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Faculteit Geneeskunde en Levenswetenschappen
School voor Levenswetenschappen

master in de biomedische wetenschappen

Masterthesis

Urinary sodium as a predictor for heart failure readmission

Frauke Somers

Scriptie ingediend tot het behalen van de graad van master in de biomedische wetenschappen, afstudeerrichting klinische moleculaire wetenschappen

PROMOTOR :

Prof. Dr. Wilfried MULLENS

De heer Pieter MARTENS

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List of abbreviations

A4C/A2C	Apical four chamber/apical two chamber
A	Late diastolic inflow velocity
ACC/AHA	American College of Cardiology/American Heart Association
ACE	Angiotensin-converting enzyme
ACEIs	Angiotensin-converting enzyme inhibitors
ADH	Antidiuretic hormone
ADHF	Acute decompensated heart failure
AHF	Acute heart failure
ANG I – II – III – IV	Angiotensin I – II – III – IV
AR1	First-order autoregressive
ARNIs	Angiotensin receptor-neprilysin inhibitors
ARBs	Angiotensin receptor blockers
CHF	Chronic heart failure
CO	Cardiac output
COPD	Chronic obstructive pulmonary disease
DBP	Diastolic blood pressure
E	Early diastolic inflow velocity
e’	Mitral annulus early diastolic velocity
ECG	Electrocardiogram
EDV	End-diastolic volume
ESC	European Society of Cardiology
ESV	End-systolic volume
GFR	Glomerular filtration rate
HF	Heart failure
HFmrEF	Heart failure with mid-range ejection fraction
HFpEF	Heart failure with preserved ejection fraction
HFrEF	Heart failure with reduced ejection fraction
HR	Heart rate
ID	Identification
IQR	Interquartile range
IV	Intravenous
IVC	Inferior vena cava
LVEF	Left ventricle ejection fraction
MRAs	Mineralocorticoid receptor antagonists
NYHA	New York heart association
NT-proBNP	N-terminal pro-B-type natriuretic peptide
Parox. noct. dysp.	Paroxysmal nocturnal dyspnea
PLAX	Parasternal long-axis
QOL	Quality of life
RAAS	Renin-angiotensin-aldosterone system
RAP	Right arterial pressure
RVSP	Right ventricle systolic pressure
SBP	Systolic blood pressure
SNS	Sympathetic nervous system
Std	Standard deviation
SV	Stroke volume
TTE	Transthoracic echocardiography
UCAI(_Osm)	Urinary chloride augmentation index
USAI(_Osm)	Urinary sodium augmentation index
ZOL	Ziekenhuis Oost-Limburg

Abstract

Background: Congestion is the main reason for hospitalization of patients with acute decompensated heart failure (ADHF). The increase in filling pressures, caused by sodium and water retention, results in typical heart failure symptoms such as peripheral edema and dyspnea. The therapeutic approach to treat congestion is the administration of diuretics, which leads to excretion of the excessive amount of sodium and water. Nevertheless, ADHF patients have a 15% to 30% chance of hospital readmission 60 to 90 days after hospital discharge, respectively. Close monitoring of reoccurrence of congestion after hospitalization can improve the patients' quality of life and survival. A promising tool to assess the development of congestion driven by sodium retention might be the assessment of urinary sodium concentration. We hypothesized that the urine composition of ADHF patients, the weeks following hospital discharge, serves as a predictor for recurrence of congestion.

Subjects and Methods: Hospitalized ADHF patients were included in a prospective observational study (MORE-RESPONSE). At baseline, subjects underwent thorough congestion evaluations consisting of transthoracic echocardiography, renal Doppler ultrasonography, bio-impedance measurement, blood sampling and a general clinical examination. Subjects were taught on the performance of urinary spot sampling, which needed to be performed at home twice a day over 14 days. At follow-up (30 ± 11 days), urinary spot samples were handed over and subjects underwent the same examinations as at baseline. The relation between recurrence of congestion and ambulatory urinary spot concentrations of sodium and chloride was assessed.

Results: A total of 14 subjects (male=11 (78.3%); age=73 ± 7 years; NYHA class II=9 (64.3%); heart failure with reduced ejection fraction=7 (50%)) prospectively collected urine spot samples twice a day on predetermined time points, resulting in one pre- and one post-diuretic urine sample. Post-diuretic urinary sodium (U_{Na+}) values were subtracted from the pre-diuretic U_{Na+} values and divided by the osmolality (Osm), resulting in a urinary sodium augmentation index (USAI_Osm) mol/Osm. On a population level, USAI_Osm levels remained stable over time (time-effect p=0.564). Usage of the worsening linear congestion scale enabled to subdivide the study population in a non-congested (n=7) and congested (n=7) group, at follow-up. Comparison of the mean USAI_Osm values of both groups illustrated two distinct urine profiles. As such, subjects of the non-congested group exceeded the USAI_Osm values of the congested group (p=0.023). Subjects who were hospitalized due to ADHF after study enrollment (n=3) showed a trend towards a USAI_Osm profile inferior to the USAI_Osm profile of the non-hospitalized subjects (p=0.158).

Discussion and Conclusion: Main findings of the urinary profiles of ADHF patients in the first weeks after hospitalization are that the urinary sodium output (USAI_Osm) remained stable during the study period, however, inter-individual differences are present as well. Subdivision of the study population in a congested and non-congested group showed that USAI_Osm output of the congested group was significantly inferior to the output of the non-congested group. Moreover, subjects hospitalized for ADHF after study enrollment had a lower USAI_Osm compared to non-hospitalized subjects. Demonstrating that urinary sodium output might be a metric to predict the risk of congestion reoccurrence and ADHF hospital readmission.

Samenvatting

Inleiding: Congestie is de belangrijkste oorzaak van hospitalisaties bij patiënten met acuut gedecompenseerd hartfalen (ADHF). Congestie wordt veroorzaakt door retentie van natrium en water, dit resulteert in stijgende vuldrukken van het hart. Deze hoge vuldrukken leiden tot typische hartfalen symptomen zoals perifere oedemen en dyspneu. De voornaamste behandelingsmethode voor congestie is de toediening van diuretica. Diuretica induceert excretie van het overtollige natrium en water dat aanwezig is in het lichaam. Desalniettemin hebben ADHF patiënten 15% tot 30% kans om heropgenomen te worden in het ziekenhuis respectievelijk 60 tot 90 dagen na ziekenhuisontslag. Door nauwe monitoring van de evolutie van congestie na hospitalisatie, zou de levenskwaliteit en de overleving van de patiënt verbeterd kunnen worden. Het meten van de urinaire natriumconcentratie kan een veelbelovende tool zijn om de ontwikkeling van congestie te bepalen. We hypothetiseren dat de urinaire natriumconcentratie van ADHF patiënten als een predictor kan dienen voor recidive congestie.

Patiënten en methoden: Gehospitaliseerde ADHF patiënten werden geïncludeerd in een prospectieve observationele studie (MORE-RESPONSE). Aan het begin van de studie werden de patiënten geëvalueerd op aanwezigheid van congestie(symptomen) doormiddel van een trans-thoracale echografie, renale Doppler echografie, bio-impedantie meting, bloedafname en een algemeen lichamelijk onderzoek. Studiedeelnemers werden opgeleid om urine-spot stalen af te nemen. Deze urinestalen werden thuis, twee keer per dag gedurende een periode van 14 dagen afgenomen. Aan het einde van de studie (gemiddelde follow-up van 30 ± 11 dagen) werden de urine-spot stalen ingeleverd en ondergingen de studiedeelnemers dezelfde onderzoeken als aan het begin van de studie. De relatie tussen recidive congestie en de ambulante urine spot concentraties van natrium en chloor werden onderzocht.

Resultaten: Een totaal van 14 patiënten (mannelijk=11 (78.3%); leeftijd=73 ± 7 jaar; NYHA klasse II=9 (64.3%); hartfalen met verminderde ejectiefractie=7 (50%)) hebben prospectief, tweedaags urine spot stalen afgenomen. Dit resulteerde in een pre- en een post-diuretisch urinestaal. Het urinair natrium van deze twee stalen werd van elkaar afgetrokken en gedeeld door de osmolaliteit (Osm) wat resulteerde in een urinair natrium verhogingsindex (USAI_Osm) mol/Osm. Gedurende de studieperiode waren de USAI_Osm waarden van de hele populatie stabiel (tijdseffect p=0.564). Gebruikmakend van de congestie schaal werd de populatie na follow-up opgesplitst in een niet-congestie (n=7) en een congestie (n=7) groep. Vergelijking van de gemiddelde USAI_Osm waarden toonden twee verschillende urineprofielen aan. Patiënten van de niet-congestie groep hadden een significant hogere USAI_Osm vergeleken met de congestie groep (p=0.023). Patiënten die werden opgenomen voor ADHF na studiedeelname (n=3) hadden een lagere USAI_Osm vergeleken met de waarden van de niet gehospitaliseerde patiënten (p=0.158).

