* pressures rise collinearly. [17, 18] Moreover, GFR and urinary production almost instantly decrease. In addition, clipping of the renal vein induces proteinuria, reflecting (pressure induced) damage to Bowman's capsule.

Two studies independently studied renal perfusion in a congested kidney model and found diminished perfusion of the renal medulla, but not the renal cortex. [18, 19] Anatomically, this means the tubules are more at risk for damage from congestion than the glomeruli. This is further supported by the notion that intrarenal expression of biomarkers of tubular damage, in particular KIM-1 and osteopontin, were increased in a murine renal congestion model. [20] Even more interestingly, the expression of these biomarkers was attenuated by removing the capsule prior to inducing congestion. [20]

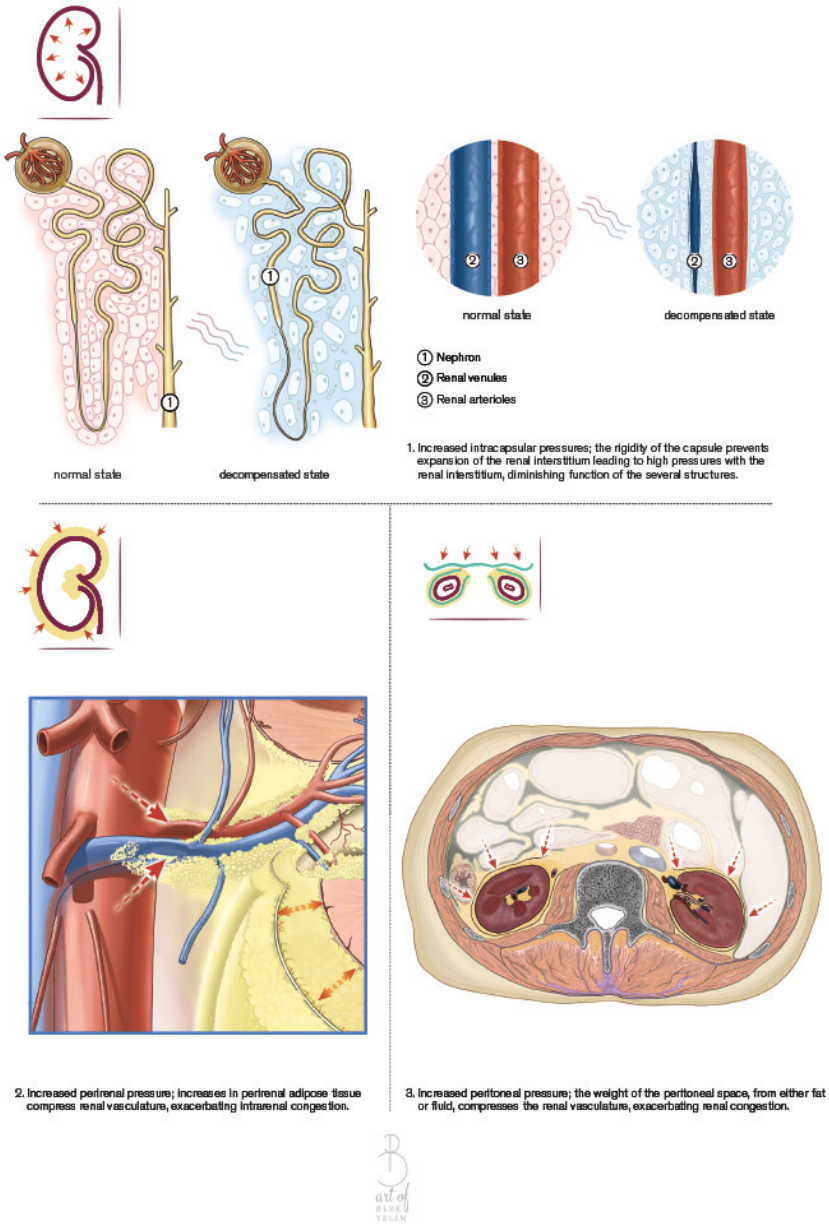

Figure 2 | The first panel shows the effect of intrarenal congestion. As fluid exits the blood stream, pressures rise within the rigid renal capsule, compressing the tubules and the intrarenal venules. The second panel shows the consequences of the perirenal adipose tissue, leading mainly to compression of renal vasculature. The third panel shows the kidneys in the retroperitoneal space. The weight of the fat or fluid in this compartment compresses the renal vasculature.

In addition to tubules and glomeruli, veins are also affected by intracapsular pressure overload, as is demonstrated in several small ultrasound studies in humans. [21-23] In the healthy kidney, venous blood flow is minimally altered by hemodynamic changes. However, increases in pressure within the renal capsule will lead to a collapsing of renal veins, as the capsule prohibits the kidney from expanding and pressures are reflected inwards. On ultrasound, a discontinuous venous flow pattern can be recognized, this pattern is correlated with clinical signs and symptoms of congestion. This indicates that in the congested kidney, blood is solely being pulled through a compressed vein during diastole.

In summary, interstitial congestion of the kidney combined with the inability for the interstitium to expand due to the renal capsule, compresses intrarenal structures such as veins, glomeruli and tubules, diminishing their function.

2- Increased perirenal pressure (Figure 2, second panel.): Increased volume of adipose tissue within the perirenal fascia may lead to increased perirenal pressure.

Both the thickness of perirenal adipose tissue surrounding the kidney and accumulation of fat in the renal sinus have been associated with chronic kidney disease, arteriosclerosis, hypertension and onset of diabetes.[24-27] This association can be explained by perirenal adipose tissue compressing the renal vasculature leading to pathologic renin-angiotensin-aldosterone system (RAAS) activation and reduced renal perfusion (*Figure 2, second panel*), as well as venous compression (further) congesting the renal interstitium.[28, 29] Alternatively, perirenal adipose tissue can cause RAAS activation though its inflammatory properties and increased local levels of TNF-α.[29, 30] Renal sinus fat is of interest in relation to the renal capsule, as the renal sinus is not protected from outside compression by the renal capsule (*Figure 1, no. 4*). This means that increases in renal sinus fat volume directly increase pressures within the renal capsule. Indeed, renal sinus volume has been correlated to both GFR and intrarenal perfusion in patients with type 2 diabetes.[31] Although this is not an acute mechanism, altered intrarenal hemodynamics resulting from perirenal fat might contribute to a decrease of renal function in the setting of interstitial congestion. Whether increases in perirenal fat and/or renal sinus fat contribute to renal congestion in heart failure is yet to be established.

3- Increased intra-abdominal pressure (Figure 2, third panel)

In patients with severe heart failure, intra-abdominal pressure (IAP) may increase because of ascites or increased fluid in the splanchnic system in the absence of ascites.[32] Presence of ascites and its severity has been associated with impaired renal function

in HF.[32] Reduction of IAP from decongestive therapies and mechanical removal of fluid restores renal function.[33] This indicates an indirect relationship between venous congestion and impaired renal function, a direct mass effect on the retroperitoneal kidneys from the weight of the fluid-filled peritoneum, or both (*Figure 2, third panel)*. In patients with (morbid) obesity IAP is similarly increased and decreases after weight-reduction surgery[34-36]. Moreover, several studies indicate weight-reduction surgery improves both renal and cardiovascular outcomes in morbidly obese patients.[34-38] Two studies from the same group indicate that renal venous compression rather than parenchymal compression is the main driver behind decreased GFR, increases in renin and aldosterone and onset of proteinuria in patients with intra-abdominal hypertension.[39, 40]

Renal decapsulation

Loss of renal function through compression of the kidney by the renal capsule suggests that renal decapsulation might improve renal function in congested heart failure patients. This was confirmed in a renal congestion rat model, where decapsulation reduced tubular damage.[20] Data on decapsulation in humans is more than 100 years old. [41] In the late 19th and early 20th century, renal capsular incision or full decapsulation was a treatment oftentimes performed for various indications, ranging from renal abscesses to pre-eclampsia and oliguria, but not congestive heart failure.[41] Better alternatives such as dialysis and antibiotic treatment, as well as contradicting results on the benefit on renal outcomes of decapsulation, eventually rendered renal decapsulation obsolete in acute kidney injury.[42, 43] However, recently decapsulation has been shown to alleviate ischemic acute kidney injury in piglets, potentially reviving the technique. [44] To date, no data on renal decapsulation in humans with heart failure exist.

The renal tamponade

We propose the renal tamponade hypothesis to explain the disproportionate impairment in renal function when central venous pressures increase in patients with heart failure. The renal capsule surrounding the kidney is very rigid and will not allow expansion when pressures rise. Increased central venous pressures lead to increased renal interstitial pressures, compressing renal structures such as the tubules, intrarenal veins and glomeruli in the encapsulated kidney. Future research is warranted to further elucidate the relationship between heart failure, congestion, obesity and impaired renal function. While decreased renal perfusion may be difficult to influence, and indeed, attempts to improve renal perfusion have shown not be associated with improved outcome [45], intrarenal congestion may possibly a treatment target. Ultimately, renal decompression therapies might be a novel therapeutic field to explore in decreasing the incidence of worsening renal function and worsening heart failure.

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Chapter 8

Perirenal Adipose Tissue is Associated with Renal Dysfunction in Patients with Heart Failure and Preserved Ejection Fraction

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Submitted

Abstract

Background and Aims | Renal dysfunction is common in heart failure with preserved ejection fraction (HFpEF) and associated with worse clinical outcomes. Since HFpEF and chronic kidney disease (CKD) share obesity related pathophysiology, we hypothesize that patients with HFpEF have more perirenal fat, which might give rise to compression of the kidney and renal vasculature, and consequently worse renal function, higher sodium avidity, and thus exercise hemodynamics.

Methods and results | Perirenal adipose tissue (PRAT) thickness was measured bilaterally in five standardized directions on abdominal computed tomography scans in 94 patients with HFpEF and 98 age-, sex-, and body mass index (BMI)-matched controls. We additionally assessed renal sinus fat volume, the adipose tissue which is situated in the renal sinus indexed to total kidney volume ratio (RSF/TK). This was estimated by measuring RSF surface area on a single slice protocol, where any pixel density between -195 and -45 Hounsfield Units was considered as adipose tissue. Patients and controls had a similar prevalence of hypertension, diabetes, and renal insufficiency. Patients with HFpEF had greater PRAT thickness compared to the controls (14.3 ± 9.0 vs. 11.7 ± 6.0 mm, P = 0.02). RSF/TK was similar between groups. Greater PRAT thickness was correlated with lower estimated glomerular filtration rate (eGFR) in patients with HFpEF (r = - 0.31, P = 0.003), but not in controls (r = -0.17, P = 0.2). A higher RSF/TK ratio was associated with a lower estimated glomerular filtration rate (eGFR) both in patients and controls. A higher RSK/TK ratio was independently associated with worse invasively measured hemodynamics and peak exercise capacity, even after adjustment for total visceral adipose tissue.

Conclusions | Patients with HFpEF have greater PRAT thickness than age-, sex-, and BMI-matched controls, which was associated with worse renal function. Increased renal sinus fat was associated with adverse hemodynamics.