Discussie en conclusie: De natriurese van de hele studiepopulatie bleef constant tijdens de studieperiode, maar individuele verschillen werden waargenomen. Opsplitsen van de populatie in een (niet)-congestie groep, toonde een significant lagere USAI_Osm output in de congestie groep. Ook patiënten die werden opgenomen voor ADHF toonden een lagere USAI_Osm output. Dit toont aan dat urinair natrium wel degelijk een tool kan zijn om het risico te voorspellen op recidive congestie.

1. Introduction

Heart failure (HF) is a clinical syndrome in which the heart is unable to pump sufficient blood to the peripheral tissues, to meet the metabolic demand of the body (1, 2). Approximately 1% to 2% of the adult population are diagnosed with HF. HF affects mostly the elderly population with a prevalence of 10% in people older than 70 years (1, 3-5). Moreover, HF is the main cause of hospitalization in patients of 65 years and older (6).

The development of HF is most often induced by a primary cardiac event which injures the heart. Cardiac events such as myocardial infarction, valve diseases, endo- pericardial abnormalities, heart rhythm disorders, or other myocardial diseases, can occur acutely or chronically and lead to structural and/or functional cardiac abnormalities. Certain risk factors (table 1) increase the occurrence of these cardiac events. If such an event arises, the heart's ability to deliver the required amount of blood to the periphery is compromised. As a result, the cardiac output (CO) will be reduced. The decrease in CO will be compensated by various compensatory mechanisms. However, these compensatory mechanisms will eventually fail and cause clinical manifestations such as peripheral edema, dyspnea, fatigue and/or tachycardia (1, 2).

Table 1| Risk factors of heart failure (1).

Risk factors		
High blood pressure	Coronary artery disease	Myocardial ischemia/infarction
Hyperglycemia	Diabetes	Hypocholesteremia
Obstructive sleep apnea	Drug abuse	Excessive alcohol consumption
Connective tissue disorders	Congenital heart defects	Family history
Smoking	Obesity	Arrhythmias

1.1 Development of heart failure

A primary injuring cardiac event causes the development of HF, as previously mentioned. Once structural and/or functional damage is present in the heart, the myocardial pump function is impaired leading to a decrease in circulatory volume (7). This 'underfilling' of the heart is sensed by baroreceptors in the aortic arch, carotid sinus, peripheral chemoreceptors, and also in ergoreceptors (7-9). To increase the CO and circulatory volume, these sensory receptors activate compensatory mechanisms which try to increase the CO and therefore, maintain the cardiac function (1, 7). An overview of the compensatory mechanisms and their effects is shown in figure 1.

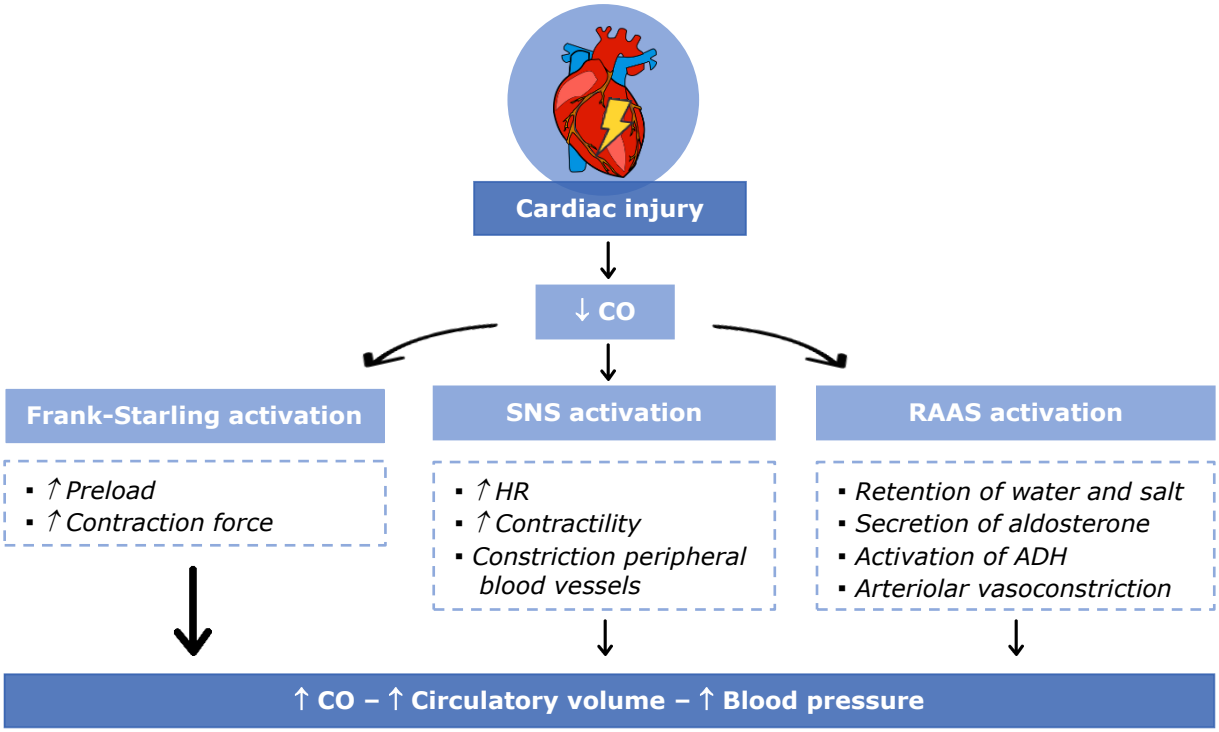

Figure 1| Heart failure compensatory mechanisms. A decrease in cardiac output (CO) induces activation of the Frank-Starling mechanism, the sympathetic nervous system (SNS), and renin-angiotensin-aldosterone system (RAAS). The effects of these mechanisms lead to an increase in CO, circulatory volume and blood pressure. HR: heart rate; ADH: antidiuretic hormone.

1.1.1 Cardiac compensatory mechanisms

The pumping mechanism of the heart is highly regulated (1). In physiologic conditions, the ventricles of the heart fill with blood until the end-diastolic volume (EDV) is reached. The EDV is defined by the preload, i.e. the stretching of the myocardial fibers. By increasing the pressure to the point where the afterload is reached, i.e. the pressure that the ventricle must overcome to eject blood, the ventricles are emptied until the end-systolic volume (ESV) is reached. Once the low pressure in the ventricles is reestablished, the cardiac pumping process restarts. This physiological process ensures an optimal CO according to the needs of the human body. By changing several factors of this process, such as the preload, the CO can be altered (10).

In pathological conditions, such as HF, alterations to the physiological pumping mechanism of the heart are carried out to improve the CO, since the contractility of the ventricle is reduced, which generates a stroke volume deficit (1). The Frank-Starling mechanism is a compensatory mechanism which acts to increase the circulatory stroke volume/CO by increasing the contraction force and preload. The increase in CO is accomplished by increasing the stroke volume (SV), as the CO is the product of SV and heart rate. The SV can be increased by elevating the preload and altering the contraction force (10). This compensation is effective in the beginning, however, over time these cardiac changes will lead to cardiac remodeling. During cardiac remodeling, irreversible macro- and microstructural changes of the heart occur such as hypertrophy, ventricular dilatation, and an increase in left ventricle compliance. These structural changes ultimately lead to cardiac dysfunction and deterioration of HF condition (11, 12).

1.1.2 Neurohumoral compensatory mechanisms

Another compensatory mechanism that aims to maintain the circulatory volume and perfusion pressure, is the neurohumoral system, which includes the renin-angiotensin-aldosterone system (RAAS), and the sympathetic nervous system (SNS) (1, 7). The responses of RAAS and SNS are defined by *Packer et al.* as the 'neurohormonal hypothesis' (13).

Physiologically, the high-pressure baroreceptors in the aortic arch and the carotid sinus, and the low-pressure cardiopulmonary baroreceptors inhibit activation of the SNS. However, since the circulating volume and contraction force of the heart is decreased in HF, the inhibitory signals of the baroreceptors on the SNS will diminish, causing activation of the SNS. Activation of the SNS has a positive inotropic effect on the heart, meaning that the cardiac contractility and heart rate increases, and a vasopressive effect, causing constriction of the peripheral blood vessels (1, 7, 14, 15).

As a consequence of the peripheral vasoconstriction at the level of the afferent renal artery, blood flow towards the kidney is reduced. The reduction in renal blood flow activates the RAAS, which acts on the decreasing circulatory volume by retention of water and salt (fig. 2 and 3). This system is initiated with the release of renin from the juxtaglomerular apparatus of the kidney into the afferent renal arteriole (1, 7, 15). The release of renin is potentiated by SNS activation of the β1-adrenergic receptors of the juxtaglomerular apparatus. In the liver, renin converts angiotensinogen to angiotensin I (ANG I) (16). Subsequently, ANG I is converted to angiotensin II (ANG II) by angiotensin-converting enzyme (ACE) in the pulmonary capillaries. ANG II can be further metabolized by aminopeptidase A into angiotensin III (ANG III) and enzymatic action of aminopeptidase N converts ANG III into angiotensin IV (ANG VI) (fig 2) (1, 7).