Introduction

Renal dysfunction is common in heart failure with preserved ejection fraction (HFpEF) and associated with worse clinical outcomes. The cause for this cardiorenal interplay is not well established, although some generally accepted theories exist.[1] Recently, we have put forward a novel angle to the cardiorenal connection called 'the renal tamponade hypothesis'.[2]

Perirenal adipose tissue (PRAT) is a layer of fat surrounding the kidney. This layer is encapsulated by the perirenal fascia. When the thickness of PRAT is increased, it compresses the kidney and the renal vasculature. Moreover, it gives rise to local inflammation, leading to endothelial dysfunction and activation of the renin-angiotensin-aldosterone (RAAS) system.[2] Renal sinus fat (RSF) is the deposition of fat within the sinus (hilum) of the kidney. This adipose tissue surrounds the renal vasculature which enters and exits the kidney in the sinus, including the thin-walled renal vein (Figure 1, panel A). The structures in the renal sinus are potentially more prone to injury from compression as this area is not covered, and thus not protected, by the collagenous renal capsule which encapsulates the rest of the kidney. Increased volume of renal sinus fat might therefore lead to even further RAAS activation and local inflammation.

Increased thickness of PRAT and/or RSF have been associated with many diseases that frequently occur alongside HFpEF, in particular diabetes, hypertension, chronic kidney disease (CKD) and atherosclerosis.[3-7] Moreover, another visceral adipose fat depot, epicardial adipose tissue (EAT), has been shown to be increased in patients with HFpEF, as compared with HFrEF and controls.[8-11] It is postulated that epicardial fat, through release of cytokines, is responsible for local inflammation and fibrosis, similar to the inflammatory properties of PRAT and RSF in those with chronic kidney disease (CKD).[12, 13]

We therefore hypothesize that patients with HFpEF have more perirenal and renal sinus fat, as compared to age-, sex, and BMI-matched controls, which sequentially is associated with worse renal function and exercise hemodynamics.

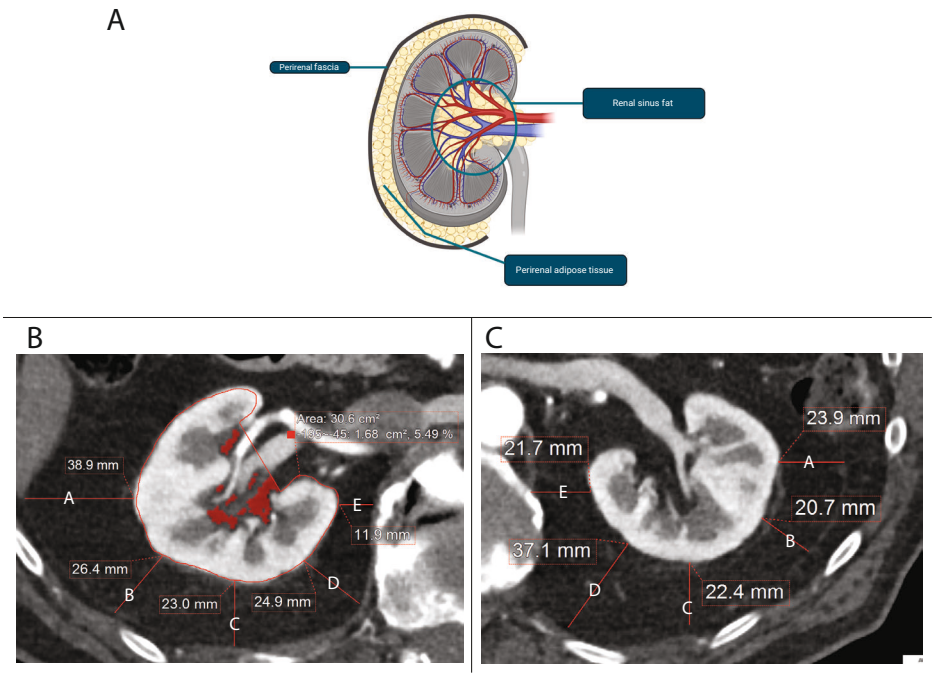

Figure 1 | summary of measuring methods. Panel A shows the anatomy of the kidney in the coronal plane, indicating the different areas of adiposity. Panel B shows the slice with both methods; lines A-E represent lateral, posterolateral, posterior, anterolateral and lateral direction of measurement, respectively. The outline of the kidney has been traced manually and all Hounsfield Units between -195 and -45 are selected to be fat (areas marked red in renal sinus). In this case the total kidney surface area is 30.6 cm^2, the total renal sinus fat surface area is 1.68 and the RSF/TK ratio is 5.49%. Panel C shows the PRAT measurements repeated on the left kidney on a slice at the level of the renal vein. Created with BioRender.com

Methods

Patient selection

The current analyses have been performed in a subset of retrospective data previously collected to investigate total visceral adipose tissue.[12] In brief, from 1100 patients who underwent right heart catheterization for the evaluation of unexplained dyspnoea at Mayo Clinic, Rochester, MN, between 2009 and 2018, 530 had a baseline pulmonary capillary wedge pressure (PCWP) of >15 mmHg and/or an exercise PCWP of ≥ 25 mmHg. Four-hundred and thirteen patients did not meet these criteria and were selected for the control group. In the HFpEF group 105 patients had an abdominal computed

tomography (CT) scan (performed for any indication) available, and 49 controls could be matched based on age, sex, BMI and availability of abdominal CT-scan. From the Rochester Epidemiology Project 54 additional cases were selected to serve as controls. From the remaining 210 scans 18 were excluded due to scan quality or macroscopic kidney disease, making measurements impossible. A total of 94 HFpEF cases and 98 control cases remained for the current analysis. A flow chart depicting patient selection can be found in Supplementary Figure 1.

CT measurements

Perirenal Fat Thickness

On CT scan, PRAT diameter was assessed by measuring the distance from the kidney to the nearest viscera or muscle. Five perirenal measurements were taken (medial, posterior, lateral, anterolateral, posterolateral) on a slice passing through the renal vein (Figure 1, Panel B).[2, 13] The most optimal slice passing through the renal vein was assessed by the reader, when several slices included imaging of the renal vein. Importantly, as the CT-scans were performed for different indications not all scans had an equal slice thickness. The current protocol to measure PRAT on a single slide has previously been shown to correlate well with total perirenal fat volume (r = 0.86).[2] All five measurements were performed on both the left and right kidney and the median of both sides was eventually used for further analysis. As PRAT on the left and right side correlate well (r = 0.84), unilateral PRAT was chosen in the event that PRAT could only be measured unilaterally. The reader was blinded to presence of HFpEF.

Renal sinus fat

Renal sinus fat volume was estimated by measuring renal sinus fat (RSF) surface area on a single slice using the Aquarius 3D Workstation (TeraRecon). Adipose tissue was identified using pixel density in Hounsfield Units (HU). Any pixel density between -195 and -45 HU was considered to be adipose tissue.[16] The reader visually assessed the range in which RSF was largest. When this range contained an odd number of slides, the middle one was selected for measurement. When the range contained an even number of slides, the more cranial of the middle 2 slides was selected.

On the selected slide the outer border of the kidney was traced. The line traced remained within the border of the kidney to exclude surrounding adipose tissue from the measurement. A straight line across the hilus of the kidney was drawn, connecting both lobes. After tracing the kidney, the Aquarius software was used to measure fat surface area within the drawn line based on the previously set HU window. This gave a ratio between the surface area of RSF and the surface area of the kidney (RSF/TK) on that

particular slide, to adjust for the potential bias of RSF measurements being related to kidney size.[17] Only the right-sided RSF/TK was chosen for analyses as this is the method previously described in literature.[16, 18] There was however a strong correlation between RSF/TK on the left and right side (r = 0.85). As with PRAT measurement, the reader was blinded to presence of HFpEF. Figure 1, Panels B and C show the measurements of both PRAT and RSF/TK on the single slice protocol

Total visceral adipose tissue area and subcutaneous adipose tissue area.
Measurements of total visceral adipose tissue area and subcutaneous adipose tissue area on a single slice protocol was established previously.14 In brief, measurements were performed using semi-automated software for body composition analysis (BodyCompSlicer).19 The automatically traced borders of both visceral adipose tissue and subcutaneous adipose tissue on a single slice at the level of the third lumbar vertebra could be manually corrected.

Invasive Hemodynamic Assessment
Subjects from the invasive cohort underwent symptom-limited supine cycle ergometry testing with simultaneous expired gas analysis as previously described (Supplementary Methods 1).[12, 18]

Plasma volume measurements
Plasma volume was calculated using the following formula: (1-haematocrit) × [a + (b × weight in kg)] where a = 1530 for men and 864 for women, and b = 41 for men and 47.9 for women.[21]

Echocardiography
Two-dimensional, M-mode, Doppler, and tissue Doppler echocardiography were performed by experienced sonographers according to the guidelines of the American Society of Echocardiography.[22] Epicardial adipose tissue thickness (EAT) was measured perpendicularly to the free wall of the RV by echocardiography in the parasternal long-axis view at end systole.[11]

Statistical analysis
Parametric data are presented as means and standard deviation, nonparametric data as medians and 25th until 75th percentile, and categorical variables as percentages and frequencies. Intergroup differences were tested using Student *t*-test for normally distributed continuous data, whereas skewed data were analyzed using Mann-Whitney. Associations of PRAT and RSF/TK-ratio with renal function and hemodynamic parameters were tested using univariable and multivariable linear regression. For the

analysis regarding hemodynamics, we have chosen to perform correlation analysis for the entire cohort, to not restrict the power of the analysis. Moreover, to give a full indication of the correlation between hemodynamics and measurements of PRAT and RSF it is imperative to analyze the full spectrum of pressures, rather than only those with elevated pressures. A two-sided P-value <0.05 was considered statistically significant. All data were analyzed using JMP14.0 (SAS Institute Inc., Cary, NC, USA).

Results

Baseline characteristics

Baseline characteristics of patients with HFpEF and their matched controls can be found in Table 1. As patients were matched for age, sex and BMI, no differences between the groups were present in any of these variables. In addition, the groups were similar in terms of prevalence of diabetes and hypertension (P for both >0.1). Estimated glomerular filtration rate (eGFR) was similar between the groups. (62 ± 18 vs. 63 ± 22 ml/min, P = 0.8), as was EAT (4.8 ± 2.6 mm vs. 5.3 ± 3.4 mm, P = 0.3).

Table 2 shows visceral fat distribution stratified for both groups. Patients with HFpEF have both a higher visceral adipose tissue and subcutaneous adipose tissue area compared to their matched controls. Additionally, they have greater overall PRAT thickness (11.7 ± 6.0 vs. 14.3 ± 9.0 mm, P = 0.02). RSF/TK ratio did not differ between the groups (P = 0.2 for the right kidney and P = 0.6 for the left kidney).

Table 1 | Baseline Characteristics

	Control (n=98)	HFpEF (n=94)	P-value
Age (years)	65±14	65±14	0.9
Female, n (%)	59 (60)	56 (60)	0.9
Body mass index (kg/m^2)	31.2±6.7	32.1±7.6	0.4
Estimated plasma volume (ml)	2978±551	3351±691	0.0005
***Comorbidities*, n (%)**			
Hypertension	57 (58)	64 (68)	0.1
Diabetes mellitus	13 (12)	22 (21)	0.2
Atrial fibrillation	3 (3)	23 (24)	<0.0001
Medications, n (%)			
Beta blocker	30 (31)	50 (53)	0.001
ACEI/ARB	20 (20)	41 (44)	0.0005
Loop diuretic	8 (8)	42 (45)	<0.0001
MRA	6 (6)	18 (19)	0.006

Table 1 *(Continued)*

	Control (n=98)	HFpEF (n=94)	P-value
Thiazide	14 (14)	5 (5)	0.04
Laboratories			
Hemoglobin (g/dL)	13.1±1.7	12.0±1.7	<0.0001
Creatinine (mg/dL)	1.0 (0.8, 1.1)	1.0 (0.8, 1.3)	0.5
Estimated GFR (mL/min/1.73m^2)	62±18	63±22	0.8
NT-pro BNP (pg/mL) (n=32/78)	106 (48, 346)	332 (109, 1230)	0.0003
Echocardiography			
LV mass index (g/m^2)	86±22	90±26	0.2
Ejection fraction (%)	63±6	62±6	0.2
E/e' ratio	10 (8, 15)	11 (8, 17)	0.1
LA volume index (ml/m^2)	28 (23, 33)	34 (27, 42)	0.002
EAT thickness (mm) (n=51/92)	4.8±2.6	5.3±3.4	0.3

Values are mean ± SD, median (interquartile range), or n (%). HFpEF, heart failure with preserved ejection fraction; ACEI/ARB, angiotensin-converting enzyme inhibitor/angiotensin receptor blocker; MRA, mineralocorticoid receptor antagonist; GFR, glomerular filtration rate; N-terminal-pro-Brain Natriuretic Peptide.

Table 2 | Visceral Fat Distribution

	Control (n=98)	HFpEF (n=94)	P-value
VAT-area (cm^2)	183±96	234±143	0.004
SAT-area (cm^2)	245±14	287±151	0.003
Right RSF volume (cm^2)	1.2 (0.5, 2.2)	1.2 (0.5, 2.7)	0.6
Left RSF volume (cm^2)	1.4 (0.5, 2.6)	1.4 (0.8, 2.4)	0.7
Right Total Kidney (TK) area (cm^2)	20.9±5.1	23.9±6.3	0.0006
Left Total Kidney (TK) area (cm^2)	19.7±4.7	20.8±4.9	0.1
Right Perirenal adipose tissue (PRAT) (mm)*	10.8±6.0	13.1±9.2	0.04
Left Perirenal adipose tissue (PRAT) (mm)*	12.6±6.8	15.7±9.4	0.01
Overall PRAT (mm)+	11.7±6.0	14.3±9.0	0.02
Right RSF volume/TK area	5.8 (2.6, 10.8)	5.9 (2.1, 10.5)	0.2
Left RSF volume/TK area	7.4 (3.2, 2.0)	6.7 (3.8, 10.5)	0.6

Values are mean ± SD, median (interquartile range), or n (%). HFpEF, heart failure with preserved ejection fraction; VAT, visceral adipose tissue; SAT, subcutaneous adipose tissue.
*PRAT: mean of A-E
+Overall PRAT; Mean of mean right and mean left of PRAT

Associations between perirenal and renal sinus fat and renal function

Both PRAT (r = -0.31, P = 0.003) and RSF/TK (r = -0.53, P <0.001) were negatively correlated with eGFR in patients with HFpEF, but only RSF/TK was also significantly negatively correlated to eGFR in controls (Figure 2).

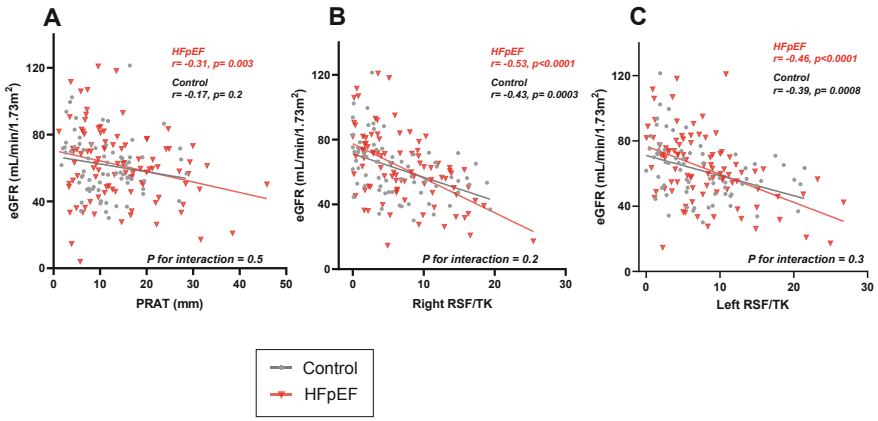

Figure 2 | Regression plots showing the correlation between PRAT (figure A) and eGFR and RSK/TK and eGFR (figures B and C). Only in patients with HFpEF does PRAT show a significant correlation with eGFR, while RSF/TK is significantly correlated to a worse renal function in both patients with HFpEF and controls.

Associations with other indices of adiposity

Perirenal adipose tissue was strongly related to total visceral adipose tissue area (r = 0.85, P <0.001, Supplementary Figure 2). RSF/TK was significantly associated with total visceral adipose tissue area, albeit of a lesser magnitude (r = 0.48, P <0.001). Both PRAT and RSF/TK were associated with BMI, although not as strongly as with total visceral adipose tissue area (r = 0.46 and 0.26, respectively, P for both <0.001).

Association between PRAT and exercise capacity and hemodynamics

Supplementary Table 1 shows baseline differences between patients with HFpEF and controls, with regards to exercise capacity and hemodynamics.

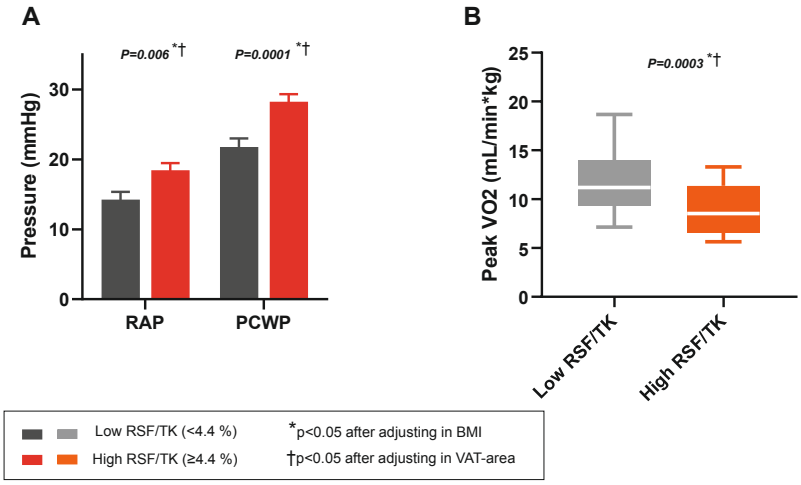

Figure 3 | Graphical depiction of the differences in right atrial pressure and pulmonary capillary wedge pressure during peak exercise (figure A) and peak oxygen consumption (figure B) between patients with those with a RSF/TK volume above and below the mean.

PRAT was univariably negatively associated with aerobic capacity (peak VO_2, Beta = -0.20, P <0.001). Moreover, PRAT was associated with invasively measured pressures during peak exercise, in particular higher PCWP (Beta = 0.26, P = 0.009) and higher right atrial (RA) pressure (Beta = 0.19, P = 0.04) (Supplementary Table 2). However, significance for these relationships was lost after adjustment for total visceral adipose tissue in multivariable linear regression analysis.

Associations between RSF/TK and hemodynamics

RSF/TK was associated with exercise hemodynamics, even after adjustment for BMI and total visceral adipose tissue (Supplementary Table 3). Patients with RSF/TK above the median had a higher PCWP (23 ± 11 vs 27 ± 8 mmHg, p = 0.02) and a lower achieved maximum workload (58 ± 38 vs 40 ± 21 Watts, P = 0.005) (Table 3). These differences remained significant after adjusting for BMI or visceral fat area. (Figure 3).

Table 3 | Hemodynamics stratified by median value of right RSF/TK in whole population (n=140) (Median value in n=140[includes only patients with RHC] used)

	Right RSF/TK (<4.4%) (n=38/70 (54%) HFpEF))	Right RSF/TK (≥4.4%) (n=56/70 (80%) HFpEF)	P-value
Baseline Hemodynamics			
RA pressure (mmHg)	8±4	10±5	0.009*†
PA systolic pressure (mmHg)	35±11	39±14	0.03*†
PA mean pressure (mmHg)	22±7	26±9	0.02*†
PCWP (mmHg)	13±5	15±6	0.02*†
Cardiac output (L/min)	5.4±1.6	5.0±1.7	0.2
O2 consumption (mL/min*kg)	2.8±0.7	2.4±0.5	0.002*
Exercise Hemodynamics			
Workload (Watts)	59±40	42±24	0.005*†
RA pressure (mmHg)	14±8	18±7	0.006*†
PA systolic pressure (mmHg)	54±18	60±16	0.02*
PA mean pressure (mmHg)	37±13	41±10	0.02*
PCWP (mmHg)	22±10	28±9	0.0001*†
Cardiac output (L/min)	9.6±3.4	9.0±3.3	0.3
O2 consumption (mL/min*kg)	12.0±4.6	9.1±3.3	0.0003*†

Values are mean ± SD, median (interquartile range). RA, right atrial; PA, pulmonary arterial; PCWP, pulmonary capillary wedge pressure.
*P<0.05 after adjusting for BMI
†P<0.01 after adjusting for VAT-area

Discussion

Here, we show that patients with HFpEF have a larger perirenal adipose fat layer, compared to age-, sex- and BMI-matched controls. The thickness of PRAT was more strongly associated with increases in total visceral adipose rather than general measures of obesity, and PRAT was further correlated with lower eGFR in the total population, as well as in a subgroup of patients with HFpEF. When examining the adipose tissue situated in the renal sinus (RSF/TK), the area where the renal vasculature enters and exits the kidney, the associations with renal function were even stronger, both for those with and without HFpEF. Additionally, RSF/TK ratio was correlated with adverse hemodynamics and increased pulmonary vascular pressures during exercise, one of the hallmark features of HFpEF, even after correction for total visceral adipose tissue.[23]These data suggest that the amount of adipose tissue situated around the kidney and in the renal sinus might have pathophysiological significance in patients with HFpEF through mechanisms including direct compression of renal and pararenal

structures, amplifying local and systemic inflammation. promoting endothelial dysfunction. or increasing neurohormonal activation.