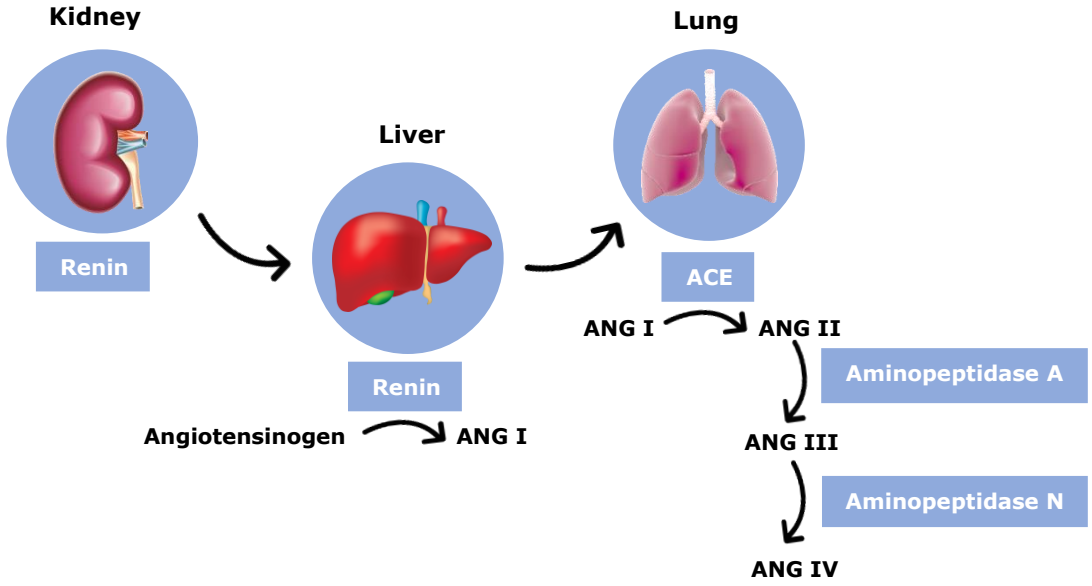

Figure 2| Angiotensin forming pathway. Renin is released from the juxtaglomerular apparatus in the kidneys. In the liver, angiotensin is converted into angiotensin I (ANG I) by renin. In the pulmonary capillaries, ANG I is converted into angiotensin II (ANG II) by angiotensin-converting enzyme (ACE). ANG II is further converted into angiotensin III (ANG III) by aminopeptidase A. Aminopeptidase N converts ANG III into angiotensin IV (ANG IV).

ANG II is the main bioactive molecule of the RAAS and has several effects on the body, shown in figure 3. Firstly, ANG II stimulates sodium retention in the proximal tubule and thick ascending loop of Henle in the kidneys. The retention of sodium is accompanied by the retention of water. Aldosterone causes resorption of sodium in the distal tubule of the kidneys also leading to fluid retention (1, 7). Thirdly, ANG II potentiates the release of antidiuretic hormone. This hormone increases free water reabsorption from the collecting tubules of the kidney (1, 17, 18). Finally, ANG II induces vasoconstriction of the renal efferent arterioles. The effects of ANG III are mainly on the aldosterone metabolism by stimulating the adrenal glands to produce aldosterone. In addition, ANG III also activates the resorption of sodium in the distal collecting duct of the kidney (fig. 3) (7, 19). ANG IV has an ANG II-like effect but with lower efficacy.

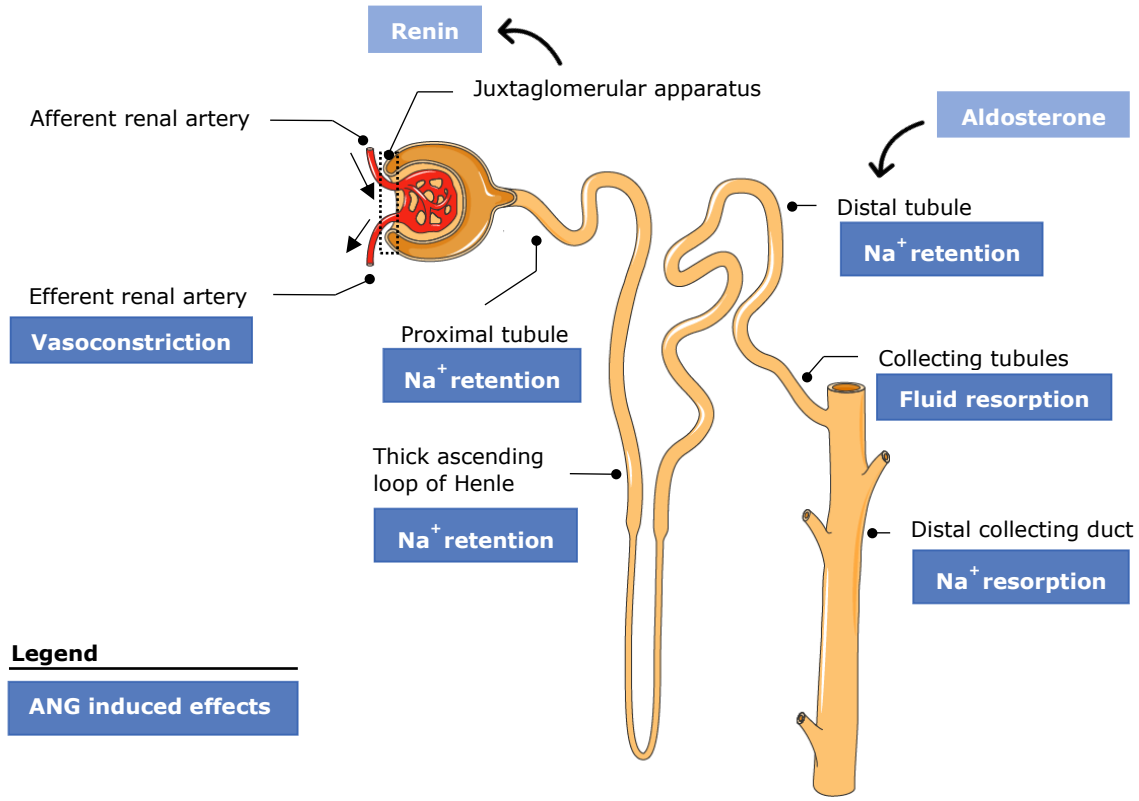

Figure 3| Angiotensin induced effects in the nephron. Angiotensin (ANG) induces sodium (Na^+) retention in the proximal tubule, the thick ascending loop of Henle, distal tubule, and distal collecting duct. ANG also causes vasoconstriction of the efferent renal artery and fluid resorption in the collecting tubules of the nephron.

As a counter-mechanism, the natriuretic peptides, brain natriuretic peptide and atrial natriuretic peptide are released from the heart chambers to counterbalance the effects induced by RAAS and SNS. These peptides promote vasodilatation and excretion of sodium in the urine, i.e. natriuresis. However, the natriuretic effect of these peptides cannot overcome the RAAS induced effects (1, 20). In conclusion, the (in)direct effects of ANG II, ANG III, and ANG IV determine the sodium-fluid regulation in the human body (1).

Although the initiation of these responses has beneficial short-term effects on the circulating volume, ultimately it contributes to the deterioration of the heart function and the circulation (21, 22).

1.2 Subtypes of heart failure

HF is a dynamic disease in which periods of clinical stability (chronic HF (CHF)) are disrupted by bouts of acute decompensation (acute HF (AHF)) (1, 23). The treatment goals in AHF and CHF are somewhat diverse. In AHF the goal is to achieve euvolemia without compromising organ function. While in CHF the goal is to beneficially influence functionality, prevent reoccurrence of congestion, and prolong life (24).

1.2.1 Chronic heart failure

Within the CHF subtype, the precise therapy is dictated by the underlying cardiac abnormality, which is typified by the left ventricle ejection fraction (LVEF) (20). Defining HF with reduced ejection fraction (HFrEF) as a LVEF of < 40%, HF with mid-range ejection fraction (HFmrEF) as a LVEF of > 40% to < 50%, and a LVEF of ≥ 50% is considered as HF with preserved ejection fraction (HFpEF), according to the European Society of Cardiology (ESC) guidelines of 2016 (2, 20, 24, 25). HFrEF and HFpEF are two distinct syndromes with definite pathophysiology, while the pathophysiology of HFmrEF is less clear (1, 24).

In HFrEF, also known as systolic HF, the ejection fraction is reduced due to impaired ventricle contraction. As a compensatory mechanism, the ventricles dilate to improve the circulatory volume. Ultimately, it causes volume overload of the heart (1, 20). HFpEF, also known as diastolic heart failure, is characterized by an impaired filling of the ventricle, impaired ventricle relaxation and an increased ventricle stiffness. As a response to these events, ventricular hypertrophy occurs, eventually causing pressure overload (1, 25). This difference in pathophysiology requires a different therapeutic approach. A recent study of *Bhambhani et al.* demonstrated that HFmrEF was more closely related to HFrEF with respect to clinical course. The potential therapies for HFmrEF could, therefore, be similar to those of HFrEF (24).