Patients with HFpEF have more perirenal adipose tissue

While an increase in thickness of perirenal fat has been described in several disease entities, including atherosclerosis, albuminuria, CKD, and hypertension, to our knowledge this is the first study to describe an increase in PRAT in patients with HFpEF.[3, 5-7, 24, 25] The current data might indicate local adiposity as a connector between HFpEF and CKD, two disease entities related to obesity that frequently coexist in one patient. [10, 26, 27] Increasing evidence suggests that in patients with HFpEF, epicardial fat is associated with worse cardiac function and exercise capacity. In patients with CKD, perirenal adipose tissue has been linked to worse renal function. [25] One meta-analysis previously indicated that patients with CKD have more epicardial adipose tissue.[28] Our data add to these studies that patients with HFpEF have more perirenal adipose tissue. Patients with HFpEF in our cohort did not have more epicardial adipose tissue, compared to controls. A shared pathophysiology of locoregional fat deposition in both HFpEF and CKD can be suggested but is not yet proven. These fat depositions would locally give compression, or potentially infiltration in the case of epicardial fat.[28] Moreover, cytokines such as adiponectin and tumor necrosis factor alpha give rise to local inflammatory processes, leading to endothelial dysfunction and fibrosis.[28, 29]

Perirenal adipose tissue is associated with a worse renal function.

In the present study, we showed that PRAT thickness was associated with worse renal function, in the overall population and in the subset of those with HFpEF, but not in the matched controls. The association between PRAT and eGFR has never been described in HFpEF, yet it has been established in several other cohorts, mainly including patients with type 2 diabetes mellitus and/or obesity. In patients with type 2 diabetes, increased PRAT measured on ultrasound correlated even better to reduced eGFR than in our data ($r = -0.414$), and this correlation was stronger than for any other adipose tissue measurement.[25] Additionally, in non-diabetic obese patients increased PRAT correlated with an increased urinary-albumin-to-creatinine ratio ($r = 0.610$).[4]

RSF/TK is associated with worse renal function

Similar to PRAT, RSF/TK was associated with worse renal function in both the HFpEF and the control population. As with PRAT, the association was stronger in HFpEF, although no significant interaction was found. Again, our study is the first to demonstrate this association in patients with HFpEF, although the association has been described in other patient categories. An earlier study regarding RSF/TK ratio indicated that in patients with type 2 diabetes mellitus and CKD, an increase in the area of renal sinus

fat on MRI was associated with adverse renal hemodynamics, in particular reduced effective renal plasma flow and increased effective renal vascular resistance. The authors hypothesize that compression of the renal vasculature and local inflammation could be responsible for these findings.[18] The adipose tissue would be accountable for release of vasoconstrictive agents, altering renal hemodynamics.[18] Altered renal hemodynamics could in turn lead RAAS- activation, as is the current main hypothesis on the strong association between PRAT and hypertension.[30] The present data do not include information on the RAAS-system however, and while neurohormonal activation is present in HFpEF, it is less dominant in the pathophysiology of HFpEF compared to that of heart failure with reduced ejection fraction (HFrEF).[31]

RSF/TK is associated with higher intra-cardiac pressures.

Finally, we found increased RSF/TK to be associated with higher intra-cardiac pressures during exercise, a prominent feature in HFpEF, but RSF/TK was similar in HFpEF and controls. One possible explanation is that it is the combination of both myocardial dysfunction and excess RSF that results in hemodynamic abnormalities, a "multi-hit" model. Another possible explanation for this finding might be that renal sinus fat is only one deposit of perivascular adipose tissue and indicative of total perivascular adipose tissue. In other words, those who have more renal sinus fat are more likely to have perivascular fat surrounding other vessels as well. Perivascular adipose tissue is defined as fat tissue surrounding the large arteries, and in healthy individuals, this has anticontractile effects through secretion of adipokines. In the diseased state however, obesity leads to adipokine dysregulation negating the anticontractile properties.[32] These pathological changes are likely mediated by local secretion of tumor necrosis factor alpha or other proinflammatory adipokines. For example, leptin, also secreted by the perivascular adipose tissue, has been shown to directly induce endothelial dysfunction. In one study investigating PRAT in obese rats, it was demonstrated that a higher PRAT thickness was associated with more endothelial dysfunction, in both the infra-renal and thoracic aorta.[33] Endothelial inflammation and dysfunction in the pulmonary arteries resulting from reduced exercise-induced vasodilation would explain the evidently increased pressures during exercise found in those with a higher renal sinus fat and, by proxy, more perivascular fat throughout the body.[34, 35] The renal vein is highly collapsible, delicate structure, and even small increases in external pressure from increased adipose deposition would be expected to increase venous pressure, which could affect renal function and sodium avidity to contribute to greater elevation in filling pressures. Finally, excess perivascular fat may contribute to alterations in sympathetic tone, leading to excessive arterial and venous vasoconstriction. In this light, it is notable that increases in body fat, estimated by BMI, are strongly correlated with increases in stressed blood volume and the ratio of stressed blood volume to

total blood volume, in patients with HFpEF, indicating more severe abnormalities in venous capacitance.[36] Because cardiac filling pressures are strongly related to stressed blood volume during exercise, the observed associations with perivascular fat provide additional potential explanations for this association.

Limitations

Several limitations to our study should be noted. First, patients are selected from a large tertiary center, and our findings might therefore not be reproducible in all patients with HFpEF. Second, although we used a standardized CT protocol to measure PRAT and RSF/TK, not all slides were equal in thickness (the slice thickness varied between 1 and 3 mm). This error should equally apply to both groups, reducing bias, and the protocol was devised to reduce these limitations by selecting a standardized location through the renal vein for the PRAT measurements and the middle slice (of several slices) on which RSF area was visually the largest. Third, because the present data was collected retrospectively, all patients had to have an indication for an abdominal CT-scan, introducing bias. Moreover, as CT-scans made solely for research purposes, were included in the healthy control population, this population may have been less sick, as compared to the patient category (Supplementary Table 1). Lastly, as a result of the retrospective nature of our study we used estimated GFR, instead of measured GFR, which is a less accurate method for establishing glomerular filtration, especially in the obese. However, as eGFR in obese individuals is at risk of *overstating* GFR, the negative correlation demonstrated might actually be more pronounced when utilizing measured GFR.[37]

Clinical implications and future perspectives:

The present data have several potentially important clinical implications. First, weight loss, either through dietary and lifestyle alterations and possibly by weight reduction surgery, could lead to lower perirenal fat and thereby improvement in renal function and hemodynamics. Furthermore, glucagon-like peptide-1 (GLP-1) agonists have recently gained attraction as the first medical treatment for obesity approved by both the FDA and the EMA. Currently, trials evaluating the GLP-1 agonist semaglutide and the combined GLP-1 agonist/glucose-dependent insulinotropic polypeptide agonist tirzepatide are underway to evaluate the use of these weight loss drugs as potential treatments for obesity related HFpEF (NCT04916470, NCT04847557). Whether weight loss improves outcomes is yet to be determined, as is whether weight loss also reduces local perirenal fat deposition.

The current results are hypothesis generating and should be interpreted as such. Naturally, these data need to be validated in a prospective study, which will also incorporate

renal hemodynamics. Currently such a study is ongoing: PErirenal Adipose tissue and RenaL hemodynamics in patients with Heart Failure with Preserved Ejection Fraction; a Pilot Study (PEARL-HFpEF, NCT05219188).

Conclusion

Patients with HFpEF have greater PRAT thickness than age-, sex and BMI-matched controls, which was associated with worse renal function. Increased renal sinus fat was associated with worse renal function and adverse hemodynamics in patients with and without HFpEF.

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Supplementary Material

Supplementary Methods 1

Invasive Hemodynamic Assessment

Right heart catheterization was performed using a 9-Fr sheath via the right internal jugular vein. Right atrial (RA) pressure, pulmonary artery (PA) pressure, and PCWP were measured at endexpiration (mean of ≥3 beats) on distinct respiratory cycles using 2-Fr high fidelity micromanometer-tipped catheters (Millar Instruments, Houston, TX) advanced through the lumen of a 7-Fr fluid-filled catheter (Balloon wedge, Arrow). Transducers were zeroed at the mid-axilla, measured using laser calipers in each patient. The PCWP position was confirmed by appearance on fluoroscopy, characteristic pressure waveforms, and oximetry (saturation≥94%). A 4-6 Fr radial arterial cannula was used to measure the arterial blood pressure and for sampling of the arterial blood gases throughout the study. The arterio-venous oxygen difference (AVO_2 diff) was directly measured as the difference between systemic arterial and PA oxygen content. Oxygen consumption (VO_2) was measured via expired gas analysis (MedGraphics, St. Paul, MN, USA), with values taken as the mean over a 30-s interval preceding arterial and venous blood sampling in each phase. Cardiac output (CO) was then calculated using the direct Fick method (CO = VO_2/AVO_2 diff). After baseline data were acquired, hemodynamic assessment and expired gas analysis were performed during supine cycle ergometry exercise, starting at 20 W for 5 min (60 rpm), increasing by 20 W increments in 3 min stages to volitional exhaustion.

Supplementary Table 1 | Clinical indication of CT scan

CT indication n, (%)	Whole population n=**192**	Control n=98	HFpEF n=94
Abdominal pain	42 (22)	9 (9)	33 (35)
Disease of kidney or urinary tract	16 (8)	3 (3)	13 (14)
Thoracic disease	16 (8)	6 (6)	10 (11)
Vascular disease	10 (5)	6 (6)	4 (4)
Hematologic disease	8 (4)	2 (2)	6 (8)
Fever of unknown origin	2 (1)	1 (1)	1 (1)
For research purpose	52 (27)	52 (53)	0 (0)
Other or unspecified	46 (24)	19 (19)	27 (29)

Supplementary Table 2 | Hemodynamics in Control and HFpEF

	Control (n=98)	HFpEF (n=94)	P-value
Baseline Hemodynamics			
Heart rate (bpm)	69±14	69±13	0.9
Systolic blood pressure (mmHg)	149±18	142±24	0.1
RA pressure (mmHg)	5±3	10±4	<0.0001
PA systolic pressure (mmHg)	27±7	41±12	<0.001
PA mean pressure (mmHg)	17±4	27±8	<0.001
PCWP (mmHg)	9±3	16±5	<0.001
Cardiac output (L/min)	5.6±1.8	5.1±1.5	0.1
O2 consumption (mL/min*kg)	2.9±0.7	2.5±0.6	0.0004
Exercise Hemodynamics			
Workload (Watts)	65±41	44±27	0.0007
Heart rate (bpm)	111±22	105±21	0.08
Systolic blood pressure (mmHg)	179±30	174±29	0.4
RA pressure (mmHg)	9±5	19±7	<0.0001
PA systolic pressure (mmHg)	45±14	63±16	<0.0001
PA mean pressure (mmHg)	29±8	44±10	<0.0001
PCWP (mmHg)	13±5	31±7	<0.0001
Cardiac output (L/min)	9.9±3.8	8.9±3.1	0.1
O2 consumption (mL/min*kg)	12.5±5.3	9.6±3.2	0.0006

Values are mean ± SD, median (interquartile range). RA, right atrial; PA, pulmonary arterial; PCWP, pulmonary capillary wedge pressure.

Supplementary Table 3 | Associations of PRAT with hemodynamics in whole population (n=140)

	R-coefficient	P-value	β (95% CI)	P-value
Baseline Hemodynamics				
Heart rate (bpm)	-0.05	0.5	-0.08 (-0.35, 0.18)	0.5
Systolic blood pressure (mmHg)	0.06	0.5	0.15 (-0.29, 0.60)	0.5
RA pressure (mmHg)	0.11	0.2	0.05 (-0.03, 0.14)	0.2
PA systolic pressure (mmHg)	0.01	0.9	0.01 (-0.24, 0.26)	0.9
PA mean pressure (mmHg)	0.02	0.8	0.02 (-0.15, 0.19)	0.8
PCWP (mmHg)	0.06	0.5	0.04 (-0.07, 0.15)	0.5
Cardiac output (L/min)	0.12	0.2	0.02 (-0.01, 0.05)	0.2
O2 consumption (mL/min*kg)	-0.38	<0.0001	-0.03 (-0.04, -0.02)	<0.0001
Exercise Hemodynamics				
Workload (Watts)	-0.18	0.049	-0.67 (-1.34, 0.00)	0.049*
Heart rate (bpm)	-0.17	0.049	-0.41 (-0.82, -0.00)	0.049*
Systolic blood pressure (mmHg)	0.10	0.3	0.34 (-0.26, 0.94)	0.3
RA pressure (mmHg)	0.20	0.04	0.19 (0.01, 0.36)	0.04

Supplementary Table 3 *(Continued)*

	R-coefficient	P-value	β (95% CI)	P-value
PA systolic pressure (mmHg)	0.09	0.3	0.19 (-0.15, 0.52)	0.3
PA mean pressure (mmHg)	0.02	0.8	0.19 (-0.15, 0.52)	0.3
PCWP (mmHg)	0.23	0.009	0.26 (0.07, 0.45)	0.009
PCWP/Watts (mmHg/W)	0.19	0.04	0.01 (0.001, 0.02)	0.04
Cardiac output (L/min)	0.01	0.4	0.03 (-0.04, 0.09)	0.4
O2 consumption (mL/min*kg)	-0.44	<0.0001	-0.20 (-0.29, -0.12)	<0.0001*

Values are mean ± SD, median (interquartile range). RA, right atrial; PA, pulmonary arterial; PCWP, pulmonary capillary wedge pressure; PCWL, ratio of PCWP at peak exercise to workload normalized to body weight

*P<0.05 after adjusting for BMI,

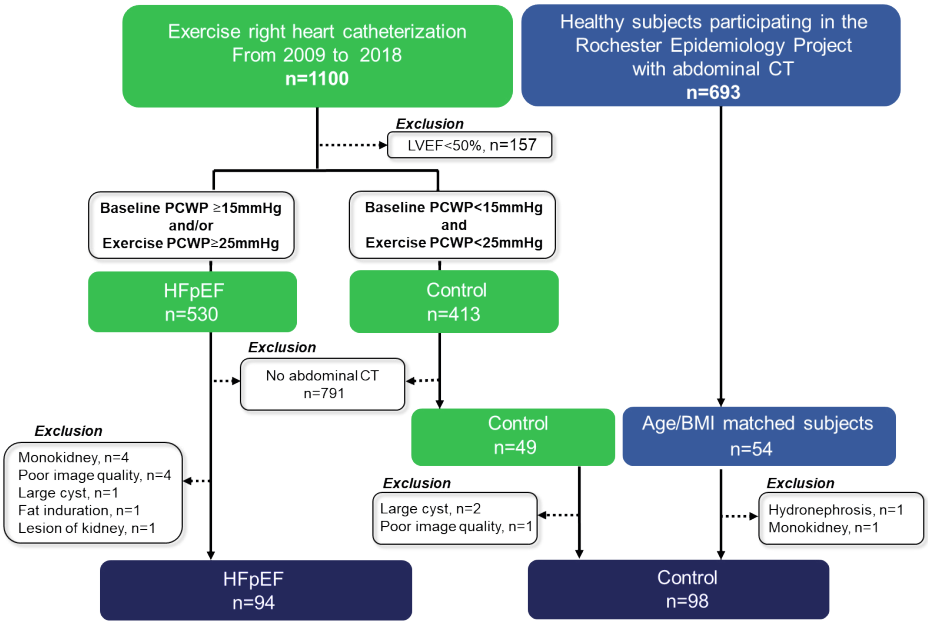
Supplementary Table 4 | Associations of right RSF/TK with hemodynamics in whole population (n=140)

	R-coefficient	P-value	β (95% CI)	P-value
Baseline Hemodynamics				
Heart rate (bpm)	-0.06	0.5	-0.16 (-0.60, 0.28)	0.5
Systolic blood pressure (mmHg)	0.04	0.7	0.16 (-0.59, 0.91)	0.7
RA pressure (mmHg)	0.15	0.08	0.13 (-0.02, 0.28)	0.08
PA systolic pressure (mmHg)	0.12	0.2	0.30 (-0.12, 0.71)	0.2
PA mean pressure (mmHg)	0.15	0.09	0.24 (-0.03, 0.52)	0.09
PCWP (mmHg)	0.12	0.2	0.13 (-0.06, 0.32)	0.2
Cardiac output (L/min)	-0.03	0.7	-0.01 (-0.06, 0.04)	0.7
O2 consumption (mL/min*kg)	-0.40	<0.0001	-0.03 (-0.05, -0.01)	0.003
Exercise Hemodynamics				
Workload (Watts)	-0.28	0.002	-0.18 (-2.92, -0.67)	0.002*†
Heart rate (bpm)	-0.21	0.01	-0.88 (-1.58, -0.19)	0.01*
Systolic blood pressure (mmHg)	0.05	0.6	0.29 (-0.74, 1.31)	0.6
RA pressure (mmHg)	0.27	0.006	0.40 (0.12, 0.69)	0.006*
PA systolic pressure (mmHg)	0.14	0.1	0.47 (-0.10, 1.04)	0.1
PA mean pressure (mmHg)	0.18	0.03	0.42 (0.04, 0.81)	0.03
PCWP (mmHg)	0.26	0.003	0.49 (0.17, 0.81)	0.003*
PCWP/Watts (mmHg/W)	0.28	0.002	0.03 (0.01, 0.05)	0.002*†
Cardiac output (L/min)	-0.05	0.5	-0.04 (-0.15, 0.08)	0.5
O2 consumption (mL/min*kg)	-0.40	<0.0001	-0.32 (-0.46, -0.18)	<0.0001*†

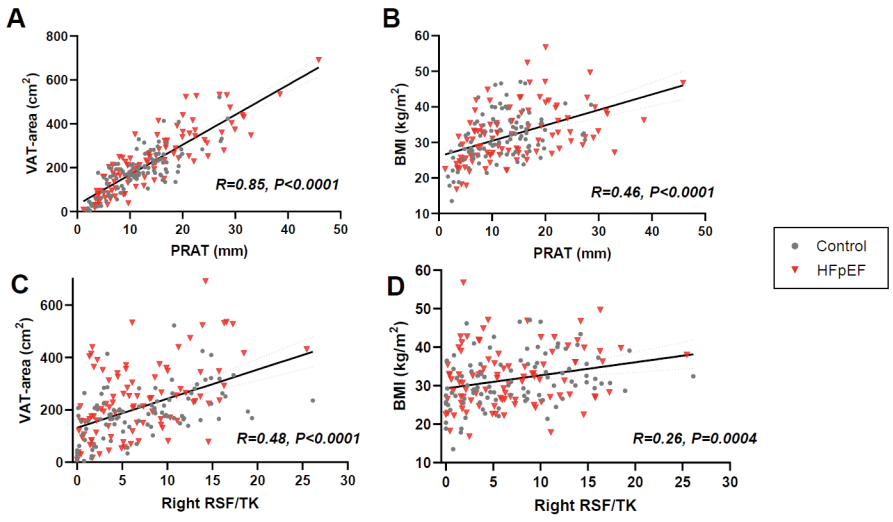
Values are mean ± SD, median (interquartile range). RA, right atrial; PA, pulmonary arterial; PCWP, pulmonary capillary wedge pressure. PCWL, ratio of PCWP at peak exercise to workload normalized to body weight

*P<0.05 after adjusting for BMI

†P<0.01 after adjusting for VAT-area



Supplementary Figure 1 | Flow diagram depicting reasons for exclusion for subjects evaluated for eligibility in the current study.



Supplementary Figure 2 | associations of perirenal adipose tissue with other indices of obesity

Chapter 9

General discussion and future perspectives

Congestion plays a central role in the pathophysiology of heart failure. The symptomatology of heart failure is mainly driven by congestion, and congestion is the most important reason for hospitalization and usually precedes further clinical decline.[1] Despite the great significance of congestion, a lot about it remains unknown.

Cardiac dysfunction leading to a decrease in cardiac output will activate neurohormonal systems (e.g., the renin-angiotensin system), eventually leading to resorption of sodium and water. However, the adaptation to fluid overload is heterogenous between patients and regions of the world.[2] Some patients present with fluid build-up primarily in the vasculature, others will have mainly pitting edema in the lower extremities. Additionally, fluid can be stored in the abdomen, both in the peritoneal space (ascites) and in the large pool of abdominal veins, called the splanchnic system.[3] While different clinical profiles in acute heart failure presentation are being recognized, the underlying pathological processes of these profiles have to date not been elucidated. Yet, these mechanisms are paramount to understand, in order to find novel tailored therapies for the different congestive subtypes. This thesis therefore aimed to study the pathophysiology of congestion using blood and urinary biomarkers. Ultimately, the information from his thesis can hopefully contribute to novel personalized diuretic treatment strategies to improve the treatment of congestive heart failure.

Discussion

Congestion and decongestion

In the first part of this thesis, we studied congestion and attempted to identify different congestive profiles, based on clinical characteristics and blood and urinary biomarkers.

In **Chapter 2** we reviewed the current knowledge and the gaps in knowledge on congestion in heart failure. First and foremost, congestion is not yet well defined and this term is used for different clinical phenomena, ranging from increased intravascular pressures to dyspnea and peripheral edema. The underlying mechanism for all these phenomena called congestion starts with increased intravascular pressures, as a result of fluid retention by the kidneys. At the level of the vasculature there are compensatory mechanisms at play which protect the interstitium from fluid overload. The lymph system plays a major compensatory role, as it can increase its output 10 to 50-fold in order to drain the interstitium of excess fluid. When this and other compensatory mechanisms no longer suffice, fluid will start to build up in the interstitial space. After initiation or intensification of a loop diuretic to treat congestion, some patients will gradually lose fluid from both the systemic circulation and the interstitial space. In

other patients, systemic venous decongestion is more rapid compared to tissue decongestion, leaving residual fluid surplus in the tissues.

From these clinical settings we can deduce two congestive subtypes: intravascular or systemic venous congestion and tissue congestion. In most patients both will be present at the same time, but in others one or the other may be more prevalent. Additionally, it is important to note that the clinical phenotype may change during treatment.