1.2.2 Acute heart failure

AHF is the stage during which acute exacerbations of congestion symptoms are present. Treatment of these symptoms, i.e. peripheral edema, dyspnea, and fatigue requires immediate hospitalization (1, 23, 26). Congestion is a typical characteristic of AHF and is defined as high left ventricular diastolic pressure or high filling pressures. Congestion arises due to an increased plasma volume and an increase in the extracellular fluid compartment, as a result of the neurohumoral activation of angiotensin and aldosterone (26, 27). These hormones favor sodium retention in the extracellular compartment, leading to fluid accumulation and retention (27). An increase in filling pressures can be present without symptoms of volume overload, this state is known as hemodynamic congestion. A further increase of the cardiac filling pressures causes symptoms of congestion such as dyspnea and peripheral edema (26, 27). This state of clinical congestion succeeds the phase of hemodynamic congestion by several days to three weeks (28). Clinical congestion always requires hospitalization to treat the symptoms (3).

AHF can also be subdivided into two subtypes, i.e. de novo AHF and acute decompensated HF (ADHF). De novo AHF is the first onset of exacerbating HF symptoms. The acute worsening of CHF symptoms, i.e. acute decompensation of CHF, is termed ADHF (2, 4).

Regarding all the HF patients, patients suffering from ADHF are most hospitalized (80%) (6, 29). Hospitalization rates of patients with de novo AHF accounts for 15% of all the HF hospitalizations. The remaining 5% are hospitalizations of patients with end-stage HF (6). Despite good clinical care, hospitalized HF patients have a 15% to 30% chance of hospital readmission 60 to 90 days after hospital discharge, respectively (29, 30). This post-discharge period is known as the vulnerable phase. In this phase, the filling pressures have elevated due to early adverse events, causing clinical congestion. In general, patients will experience an improvement in symptoms of congestion after treatment at the hospital. Nevertheless, most patients are discharged from the hospital without complete decongestion. At home, the patient should completely decongest with the correct therapy. However, if a patient's diuresis is incomplete at home, the filling pressures fail to normalize. As a result, an incomplete decongestion of the patient at home might correlate to these early readmission rates. An early post-discharge follow-up in de vulnerable phase could improve patients' outcomes and reduce hospital readmission rates (30).

1.3 Management of heart failure

There are several aspects for optimal HF management. The main goal of HF treatment is to maintain a stable disease state and to relieve the patient from worsening HF symptoms if these are present (2). This will improve the patient's quality of life (QOL) and prevent hospital (re)admission. Another significant factor of the therapeutic approach are the patients themselves. HF patients are advised to perform certain self-management strategies which contribute to optimal HF management (31).

1.3.1 Pharmacological heart failure management

HF medication targets the two main systems, responsible for the HF-related symptoms. The positive ionotropic effects of the SNS is counterbalanced by beta-blockers which lower the heart rate. The RAAS induced effects on the volume status of the patient are targeted by variant drugs, which each reduce the activity of RAAS on a different level. These are ACE-inhibitors (ACEIs), angiotensin receptor blockers (ARBs), mineralocorticoid antagonists (MRAs) and angiotensin receptor-neprilysin inhibitors (ARNIs). They inhibit the conversion of ANG I to ANG II, intervene with the binding of angiotensin to its receptor, and block the binding of aldosterone to its receptors, respectively (2, 32). ANRIs are a combination of the ARB valsartan and a neprilysin inhibitor prodrug sacubitril. Besides blocking the angiotensin receptor, ARNIs also inhibit the enzyme responsible for breakdown of natriuretic peptides and other small biologic peptides (33). A correct combination and dosage of these drugs, according to the patient's needs, improves the clinical status of the patient (2, 32).

ACEIs, ARBs, MRAs and ARNIs attempt to prevent volume overload (2, 32). Nevertheless, congestion is a frequent HF symptom. The main treatment option of volume overload is the administration of diuretics. The goal of diuretic treatment is to excrete the excessive amount of fluid and to achieve decongestion in a patient by enhancing the renal natriuresis, i.e. sodium excretion (34, 35). Loop diuretics enhance sodium excretion by inhibiting the sodium-potassium-chloride symporter in the thick ascending loop of Henle. This symporter is responsible for up to 25% reabsorption of the filtered salt. Thus, by blocking this symporter, a significant natriuretic effect is achieved. If loop diuretics are inadequate to achieve decongestion, other diuretics can be administered to the patient (36, 37) such as thiazides, which affect the sodium-chloride cotransporter in the ascending loop of Henle and in

the distal nephron (34, 38). However, despite the use of the therapeutically maximal recommended (loop) diuretic doses, some patients fail to achieve decongestion. This failure to reduce the excessive amount of salt and free water is termed as diuretic resistance, which will ultimately result in the (re)occurrence of congestion and clinical deterioration (36).

1.3.2 Self-management

HF management is partially dependent on the actions of the HF patient. HF self-management aims to improve the patients' outcome and to lower the recurrence of congestion (31). To optimize the fluid balance in the body, HF patients are advised to frequently measure their weight and to restrict their dietary salt and fluid intake (27, 34). Changes in fluid balance can be monitored by weight measuring. To minimize changes in fluid balance or to tip the balance towards fluid excretion, the American College of Cardiology/American Heart Association (ACC/AHA) and ESC set guidelines for dietary fluid and salt restriction. The guidelines recommend a fluid restriction of 1.5 – 2 liter/day and a sodium restriction of 2 – 3 g/day (2, 39).

1.3.3 Facing problems

Management of HF is a combination of both an appropriate pharmacological treatment and patient self-management. A correct patient self-care by salt and fluid restriction, and frequent weight measuring should improve the patients' outcome. However, the accuracy of this metric, and the positive effect of fluid and salt restriction is disputed (34).

Dietary salt restriction is the main self-care recommendation in HF patients since they are unable to excrete sodium and water due to neurohumoral activation of RAAS. However, the beneficial effect of salt restriction is contradicted in literature (34). Some reports indicate that salt restriction has a neutral (40-42) or a disadvantageous (43-45) effect on the patients' outcome. The disadvantageous effects of dietary salt restriction in certain HF patients might relate to an abundant diuretic use and too strict salt restriction, which causes 'underfilling' of the patient followed by neurohumoral activation of RAAS (46). Therefore, diuretic use and dose should be tailored to the patients' individual needs (27).

Fluctuations in body weight can reflect changes in volume status (26). Therefore, the ACC/AHA and ESC guidelines advice to frequently measure body weight at home (2, 39). Nevertheless, these fluctuations in body weight may not be representative of changes in intravascular volume or filling pressures (26, 47). In the hospital, fluctuations in body weight are also measured to assess the process of decongestion in HF patients. However, accurate measurements of changes in weight are challenging in the hospital. Furthermore, body weight measurement and administration of diuretics occur non-simultaneously. This delay is not beneficial when assessing for the status of decongestion and cannot be reliably used to asses for rising filling pressures (48-50). Therefore, a new method should be implemented to correctly asses the state of congestion.

1.4 Research aims

Congestion is a typical HF symptom especially in patients with ADHF (6). Increase in filling pressures, caused by sodium and water retention, has an unfavorable impact on the patients QOL. Patients suffer from peripheral edema, dyspnea, and fatigue. To treat these symptoms, ADHF patients need to be hospitalized and diuretics are administered (1, 26). Diuretics block important symporters in the kidney responsible for sodium and fluid (re)adsorption. By blocking these symporters, the excessive amount of sodium and water is excreted via the urine and decongestion is achieved (36). Once patients are clinically free from congestion symptoms, they are discharged from the hospital.

In the period after discharge, i.e. the vulnerable phase, HF patients have a 15% to 30% chance of hospital readmission 60 to 90 days after discharge, respectively. Despite HF medication and self-care actions such as salt and fluid restriction, congestion reoccurred in this period (29, 30). Therefore, a new method is welcomed, which could identify patients at risk for the development of congestion after hospital discharge.

Previous studies have investigated the role of urinary sodium concentration during episodes of ADHF as a new metric for the assessment of congestion. They demonstrated that a low urinary sodium excretion correlates with a diminished response to diuretic treatment, causing progression of congestion and an increased risk for hospital readmission (48, 49, 51-56). However, little is known about the urinary sodium concentration in relation to the congestion status in HF patients' transition from ADHF to CHF in the vulnerable phase.

The hypothesis of this investigation is that the urine composition of ADHF patients, in the weeks following hospital discharge, serves as a predictor for congestion reoccurrence. The objectives are to examine the increase in sodium excretion, following diuretic administration. This is done by a urinary sodium augmentation index. The second objective is to investigate the correlation of urinary sodium concentration and the degree of congestion. Monitoring of the diuretic efficacy at home helps to better understand the development of congestion driven by sodium retention. The aim is to set an optimal range of urinary sodium concentration, associated with the lowest rate of recurrence of congestion and hospital readmission. In the future, this range can be used in the clinic to identify patients at risk for the recurrence of ADHF, which enables to take early measures to halt the development of congestion. This early identification will improve patients' QOL and survival.

2. Subjects and methods

A prospective observational study (MORE-RESPONSE study) was executed from November 2018 until August 2019 at the Department of Cardiology of the hospital 'Ziekenhuis Oost-Limburg' (ZOL, Genk, Belgium). The study complied to the Declaration of Helsinki and an ethical approval was given by the local institutional review board (B371201526234). Written informed consent was provided by all patients before study enrollment.

2.1 Study population

A total of 14 patients were prospectively enrolled in the hospital (ZOL, Genk) between January 2019 and June 2019. Subjects were eligible if they were able to provide a written consent and were hospitalized with AHF, i.e. ADHF, regardless of ejection fraction. Table 2 indicates the inclusion and exclusion criteria of eligible patients.

Table 2| Inclusion and exclusion criteria of MORE-RESPONSE study population.

Inclusion criteria	Exclusion criteria
Age: 18 years ≥ 85 years	Patient is unable to correctly gather urine samples or adhere to the protocol
AHF hospitalization > 48 hours requiring IV diuretics	Renal replacement therapy
Medication: loop diuretics	Absence of a freezer at home (-18°C)

AHF: acute heart failure; IV: intravenous.

2.2 Study procedure

Eligible hospitalized ADHF patients were informed about the study by the investigator. If patients agreed to participate they were enrolled in the study. The study procedure consisted of several parts, i.e. baseline examination protocol, at home performed protocol, and a follow-up examination protocol, visually shown in figure 4.

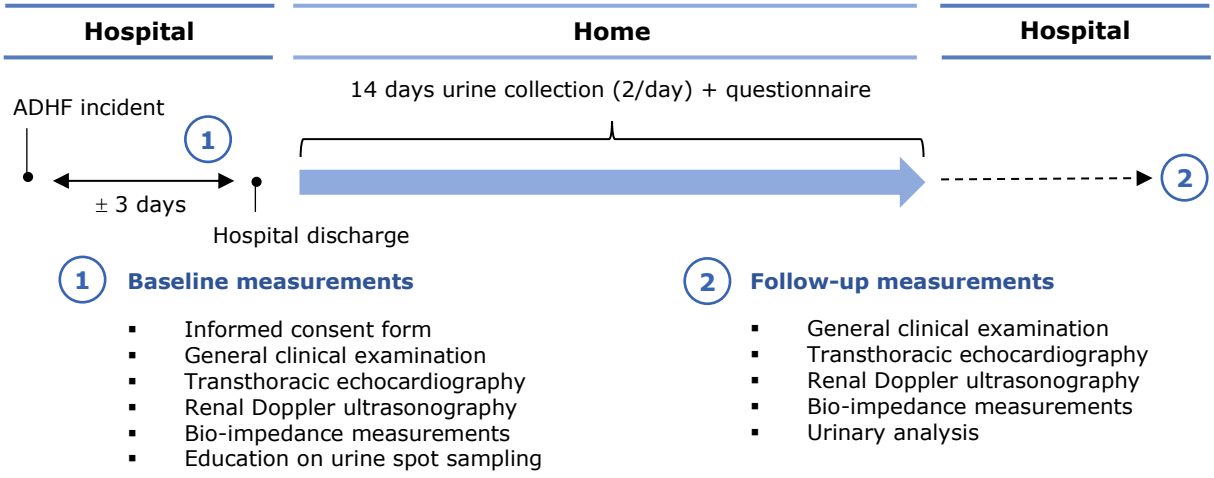

Figure 4| Study procedure of MORE-RESPONSE study. Study subjects underwent a baseline and follow-up examination at the hospital. At home, study subjects performed a urine collection twice a day and filled in a daily questionnaire for 14 days. ADHF: acute decompensated heart failure.

2.2.1 Baseline examination

Baseline measurements were executed at the date closest to hospital discharge. At baseline, a general clinical examination, transthoracic echocardiography (TTE), renal Doppler ultrasonography, bio-impedance measurement and blood collection were executed. The subjects were also educated on the correct performance of urinary spot sampling.

- **General clinical examination**

Baseline demographics were collected through a case report form and via the subjects' electronic medical file and direct questioning. Hence, data concerning the dyspnea status, functional status, medical history and baseline medication use of the subject was gathered. Blood sampling was executed by the nursing staff with blood collection tubes (BD, UK).

- **Transthoracic echocardiography**

Utilizing TTE, the hemodynamic and myocardial pump function of the heart was assessed. The commercially available EPIQ 5 ultrasound system (Philips Healthcare, Best, The Netherlands) was used to perform a two-dimensional TTE examination. Subjects were laying down in supine position while three adhesive monitoring electrodes (3M™, MN, USA) were applied on the subject's thorax and were connected with the electrocardiogram (ECG) wires of the ultrasound system. Consequently, an ECG was obtained during the procedure. The positioning of the monitoring electrodes is shown in figure 5.

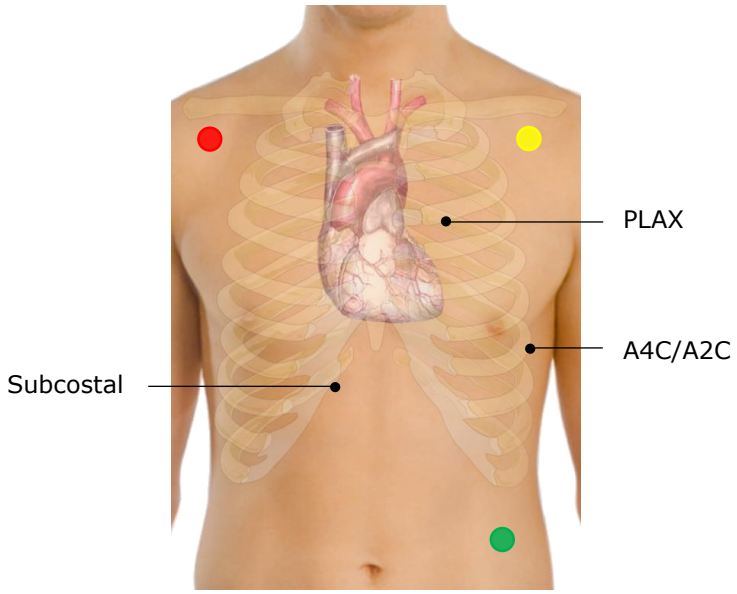

Figure 5| Positioning of electrocardiogram (ECG) electrodes and transducer. The red, yellow and green dot indicates the positioning of the ECG electrodes. Transthoracic echocardiography images are obtained by positioning the transducer at the parasternal long-axis (PLAX), apical four and two chamber (A4C/A2C) and subcostal position.

To start the TTE protocol, subjects were asked to turn on their left side, while positioning their right arm on their legs and their left arm alongside their head. This positioning enabled an optimal echocardiography condition. By positioning the transducer at defined locations, TTE images could be obtained (fig. 5). All images were averaged over three cardiac cycles. Aquasonic ultrasound gel

(Parker Laboratories, CT, USA) was applied to the S5-1 transducer (Philips Healthcare) for optimal ultrasound transmission. The transducer was placed at the parasternal long-axis (PLAX), apical four and apical two chamber (A4C/A2C) position. Subcostal images were realized when subjects were in a supine position.

- **Renal Doppler ultrasonography**

Intrarenal vascular flow patterns were visualized with renal Doppler ultrasonography. Subjects laid down and were positioned on their left side while an intrarenal Doppler ultrasonography was recorded of the right kidney in the right subcostal region with the C5-1 transducer (Philips Healthcare). In case of unsatisfactory image quality, Doppler ultrasonography of the left kidney was recorded. The ECG was simultaneously recorded by the EPIQ 5 ultrasound system (Philips Healthcare). Intralobar renal vessels were visualized by color Doppler images, followed by transmission of pulsed waveforms to determine flow velocity. Renal Doppler ultrasonography measurements and flow patterns were analyzed as recommended by *Nijst et al.* (57). As for the TTE, images were averaged over three cardiac cycles.

- **Bio-impedance**

Volume (overload) status was measured by means of bio-impedance with the commercially available BioScan 920 v1.1 (Maltron International Limited, UK). A single-sided hand to foot assessment was conducted. Subjects were placed in a supine position while placing the hand palms down, parallel to the body. Legs were placed apart from each other while the subjects' body remained symmetrical and still during the measurement. Four Ambu® WhiteSensor 0415M tap electrodes (Ambu®, Denmark) were applied on the subject, on which cables of the BioScan 920 v1.1 (Maltron International Limited) could be connected. The first two electrodes were placed centrally below the third knuckle of the right middle finger and on the crease of the right wrist, connecting cable 1 and 2, respectively. Electrode three and four were applied centrally on the right foot below the third toe and at the crease of the ankle connecting cable 3 and 4, respectively.