Systemic venous congestion is the buildup of fluid within the venous circulation. In every case this is the first type of congestion and should thus be present in every untreated congested patient. An interesting subset of intravascular congested patients are those who very suddenly become dyspneic and severely congested because of redistribution of fluid, rather than of excess fluid. This redistribution comes from the splanchnic vasculature; the veins in the abdomen, which are able to sequester a large pool of the blood volume as a reserve in the case of hypovolemia.[4] In the case of neurohormonal activation in acute heart failure this reserve likewise empties itself into the thoracic vasculature, creating a very sudden onset of acute heart failure.

Tissue congestion is the result of loss of compensatory mechanisms at the level of the interstitial space, mainly due to a loss of balance between intravascular and interstitial pressures and an inability of the lymph vessels to adequately drain the interstitium. Tissue congestion results from, likely longer standing, intravascular congestion and can be exacerbated by inflammation, ischemia and endothelial dysfunction. [5, 6]

We furthermore hypothesize that both types of congestion may require a different kind of treatment, based on the potential of the drug to either excrete sodium (natriuresis) or free water (aquaresis). Natriuresis might be best suited to treat intravascular congestion, while aquaresis might assist in drawing residual congestion from the interstitium, by increasing plasma osmolality.

In **Chapter 3**, we prospectively studied the effects of *empagliflozin* on clinical outcomes in patients with acute decompensated heart failure. This randomized, double-blind, placebo-controlled, multicenter pilot study on the safety and efficacy of the sodium glucose cotransporter-2 (SGLT-2) inhibitor empagliflozin (EMPA-RESPONSE-AHF) in 79 patients with acute decompensated heart failure showed no difference between empagliflozin and placebo in the combined primary endpoint, which consisted of change in dyspnea, reduction in NT pro-BNP, diuretic response, and length of in-hospital stay. Administrating empagliflozin on top of standard of care in this sick population was well tolerated and safe, based on the low number of adverse events. Empagliflozin

did increase cumulative urinary volume and net negative fluid balance. Additionally, a significantly lower rate of deaths, in-hospital worsening heart failure and HF readmissions through day 60 were observed in the empagliflozin arm compared with placebo. Interestingly, despite an increment in urinary output, this additional fluid loss did not lead to less dyspnea or a shorter length of hospital stay. This disconnect may suggest that absolute fluid loss is not the only factor needed to adequately decongest and discharge a patient. Alternatively, there may have been an issue with statistical power. The publication of the pilot study presented in this Chapter was followed by a larger clinical trial investigating the use of empagliflozin in acute heart failure: A Study to Test the Effect of Empagliflozin in Patients Who Are in Hospital for Acute Heart Failure (EMPULSE[7]). EMPULSE found a clinical benefit of the use of empagliflozin when compared with placebo on the hierarchical endpoint of time to death, frequency of heart failure events, time to first heart failure event and change in KCCQ-TSS. Interestingly, the secondary endpoint of diuretic response in this trial was significantly different between those with and without empagliflozin, as patients with empagliflozin had a larger diuretic response, both after 15 days of treatment and 30 days of treatment. Combining the data from our study and the larger EMPULSE trial indicates that use of empagliflozin in acute heart failure is safe and associated with better clinical outcomes. The increase in net fluid loss and likely also diuretic response, might make it especially useful for patients with overt fluid overload, such as those with tissue congestion, rather than those with strictly intravascular congestion. The notion that SGLT-2 inhibitors are especially suited for treatment of patients with tissue congestion is further supported by a mathematical model which calculates the reduction of interstitial fluid volume to be much larger with the use of the SGLT-2 inhibitor dapagliflozin, when compared with the loop diuretic bumetanide. [8] Yet, it is unlikely that SGLT-2 inhibitors will ever be prescribed to only a specific subset of patients, after the overwhelming evidence of their benefit in all heart failure subtypes, whether HFpEF, HFrEF, acute or chronic heart failure.[7, 9-12]

Chapter 4 added to the findings in **Chapter 3**. In this chapter we assessed the effects of empagliflozin on renal handling of glucose and sodium in patients admitted with acute decompensated heart failure. We found that patients randomized to empagliflozin had an increase of fractional glucose excretion, as expected. However, their fractional excretion of sodium remained similar to those treated with placebo. Despite an increase in urinary output, as demonstrated in **Chapter 3**, urinary osmolality did not change. From these findings we concluded that in (acute) heart failure the SGLT-2 inhibitor empagliflozin acts as neither a natriuretic nor an aquaretic drug, but rather an osmotic diuretic drug. The excretion of glucose draws water from the renal interstitium to the urine, keeping urinary osmolality constant by increasing its volume to

match the increase in molecular particles. This would theoretically eventually lead to a (moderate) increase of plasma osmolality, which we also demonstrated. The increase in plasma osmolality would make empagliflozin a candidate for the treatment of (residual) tissue congestion.

Chapter 5 focusses on dipeptidyl peptidase 3 (DPP3), which is a peptide responsible for cleavage of several enzymes in the renin angiotensin aldosterone (RAAS) system. DPP3 counteracts the antagonistic pathway of the RAAS-system, which is called the angiotensin-converting enzyme 2 (ACE2)–Ang(1–7)–Mas receptor (AAM) axis. This axis might be beneficial in heart failure, as it does the opposite of the classic RAAS-system, which is a central and detrimental part of heart failure pathophysiology. Predictors of DPP3 concentrations were related to either cell decay or to the RAAS-system. DPP3 was univariately associated with worse outcomes, however significance was lost after adjustment for confounders. While DPP3 was correlated to biomarkers and clinical signs of congestion, the correlations were weak and a clear distinction between intravascular and tissue congestion could not be made. As DPP3 is an actionable biomarker with an available monoclonal antibody, it might still be a treatment target in a subset of congested patients. Which specific subset of patients could benefit from DPP3-antagonism, is yet to be investigated.

In **Chapter 6** we investigated the clinical characteristics and biomarker profiles of patients with albuminuria and heart failure, and compared these to patients with heart failure, but without albuminuria. We found that in patients with new-onset or worsening heart failure, albuminuria was consistently related to clinical, echocardiographic, and circulating biomarkers of congestion. One potential explanation for the correlation between albuminuria and heart failure could be increased (central) venous pressures. This hypothesis is further supported by evidence from several preclinical studies. In these animal models clipping of the renal vein induced protein excretion in the urine.[13-15] Moreover, the clipping of the vein gave rise to microscopic podocyte injury, explaining the increase in urinary albumin in these animals.[15] The relationship between increased renal vascular pressures can also be found in clinical settings, such as the increase in urinary albumin excretion in the event of a renal vein thrombosis.[16] Alternatively, the correlation between albuminuria and congestion might be explained by endothelial dysfunction, underlying both destruction of the glycosaminoglycans (GAGs) at the level of the peripheral veins and the glomerular endothelium. As described in **Chapter 2**, increasing evidence indicates that destruction of GAGs in the interstitium lowers the threshold for fluid exiting the bloodstream into the interstitium.[17] GAGs also play a significant part in upholding the barrier function of the glomerulus and destruction has been described to initiate albuminuria.[18] Whether albuminuria might serve as a

biomarker specifically for intravascular or tissue congestion remains to be determined. If these findings are confirmed urinary albumin might serve as an early detection for congestion in patients with heart failure, although its (strong) relationship with co-morbidities, such as diabetes and hypertension, complicates its interpretation in a clinical setting.

Renal Compression

The relationship between albuminuria and congestion and the understanding of the detrimental local effects of congestion on tissues, led to the concept of intrarenal congestion as a potential explanation for the high prevalence of renal dysfunction observed in heart failure. In the second part of this thesis, we studied the effects of compression of the kidney and the renal vasculature. The increasing worldwide prevalence of obesity, and the shown detrimental local and systemic effects of adipose tissue on both the heart and the kidney completed the renal tamponade hypothesis. [19-23]

Chapter 7 provides this concept of a renal tamponade in patients with heart failure and renal disease. The concept builds the bridge between congestion and perirenal adipose tissue as important pathophysiological mechanisms in cardiorenal interaction. We propose the renal tamponade hypothesis to explain the disproportionate impairment in renal function when central venous pressures increase in patients with heart failure. The renal capsule surrounding the kidney is very rigid and will not allow expansion when pressures rise. Increased central venous pressures lead to increased renal interstitial pressures, compressing renal structures such as the glomeruli, tubules and intrarenal veins in the encapsulated kidney. Additionally, perirenal adipose tissue (PRAT) might likewise give rise to a different type of renal compression, further worsening renal function in these patients. While decreased renal perfusion may be difficult to influence, and indeed, attempts to improve renal perfusion have shown not be associated with improved outcome, intrarenal congestion may be a treatment target.[24] Future research is warranted to further elucidate the relationship between heart failure, congestion, obesity and impaired renal function. Ultimately, renal decompression therapies might be a novel therapeutic field to explore in decreasing the incidence of worsening renal function in the context of heart failure.

Chapter 8 tests the hypothesis formed in **Chapter 7** regarding perirenal adipose tissue (PRAT) in patients with heart failure and preserved ejection fraction. We found that patients with HFpEF have greater PRAT thickness than age-, sex and BMI-matched controls, which was associated with worse renal function. Increased renal sinus fat, the fat in the hilum of the kidney, was associated with worse renal function and adverse hemodynamics. These findings build on the hypothesis that compression of the renal

vasculature is an important mechanism, and perhaps even the missing link, between HFpEF and chronic kidney disease (CKD).

As obesity is a worldwide growing epidemic, the effects of excess adipose tissue on the entire body cannot be understated. [19-23] Adiposity leads to low grade local and systemic inflammation, resulting in fibrosis throughout the body.[25] The findings in this chapter again underline the local effects of adiposity and might further elucidate the complex interplay between the heart and the kidney.

A summary of the main findings of the different chapters can be found in Figure 1.