2.2.2 Home-based urine spot sampling

At baseline, subjects were thoroughly educated on the performance of urine spot sampling by the usage of a disposable urine collection cup (BD) and urine test tube (BD), shown in figure 7. Subjects were instructed to collect ambulatory spot urine samples twice a day, over 14 days starting the day after hospital discharge. The first urine spot samples were of the subjects' first-morning void. Thereafter, subjects took their diuretics and set an alarm for 1 hour and 30 minutes. After 1 hour and 30 minutes, the second urine sample was collected (figure 6).

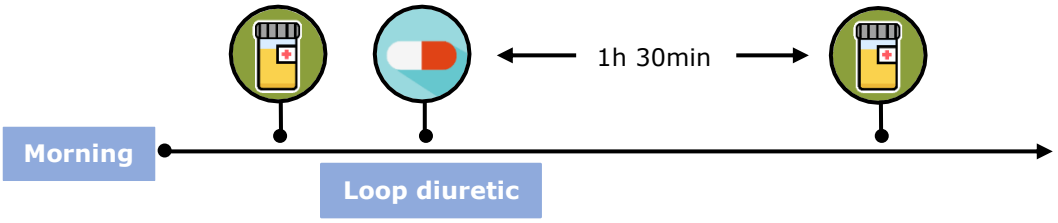

Figure 6| Urine spot sampling protocol. In the morning the first urine sample was taken followed by intake of loop diuretics. 1 hour and 30 minutes after diuretic intake, the second urine sample was taken.

Urine was collected in a disposable urine collection cup (fig. 7), containing a cap with an aspiration port. By sealing the collection cup with the specialized cap, urine could be aspirated with a urine test tube via the aspiration port. These test tubes were labeled according to the day (day 1 to 14) and sample (sample 1 or 2) of the urine collection. Afterwards, the labeled urine tube was stored in the subjects' freezer (commercial standard of -18°C) at home.

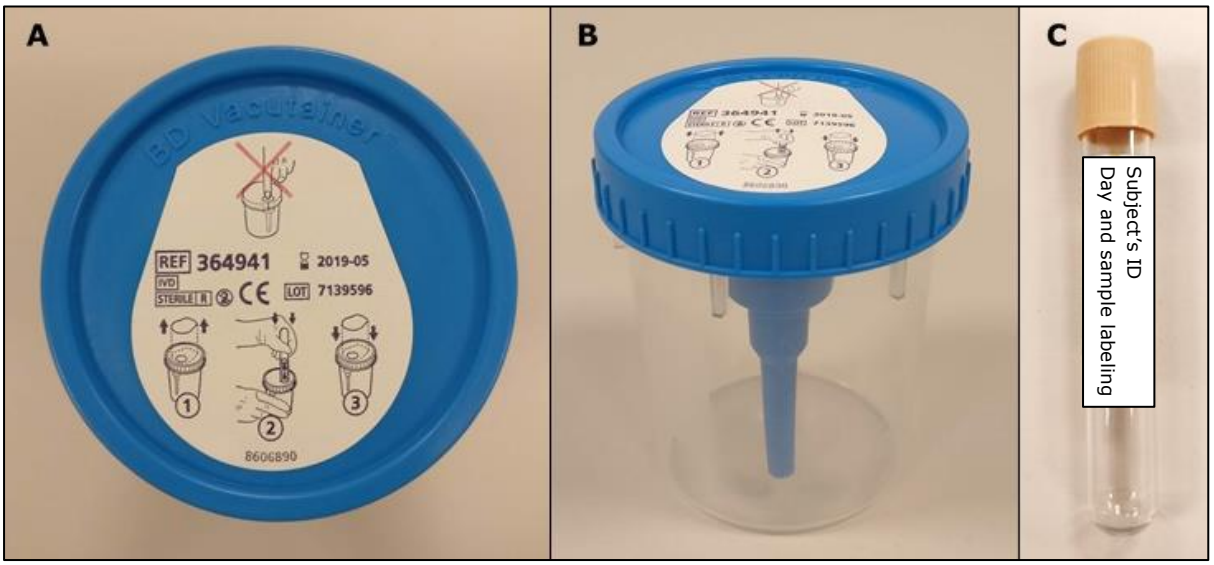

Figure 7| Urine spot sampling tools. **A**: Top view of disposable urine collecting cup, indicating the method for urine aspiration via the aspiration port. **B**: Front view of disposable urine collecting cup and blue aspiration port. **C**: Urine test tube labeled with the subject's identification (ID), day and sample number.

Subjects were also instructed to fill in a checklist and a daily biometrical data questionnaire (Appendix). The checklist served as a control for both the subjects and study investigator, indicating which urine samples were collected. The questionnaire, identified with the corresponding day, assessed for the subjects' blood pressure (if a blood pressure monitor was available), weight, dyspnea scoring with the visual analogue scale, diuretic dose, edema scoring with the Likert scale and if any changes in diet were present that day.

2.2.3 Follow-up examination

At approximately one month after hospital discharge, subjects returned for follow-up. As compensation for the trip to the hospital, subjects received a free parking ticket. If a predefined consultation was scheduled with the subjects' cardiologist, study follow-up was combined with this appointment. Upon arrival in the hospital, frozen urine samples and study documentation (daily questionnaire and checklist) were handed over to the study investigator. Urine samples were immediately stored in the freezer (-18°C) at the hospital until the day of analysis. Urine samples were assessed for urinary sodium and chloride content with the Cobas-800 (Roche Diagnostics, Switzerland) in batch. Additionally, subjects underwent a TTE, renal Doppler ultrasonography, bio-impedance measurement and blood sampling at follow-up. Medication use, dyspnea and functional status were assessed at follow-up as well. Protocols of follow-up examinations were analogously executed as at baseline (see 2.2.1 Baseline examination). All subjects' electronic medical files were censored on the 23rd of July for prospective events. These events were loop diuretic increase, (ADHF) hospitalization and all-cause mortality.

2.3 Sample analysis

Subjects collected pre- and post-diuretic urine samples over 14 days. To obtain knowledge on their diuresis efficiency, i.e. sodium and chloride excretion, several indexes were set up. Additionally, a congestion scale was put together to determine whether congestion reoccurrence was present at follow-up.

2.3.1 Urine samples indexes

Urine samples indexes enabled to analyze the daily sodium and chloride excretion, induced by loop diuretics. In total, four indexes were set up.

- **Urinary sodium augmentation index**

The first urine sample index is the urinary sodium augmentation index (USAI). This index is defined as the difference in urinary sodium concentration of the pre-diuretic (morning) urinary sample ($[U_{Na+}]_{pre\text{-}diuretic}$) and the post-diuretic sample ($[U_{Na+}]_{post\text{-}diuretic}$), collected 1 hour and 30 minutes after diuretic administration.

However, a side effect of the use of diuretics that should be kept into consideration, is the hypertrophy of the distal tubule cells of the nephron. Due to this adaptation of the nephron, sodium retention occurs in the distal tubule combined with increased water reabsorption (37). This event can be present in the post-diuretic urine samples. To normalize this event, both the pre- and post-diuretic urine samples are divided by the creatinine concentration ([creatinine]), since this is a substance that is produced at a constant velocity and it is absorbed in a low, constant rate by the kidneys.

$$USAI = \frac{[U_{Na+}]_{post\text{-}diuretic}}{[creatinine]_{post\text{-}diuretic}} - \frac{[U_{Na+}]_{pre\text{-}diuretic}}{[creatinine]_{pre\text{-}diuretic}} \text{ (mmol/dg)}$$

An alternative for normalizing the event of increased water reabsorption in the post-diuretic sample is dividing the urinary sodium concentrations by the osmolality. The osmolality is a measure on the urine concentration and results in the second index, i.e. USAI_Osm.

$$USAI\_Osm = \frac{[U_{Na+}]_{post\text{-}diuretic}}{osmolality_{post\text{-}diuretic}} - \frac{[U_{Na+}]_{pre\text{-}diuretic}}{osmolality_{pre\text{-}diuretic}} \text{ (mol/Osm)}$$

- **Urinary chloride augmentation index**

The third urine sample index is the urinary chloride augmentation index (UCAI) and is set up correspondingly as the USAI. The difference in urinary chloride concentration of the pre-diuretic ($[U_{Cl-}]_{pre\text{-}diuretic}$) and post-diuretic ($[U_{Cl-}]_{post\text{-}diuretic}$) sample, normalized by creatinine concentration is known as the UCAI. Division of the chloride concentration by the osmolality results in the fourth index, i.e. UCAI_Osm. Formula of the third and fourth index is shown below.

$$UCAI = \frac{[U_{Cl-}]_{post\text{-}diuretic}}{[creatinine]_{post\text{-}diuretic}} - \frac{[U_{Cl-}]_{pre\text{-}diuretic}}{[creatinine]_{pre\text{-}diuretic}} \text{ (mmol/dg)}$$

$$UCAI\_Osm = \frac{[U_{Cl-}]_{post\text{-}diuretic}}{osmolality_{post\text{-}diuretic}} - \frac{[U_{Cl-}]_{pre\text{-}diuretic}}{osmolality_{pre\text{-}diuretic}} \text{ (mol/Osm)}$$

2.3.2 Congestion reoccurrence

By examination and comparison of certain variables measured at baseline and follow-up, a general linear congestion scale and a worsening congestion scale could be obtained. The general congestion scale indicates the change in subjects' degree of congestion during the study period. Negative or positive values of the general congestion scale correspond to improvement or worsening in congestion, respectively. The worsening congestion scale illustrates whether subjects had a higher degree of congestion at follow-up compared to baseline.

The scales enabled to subdivide the study population at follow-up in congested and non-congested group. Subjects with a congestion scale of ≥5 or <5 were assigned to the congested group or non-congested group, respectively. Included variables together with corresponding point(s) are shown in table 3.

Table 3| Linear congestion scale set-up.

Variable	Worsening congestion scale		General congestion scale	
NYHA class	↑:	+1	↑: =: ↓:	+1 0 -1
Orthopnea/bendopnea	New-onset:	+1	New-onset: =: Absence:	+1 0 -1
Body weight	↑ 4 kg: ↑ 2 kg:	+2 +1	↑ 4 kg: ↑ 2 kg: ↓ 2 kg: ↓ 4 kg:	+2 +1 -1 -2
Likert edema	↑:	+1	↑: =: ↓:	+1 0 -1
NT-proBNP	↑ and > 900 pg/mL	+1	↑ and > 900 pg/mL > 900 pg/mL < 900 pg/mL	+1 0 -1
Deceleration time	< 150 ms > 240 ms	+1 +1	< 150 ms > 240 ms 150 ms – 240 ms	+1 +1 0
E/e'	≥ 13	+1	≥ 13 < 13	+1 0
IVC collapse (RAP)	RAP of 15 RAP of 8	+2 +1	RAP of 15 RAP of 8 RAP of 3	+2 +1 0
Discontinuous flow	New-onset: = and discontinuous: Absence:	+2 +1 0	New-onset: = and discontinuous: Absence:	+2 +1 0

Table 3 continued| Linear congestion scale set-up.

Variable	Worsening congestion scale		General congestion scale	
Excess fluid	↑ > 4 kg:	+2	↑ > 4 kg:	+2
	↑:	+1	↑:	+1
			=:	0
			↓:	-1
Scale range	0 to 13		-8 to 13	

E/e': early diastolic inflow velocity/mitral annulus early diastolic velocity; IVC: inferior vena cava; NT-proBNP: N-terminal pro-B-type natriuretic peptide; NYHA: New York Heart Association; RAP: right arterial pressure.

2.4 Data analysis

Continuous variables are presented as mean ± standard deviation (std) if normally distributed. If variables failed normality, they are presented as median and interquartile range (IQR). Normality was tested by the Shapiro-Wilk test, Q-Q plots, mean and std, and skewness and kurtosis. Discrete variables are presented as numbers and percentages (n(%)) and were tested with the χ^2 -, McNemar – or Wilcoxon signed rank test. Continuous variables were tested with the (paired) Student's t-test or Mann-Whitney U test as appropriate. Homogeneity of variance was tested with the Levene's test.

Longitudinal urinary sodium profiles, USAI, USAI_Osm, UCAI and UCAI_Osm were assessed using linear mixed modeling for repeated measures. This statistical model is preferred to repeated measures ANOVA, as it was expected that missing values would be present in a repeated measure design. First-order autoregressive (AR1) was used to model the time-effect since it can be expected that sodium measurements taken one day after the other are more correlated to one-and-another. Models were built investigating the fixed effect time, worsening congestion scale, HF episode, and their interaction in different mixed models. Additionally, systolic blood pressure, heart rate, weight, aldosterone antagonist and chronic obstructive pulmonary disease (COPD) were included in the model as random effects. The time variable was assessed in a linear and quadric manner to assess the appropriateness of a linear mixed meddles compared to a non-linear mixed model. Fixed effects were analyzed using sum of squares type III. Statistical significance level was set with a 2-tailed probability level of α=0.05, except for the variable "Age". Statistics were executed by the usage of SPPS statistics® version 25 (IBM company, NY, USA) and subjects' data was coded.

3 Results

From January 2019 until June 2019 a total of 142 patients were hospitalized due to an AHF episode. Of these patients, 95 were not eligible and 23 were not interested to participate. In total 24 patients provided a written consent and were included in the MORE-RESPONSE study. However, 10 subjects dropped-out during the study protocol. These subjects ended study participation voluntarily (n=5), because of general worsening of health status (n=2), due to a urinary tract infection (n=1) and incontinence (n=1). One subject was excluded at follow-up as the subject halted the intake of loop diuretics. Data analysis was carried out of 14 patients who correctly performed urinary spot samples at home and were examined at follow-up. Study population flowchart is shown in figure 8.

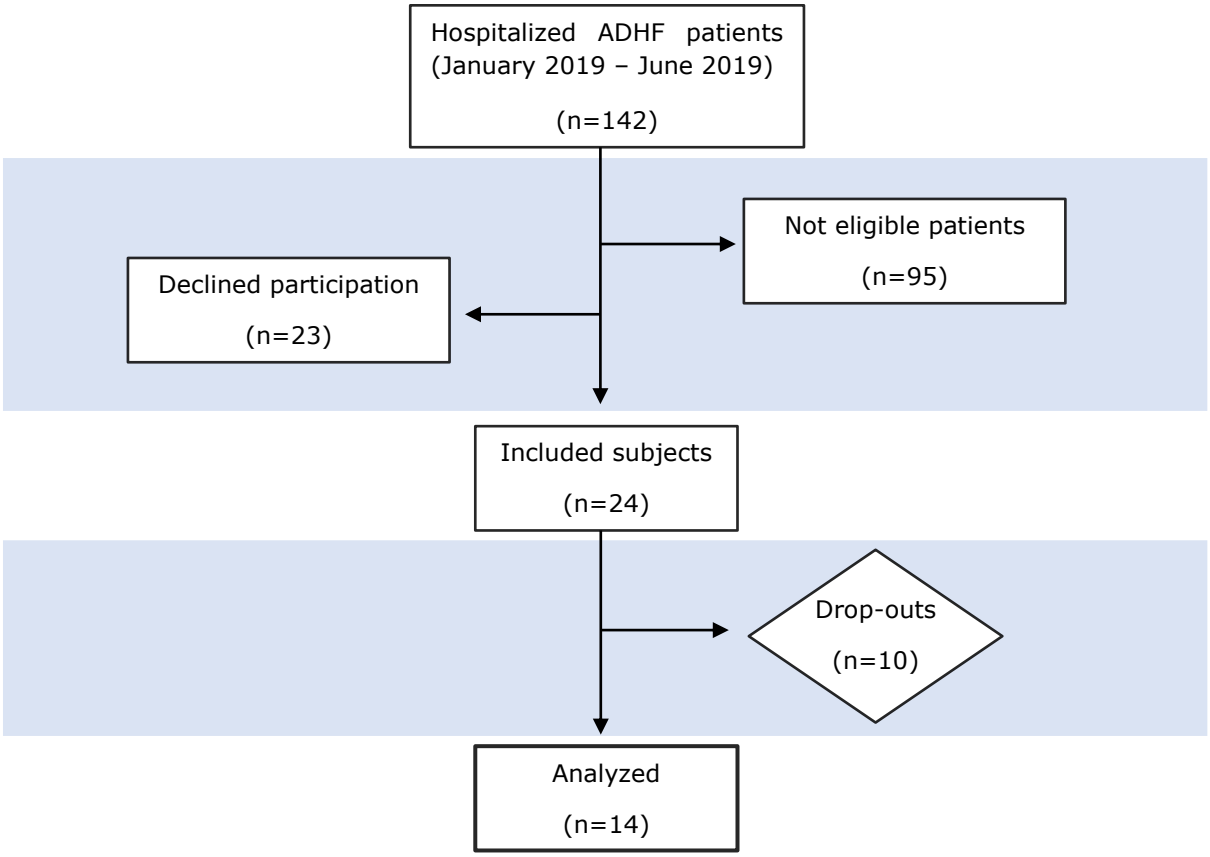

Figure 8| Study population flowchart. ADHF: acute decompensated heart failure.

3.1 Subject characteristics

The MORE-RESPONSE study population consisted in total of 14 patients. Subjects' baseline characteristics are summarized in the left-side column of table 4. Subjects had a mean age of 73 ± 7 years and were predominantly male (78.6%). Both HFpEF and HFrEF was in equal amounts present in the study population, the vast majority of the HFrEF subjects had an ischemic etiology (85.7%). The most frequent comorbidity was a history of atrial fibrillation (71.1%) followed by hypertension (57.1%) and diabetes (50%). Subjects were mildly symptomatic as illustrated by the high frequency of NYHA class II (64.3%). The vast majority of the subjects administered a loop diuretic dose of 40 mg furosemide equivalent (64.3%).