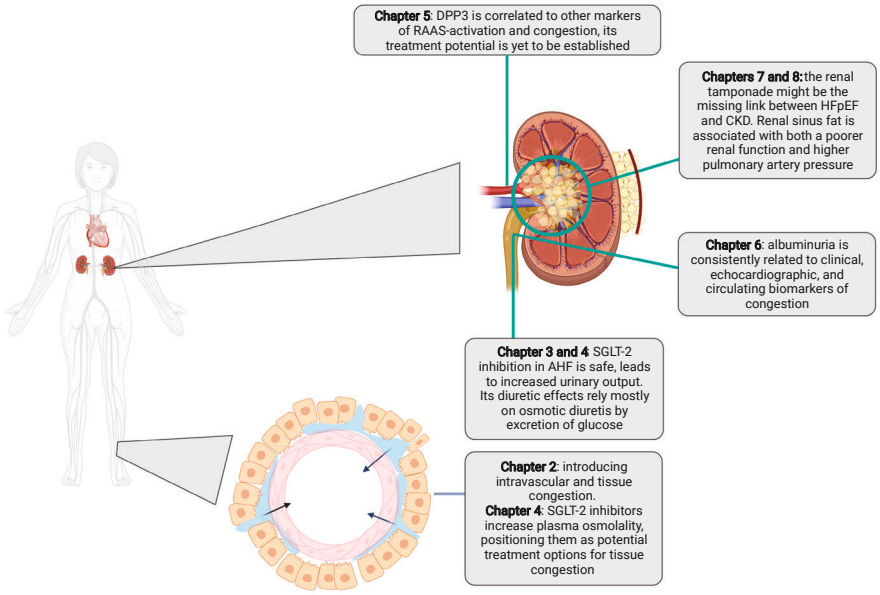


Figure 1 | Summary of the main findings of this thesis. Abbreviations: DPP3: dipeptidyl peptidase-3; RAAS: renin-angiotensin-aldosterone-system; HFpEF: heart failure with preserved ejection fraction; CKD: chronic kidney disease; SGLT-2: sodium glucose cotransporter-2; AHF: acute heart failure. Made with biorender.com

Future perspectives

Recognizing congestion

The results presented in this thesis pave the way for future research questions. While central to heart failure pathophysiology, a lot about congestion is still unknown. Two large limitations in heart failure database research are first the fact that information on onset of complaints is often missing. Based on our current understanding of onset of congestion, this can either be slow and gradual, oftentimes in the onset of tissue congestion. Several studies indicate that already two weeks before presentation at an emergency department, intravascular pressures start to rise. [26, 27] On the other hand, systemic venous congestion can have a very rapid onset and is likely caused by prompt emptying of the splanchnic vasculature. These two scenarios are decidedly different from each other and are likely both accompanied by different mechanisms, blood and urinary markers and optimal treatment strategies. Yet, these data are not usually captured and therefore the distinction between both is hard to make in a retrospective setting. The other limitation of this type of research is the fact that these data are usually collected in the setting of an investigational product, and the type of patients that suffer from rapid onset intravascular congestion are likely deemed too sick to participate. Combining these two limitations highlights the difficulties faced when attempting to retrospectively study different congestive subtypes. Future, prospective, research should therefore focus on differentiating between congestive subtypes based on duration between first onset of complaints and admission to hospital. After we succeed in identifying the right congestive subtypes based on clinical characteristics such as main site of fluid build-up and duration of onset, we should use large panels of biomarkers for network analyses, in order to understand the underlying pathways of both (or more) congestive subtypes. These pathways may then guide us towards novel treatment targets or to fine-tune our current treatments to the underlying biological disruptions.

Treating Congestion

When the (molecular and hormonal) pathophysiology underlying the different congestive subtypes is fully known, research should focus on treatment of the different types. Based on our findings in **Chapters 3 and 4**, we hypothesize that SGLT-2 inhibitors might be best suited to treat (residual) tissue congestion, a theory that is further supported by dr. Mullens and dr. Martens, who wrote an editorial about **Chapter 4**. They postulate that SGLT-2 inhibitors increase plasma refill rate, making them a very suitable agent to treat residual congestion in the interstitial space.[28] Whether this is indeed the case should be further evidenced by a decrease in biomarkers of tissue congestion after initiation of SGLT-2 inhibitors.

Additionally, the concept of congestion arising from the splanchnic vasculature provides starting points for future understanding and treatment of congestion. Preliminary research on the use of splanchnic nerve blocks in patients admitted with acute, therapy resistant heart failure shows promising results in creating vasodilation in the splanchnic vasculature to redistribute fluid back into this sequester.[29, 30] A large and obvious limitation of this treatment is its invasive nature, and although it may provide a treatment opportunity in a subset of patients, a non-invasive option would be preferable.

The high density of alpha receptors in the splanchnic vascular bed might be this non-invasive treatment target in the case of congestion originating from the splanchnic system. The use of alpha receptor blockers, such as doxasozin and tersozin, for patients in acute heart failure to alleviate symptoms, would be an interesting topic for research. In that same vein, patients with chronic heart failure who are prone to sudden onset vascular congestion might benefit from alpha blockage to prevent exacerbation. It should be noted however, that in the Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial (ALLHAT), designed to investigate the most optimal treatment regimen for patients with hypertension, the doxasozin arm was terminated prematurely due to a higher incidence of new onset heart failure.[31, 32] No trials on the use of non-selective alpha-1 receptor blockers in heart failure have been conducted to date.

Lastly, the lymph system plays a very important part in tissue congestion, as it is largely responsible for the draining of excess fluid from the interstitium. Novel therapies focusing on utilizing the lymph system in order to better drain the interstitium would be a very interesting and novel field in heart failure research. As all lymphatic vessels eventually drain in the thoracic ducts, which empties its contents in the left subclavian vein, reintroducing excess fluid into the blood stream, the thoracic duct could be a target for congestion treatment.[33] To date, published data on this topic is scarce. One older clinical study in twelve patients with therapy resistant heart failure indicated that external cannulation and drainage of the thoracic duct renewed lymph flow in the oversaturated lymph system, which led to a prompt reduction in clinical signs and symptoms of congestion.[34] As with splanchnic nerve blocks, this treatment is highly invasive and not suitable for all heart failure patients. However, it does provide a proof of concept for the use of the thoracic duct in congestion treatment. Future research should focus on medical or minimally invasive treatment of the thoracic duct to improve decongestion.

Renal Compression

In this thesis we introduce the concept of a renal tamponade responsible for the interaction between the heart and the kidney in heart failure. This renal tamponade can either be the result of congestion, or could result from adipose tissue around the kidney or within the sinus of the kidney. The first lines of evidence for this theory were provided in **Chapter 8**, in which we showed that patients with HFpEF on average had a larger perirenal adipose tissue thickness, compared to matched controls. Still, we should acknowledge that these data were collected retrospectively and while perirenal adipose tissue and renal sinus fat were associated with a worse renal function, we could only hypothesize that this was due to compression, RAAS-activation and diminished renal perfusion. To continue collecting evidence to support our renal tamponade hypothesis, a prospective study is currently ongoing, which is called PErirenal Adipose tissue and RenaL hemodynamics in patients with Heart Failure with Preserved Ejection Fraction; a Pilot Study (PEARL-HFPEF, NCT05219188). The PEARL-HFPEF is a single-center, cross-sectional, case-control observational study, including patients with HFpEF and healthy controls.

Since obesity is strongly related to increased PRAT thickness, but PRAT can also be increased in lean patients, we aim to include both patients with obesity (BMI >30 kg/m^2) and lean patients (BMI <25 kg/m^2). The same BMI -categories will be maintained for the healthy controls. Moreover, this allows us to distinguish whether our findings are more strongly related to obesity or to HFpEF.

Patients and healthy controls will be considered a match when they fulfill all 3 criteria below:

1. Are of the same sex
2. Are within 5 kg/m^2 (BMI) of each other, either higher or lower.
3. Are within a five-year age difference of each other, either older or younger.

During the testing visit outpatient HFpEF patients and healthy age, sex and BMI matched controls will undergo dynamic contrast enhanced computed tomography (DCE-CT) to assess renal perfusion and to measure perirenal and renal sinus fat according to the methods used in **Chapter 8**. Moreover, renal and cardiac ultrasonography will be performed on the same day. During 24 hours prior to the testing visit urine will be collected to evaluate natriuresis, which typically has a circadian rhythm and can therefore not be adequately evaluated from a spot urine sample. Moreover, a 24-hour urine sample will allow for calculation of creatinine clearance, as estimation of

glomerular filtration rate using solely serum creatinine typically underestimates GFR in obese subjects. On the day of DCE-CT and ultrasound, blood and spot urine samples will be collected as well from both the HFpEF patients and the healthy controls. A clinical congestion score, comprised of jugular venous distention, presence of rales, peripheral edema and orthopnea will also be taken. The primary objective is to confirm whether perirenal adipose tissue thickness is increased in patients with HFpEF compared with age, sex and BMI-matched healthy controls. The main secondary objective is to determine whether a greater PRAT volume correlates to impaired kidney perfusion on DCE- CT and on renal venous ultrasound in patients with HFpEF. A summary of the study design can be found in Figure 2.

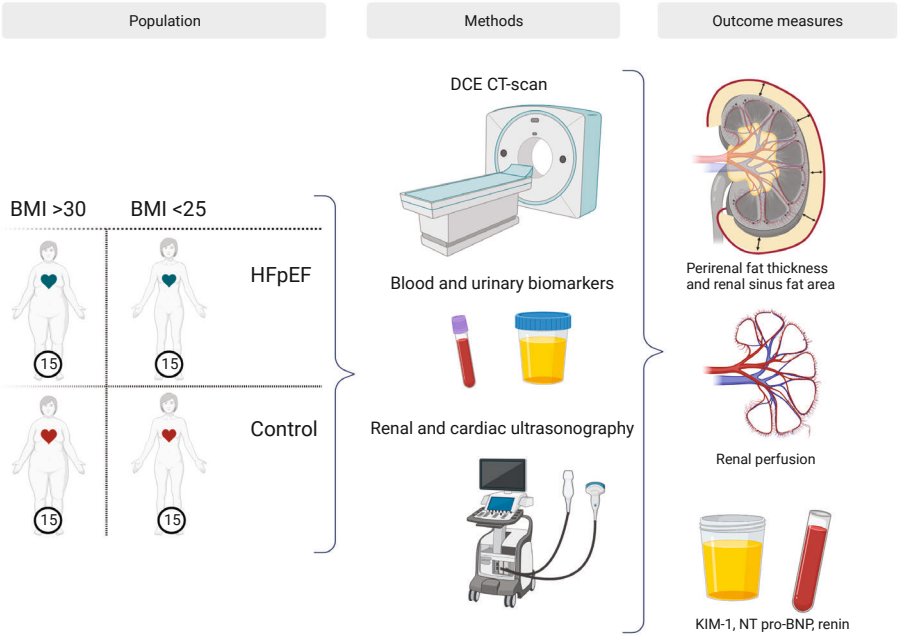


Figure 2 | Summary of the PEARL-HFPEF study protocol. Abbreviations: BMI: body mass index; HFpEF: heart failure with preserved ejection fraction; DCE CT-scan: dynamic contrast enhanced computed tomography scan; KIM-1: kidney injury marker-1; NT pro-BNP: N-terminal pro-brain natriuretic peptide.

Taken together, the studies presented in this thesis have advanced our knowledge about the pathophysiology of congestion in heart failure. For several decades heart failure has been thought of as a disease which leads to damage to other organs, in

particular the kidney, as a result of reduced cardiac output.[35, 36] Nevertheless, increasing evidence emerges which guides us to the conclusion that congestion might be as important, if not more important, in organ dysfunction in heart failure.[37-39] This thesis highlights the importance of recognizing the underlying mechanisms of congestion, in order to help us differentiate between different congestive phenotypes. Moreover, awareness of the detrimental effects of congestion and adipose tissue on organs outside of the heart is of the highest importance in optimally treating our patients. Ultimately, the goal is to improve outcomes in patients with congestive heart failure, as these outcomes to date are still very poor.[40]

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Appendices

Nederlandse Samenvatting

Hartfalen is een klinisch syndroom dat optreedt wanneer het hart onvoldoende in staat is het bloed rond te pompen. De onderliggende redenen hiervoor zijn talrijk en lopen uiteen van het doormaken van een hartinfarct, een ritmestoornis, een onderliggende genetische afwijkingen of een aandoening aan een van de hartkleppen.

Wanneer het hart onvoldoende bloed rondpompt, leidt dit tot een afname van het hartminuutvolume, wat vervolgens activatie van tal van hormonale systemen tot gevolg heeft. Activatie van het renine-angiotensine-aldosterone-systeem (RAAS) leidt tot vasthouden van natrium en water door de nieren, hetgeen uiteindelijk leidt tot 'overvulling': een overmaat aan vocht in het lichaam. Deze ophoping van vocht wordt ook wel congestie genoemd.

Congestie kan plaatsvinden in de bloedvaten, maar het overtollige vocht kan ook uittreden naar diverse weefsels. De bekendste locaties voor uittreden van vocht zijn de (onder)benen, de longen, en recenter ook de nieren. Deze ophoping van vocht wordt ook wel oedeem genoemd.

Congestie is van centraal belang in hartfalen. Niet alleen is dit fenomeen de veroorzaker van de meeste klachten en symptomen, het is tevens de belangrijkste reden voor ziekenhuisopname in patiënten met hartfalen. Bovendien is het zo dat als iemand eenmaal is opgenomen met hartfalen, de kans opnieuw opgenomen te worden voor hartfalen verdubbelt en de kans om te overlijden eveneens groter wordt. Ziekenhuisopnames voor hartfalen richten zich in het merendeel van de gevallen dan ook op het verlichten van congestie. Toch lukt het in een aanzienlijk deel van de patiënten niet om het overtollige vocht volledig kwijt te raken. Patiënten die worden ontslagen met persisterende congestie, hebben een nóg grotere kans om opnieuw opgenomen te worden.

Ondanks het belang van congestie in symptomatologie en behandeling van hartfalen, is er nog veel over onbekend. Momenteel wordt iedereen met congestie op dezelfde manier behandeld, ongeacht of het vocht zich primair in de vaten, de longen of de nieren bevindt. Het onderscheiden van verschillende profielen zou kunnen bijdragen bij het beter en gerichter behandelen van patiënten met hartfalen en de kans opnieuw opgenomen te worden in positieve zin kunnen beïnvloeden.

Deel 1: Congestie

In het eerste deel van dit proefschrift wordt congestie bestudeerd en worden op basis van klinische kenmerken en biomarkers uit het bloed en de urine verschillende congestieve profielen geïdentificeerd.

Hoofdstuk 2 presenteert een concept waarin onderscheid wordt gemaakt tussen twee vormen van congestie: systemische veneuze congestie en weefselcongestie. Systemische veneuze congestie is vochtophoping in de bloedvaten, weefselcongestie is ophoping van vocht in de verschillende weefsels van het lichaam. Het onderscheid tussen deze beide vormen van congestie is belangrijk voor de klinische praktijk, omdat beide vormen in theorie een unieke behandeling vergen. Systemisch veneuze congestie dient behandeld te worden door de nieren aan te zetten tot uitscheiding van met name zout (natriurese). Water volgt dan passief. Voor weefselcongestie is het van belang de samenstelling van het bloed zo te beïnvloeden dat vocht uit de weefsels terug de vaten in vloeit. In theorie kan dit bereikt worden door de uitscheiding van vrij water, of aquarese, waardoor de osmolaliteit van het bloed verhoogd wordt.

In **Hoofdstuk 3** zijn de effecten van empagliflozine op klinische uitkomsten bestudeerd bij 79 patiënten met acuut gedecompenseerd hartfalen. Ondanks dat empagliflozine geen effect had op de klachten van kortademigheid, diurese en NT pro-BNP, was er wel een gunstig effect van empagliflozine op verslechtering van hartfalen, heropnames of vroegtijdig overlijden. In **Hoofdstuk 4** bestuderen we de effecten van empagliflozine op de nier en de uitscheiding van glucose en natrium. De resultaten toonden dat patiënten met acuut hartfalen die werden behandeld met empagliflozine een toename van de uitscheiding van glucose lieten zien, maar geen toename van uitscheiding van natrium. Uit deze bevindingen concludeerden we dat bij (acuut) hartfalen de SGLT-2-remmer empagliflozine niet werkt als een natriureticum, noch als een aquareticum, maar als een osmotisch diureticum.

Hoofdstuk 5 richt zich op het peptide dipeptidyl peptidase 3 (DDP3). DPP3 is een peptide dat verantwoordelijk is voor het splitsen van verscheidene enzymen in het RAAS-systeem. Hoewel DPP3 een correlatie had met biomarkers en klinische verschijnselen van congestie, waren de correlaties zwak. Een duidelijk verschil tussen intravasculaire en weefselcongestie kon hierbij niet gemaakt worden.

In **Hoofdstuk 6** worden de de klinische karakteristieken en biomarker profielen van patiënten met albuminurie en hartfalen bestudeerd. De belangrijkste bevinding is dat in patiënten met hartfalen albuminurie consequent was geassocieerd met klinische, echocardiografische en circulerende biomarkers van congestie. Een mogelijke ver-

klaring voor deze correlatie zou verhoogde centraal veneuze druk kunnen zijn. Als alternatieve verklaring kan endotheeldysfunctie aangevoerd worden. Als onze bevindingen worden bevestigd, zou urine albumine een biomarker kunnen zijn voor de vroege detectie van congestie in patiënten met hartfalen. Hierbij is het van belang de sterke relatie met comorbiditeiten, zoals diabetes en hypertensie, in het achterhoofd te houden, aangezien deze relatie de interpretatie bemoeilijkt.

Deel 2: Compressie

In het tweede deel van dit proefschrift worden de effecten van externe druk op de nier en de renale vasculatuur bestudeerd: de renale compressie of tamponade. Verhoogde druk kan veroorzaakt worden door congestie in de nier of door (vet-) massa buiten de nier.

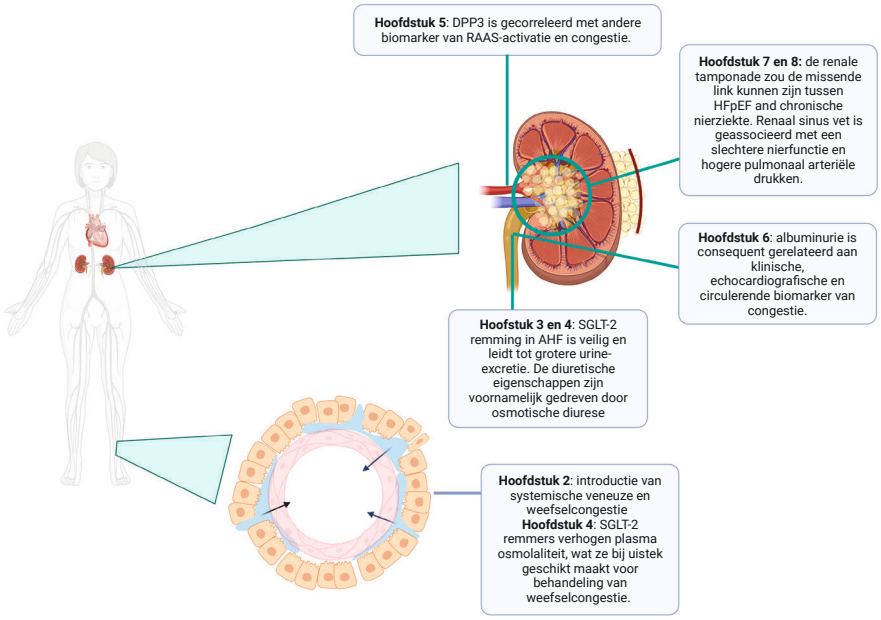
Hoofdstuk 7 introduceert het concept van deze renale tamponade in patiënten met hartfalen en nierfunctiestoornissen. Het renale kapsel dat de nier omsluit is erg stug en is niet in staat op te rekken zodra de druk hoger wordt. Verhoogde centraal veneuze druk leidt tot verhoogde renale interstitiële druk in de gekapselde nier, waarna intrarenale structuren, zoals de glomeruli, tubuli en intrarenale venen gecomprimeerd raken. De wereldwijde obesitasepidemie, gecombineerd met de schadelijke lokale en systemische effecten van vetweefsel op zowel het hart als de nier hebben de hypothese van de renale tamponade voorts gecompleteerd. In de toekomst zou behandeling gericht op renale decompressie een nieuwe therapeutisch veld kunnen zijn in de strijd tegen verslechterende nierfunctie in de context van hartfalen.

Hoofdstuk 8 onderzoekt de hypothese dat perirenaal vet is geassocieerd met een minder goede nierfunctie. We vonden dat patiënten met HFpEF meer perirenaal vet hebben dan leeftijd- geslacht, en BMI-gematchte controles, hetgeen geassocieerd was met een slechtere nierfunctie en nadelige hemodynamiek. Deze bevindingen versterken de hypothese dat compressie van de renale vasculatuur een belangrijke mechanisme en mogelijk zelfs de missende schakel is in de relatie tussen HFpEF en chronische nierziekte.

Een samenvatting van de belangrijkste bevindingen van de verschillende hoofstukken is grafisch afgebeeld in **Figuur 1.**

Concluderend heeft het huidige proefschrift de kennis omtrent de onderliggende ziektemechanisme bij congestie vergroot. Dit proefschrift benadrukt verder het belang van het onderkennen van verschillende soorten congestie. Bovendien creëren de huidige studies bewustwording van de schadelijke effecten van zowel congestie als vetweefsel

op organen buiten het hart. Het doel van al deze studies is om patiënten met hartfalen uiteindelijk beter te kunnen behandelen.



Figuur 1 | Samenvatting van de belangrijkste bevindingen van dit proefschrift. Afkortingen: DPP3: dipeptidylpeptidase-3; RAAS: renine-angiotensine-aldosteron-systeem; HFpEF: hartfalen met behouden ejectiefractie; SGLT-2: natriumglucose-cotransporter-2; AHF: acuut hartfalen.

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About the author

Eva Maria Boorsma was born on August 28th, 1992 in Leermens, the Netherlands. At 1 year of age she moved to Groningen. In 2010 she graduated high school and started studying medicine. During her Bachelor in Medicine at Groningen University she was part of the organizing committee for the 2012 Summer School on Global Health, during which she met many medical students from across the globe. She did her clinical rotations partly on Curacao, where she first got in touch with and got excited about clinical cardiology. Moreover, on Curacao she was chair of the intern representation committee. After returning to the Netherlands, she conducted her research fellowship on intracutaneous and hepatic amyloidosis, with the department of Rheumatology in the University Medical Centre Groningen (UMCG). After obtaining her MD, Eva started working in the department of Cardiology in Leeuwarden Medical Centre, where her interest in cardiology was further established.

Eva started as a junior physician in the UMCG in September 2018. Here she met prof. dr. Adriaan Voors, who got her excited about research and later became her first promotor. She started her PhD research in April 2019, under the supervision of prof. dr. Voors, prof. dr. van Veldhuisen and dr. ter Maaten, several chapters of which she presented at national and international congresses. Her PhD research resulted in this thesis, which she plans to defend on February 15th, 2023.

Eva has started her clinical Cardiology Residency on September 1st 2022 and is currently doing her internal medicine rotations at the Martini Hospital, Groningen.