Baseline characteristics of the (non-)congested study population, determined at follow-up by the worsening linear congestion scale, are shown in the middle- and right-side column of table 4. Congested subjects were older (76 ± 8 years) and had predominantly HFpEF (71.4%) compared to the non-congested subjects. A significant difference in systolic blood pressure (p=0.032) and heart rate (p=0.015) was present between the two groups. Besides these variables, no significant differences between the two groups were present.

Table 4| Baseline characteristics of MORE-RESPONSE study population (n=14).

Variable	Total population (n=14)	Non-congested group (n=7)	Congested group (n=7)	*p*-value
Demographics				
Age, years	73 ± 7	71 ± 6	76 ± 8	0.282
Male, n(%)	11 (78.6)	5 (71.4)	6 (85.7)	0.515
Active smokers, n(%)	3 (21.4)	3 (42.9)	0 (0)	0.053
Heart failure type, n(%)				0.109
HFpEF	7 (50)	2 (28.6)	5 (71.4)	
HFrEF	7 (50)	5 (71.4)	2 (28.6)	
Heart failure etiology if HFrEF, n(%)				0.495
Ischemic	6 (85.7)	4 (80)	2 (100)	
Non-ischemic	1 (14.3)	1 (20)	0 (0)	
Physical features				
SBP, mmHg	116.2 ± 19.8	104.7 ± 8.1	127.7 ± 21.9	**0.032***
DBP, mmHg	69 (61.8 – 78.3)	69 (62 – 73)	69 (61 – 79)	1
Weight, kg	91.4 ± 22.1	91 (81 – 121)	78 (69.8 – 115)	0.259
BMI, kg/m²	31.6 ± 7.5	33.9 (30.6 – 39.6)	25.5 (23.5 – 39.5)	0.128
Heart rate, beats/min	67 ± 12.6	59.3 ± 10.1	74.7 ± 10.3	**0.015***
Comorbidities, n(%)				
Atrial fibrillation	10 (71.4)	4 (57.1)	6 (85.7)	0.237
COPD	3 (21.4)	1 (14.3)	2 (28.6)	0.515
Hypertension	8 (57.1)	4 (57.1)	4 (57.1)	1
Diabetes	7 (50)	3 (42.9)	4 (57.1)	0.593
NYHA class, n(%)				0.574
Class I	4 (28.6)	2 (28.6)	2 (28.6)	
Class II	9 (64.3)	4 (57.1)	5 (71.4)	
Class III	1 (7.1)	1 (14.3)	0 (0)	

Table 4 continued| Baseline characteristics of MORE-RESPONSE study population (n=14).

Variable	Total population (n=14)	Non-congested group (n=7)	Congested group (n=7)	*p*-value
Guideline directed heart failure-therapy, n(%)				
ACE-I (if HFrEF)	1 (14.3)	0 (0)	1 (20)	0.495
ARB (if HFrEF)	1 (14.3)	1 (20)	0 (0)	0.495
Beta-blocker (if HFrEF)	6 (85.7)	4 (80)	2 (100)	0.495
Aldosterone antagonist	11 (78.6)	7 (100)	4 (57.1)	0.051
Furosemide equivalent				0.574
40 mg	9 (64.3)	4 (57.1)	5 (71.4)	
60 mg	1 (7.1)	1 (14.3)	0 (0)	
80 mg	4 (28.6)	2 (28.6)	2 (28.6)	

Data is presented as mean ± standard deviation (std) if normally distributed or by median and interquartile range (IQR). Discrete variables are expressed as sample size (n) and percent. A p-value of <0.05 was considered as statistical significant (*). ACE-I: angiotensin-converting enzyme inhibitor; ARB: angiotensin receptor blocker; COPD: chronic obstructive pulmonary disease; DBP: diastolic blood pressure; HFpEF: heart failure with preserved ejection fraction; HFrEF: heart failure with reduced ejection fraction; NYHA: New York heart association; SBP: systolic blood pressure.

3.2 Congestion status

At baseline, i.e. ADHF hospitalization, and at one month follow-up (mean follow-up of 30 ± 11 days) subjects underwent a general clinical examination, blood sampling, TTE examination, renal Doppler ultrasonography and bio-impedance measurement. These techniques enabled to gather information on the subjects' congestion status and are illustrated in table 5.

Clinical characteristics, obtained by general clinical examination and direct questioning, show that no significant differences are present at baseline and follow-up. Orthopnea, bendopnea and paroxysmal nocturnal dyspnea indicate whether the subjects had difficulty breathing while laying down, bending forward or during the night, respectively. These symptoms are overall less experienced at follow-up. Grade of edema, with the severity of edema directly related to edema score, also lowers at follow-up with 28.6% experiencing grade 0 edema at baseline, compared to 50% at follow-up. A minor increase (7.1%) in frequency of edema grade 2 and 3 is present at follow-up compared to baseline.

Laboratory analysis demonstrates a significant difference in blood potassium levels (p<0.001) at follow-up compared to baseline. Estimated GFR, a measure for kidney function, indicates that subjects had a moderate kidney dysfunction at baseline (38.5 mL/min/1.73m^2) and follow-up (38 mL/min/1.73m^2). NT-proBNP, a marker for HF, is lower at follow-up 1733 ng/L (1318 – 2287) compared to baseline 2283 ng/L (1086 – 4528) but still above the threshold of 900 pg/mL.

Cardiac pump function and diastolic dysfunction were assessed by TTE. Subjects' cardiac function remained predominantly constant during study investigation. A significant difference was observed at follow-up compared to baseline in medial e' (mitral annulus early diastolic velocity) (p=0.001). Discontinuous flow of the renal veins was both at baseline and follow-up present in 50% of the study population.

Bio-impedance data shows a tendency towards statistical significance (p=0.074) of an increased amount of excessive fluid present at follow-up compared to baseline.

Table 5| Examination characteristics at baseline and follow-up.

Variable	Baseline characteristics (n=14)	Follow-up characteristics (n=14)	*p*-value
Clinical characteristics, n(%)			
Orthopnea	5 (35.7)	2 (14.3)	0.250
Bendopnea	5 (35.5)	1 (7.1)	0.125
Parox. noct. dysp.	1 (7.1)	1 (7.1)	1
Edema			0.931
Grade 0	4 (28.6)	7 (50)	
Grade 1	8 (57.1)	3 (21.4)	
Grade 2	1 (7.1)	2 (14.3)	
Grade 3	1 (7.1)	2 (14.3)	
Laboratory analysis			
Sodium, mmol/L	140.2 ± 3.9	138.5 ± 3.1	0.142
Potassium, mmol/L	4 ± 0.6	4.7 ± 0.6	**<0.001***
Hemoglobin, g/dl	13 ± 1.8	13.2 ± 1.5	0.573
Estimated GFR, mL/min/1.73m²	38.5 (27.8 – 47.5)	38 (31.5 – 46.5)	0.638
NT-proBNP, ng/L	2283 (1086 – 4528)	1733 (1318 – 2287)	0.249
Echocardiography			
E, m/s	1.1 ± 0.4	0.9 ± 0.3	0.114
A, m/s	0.6 ± 0.1	0.7 ± 0.3	0.712
E/A	1.8 (0.9 – 2.6)	1.2 (0.7 – 2.8)	0.917
Deceleration time, ms	187 (163 – 217)	195 (171 – 216)	0.308
Medial e', m/s	0.07 ± 0.02	0.05 ± 0.01	**0.001***
E/e'	15.8 (11.4 – 24)	17.8 (12 – 21.9)	0.279
RVSP, mmHg	38.3 ± 10.8	31.5 ± 13.3	0.231
RAP, mmHg			1
3	1 (7.1%)	1 (7.1%)	
8	4 (28.6%)	4 (28.6%)	
15	4 (28.6%)	5 (35.7%)	
Renal Doppler Ultrasonography, n(%)			
Discontinuous flow	7 (50)	7 (50)	1

Table 5 continued| Examination characteristics at baseline and follow-up.

Variable	Baseline characteristics (n=14)	Follow-up characteristics (n=14)	*p*-value
Bio-impedance			
Excess fluid, L	1.6 (-0.1 – 4.4)	4 (0 – 6.9)	0.074

Data is presented as mean ± standard deviation (std) if normally distributed or by median and interquartile range (IQR). Discrete variables are expressed as sample size (n) and percent. A p-value of <0.05 was considered as statistical significant (*). A: late diastolic inflow velocity; E: early diastolic inflow velocity; e': mitral annulus early diastolic velocity; GFR: glomerular filtration rate; NT-proBNP: N-terminal pro-B-type natriuretic peptide; Parox. noct. dysp.: paroxysmal nocturnal dyspnea; RAP: right arterial pressure; RVSP: right ventricle systolic pressure.

3.2.1 Congestion status according to the worsening congestion scale

Individual analysis of subjects' parameters at baseline and follow-up allowed to distinguish the study population in two groups, a non-congested and a congested population. Used method to divide the population was the linear congestion scale. Subjects that scored ≥5 on the worsening linear congestion scale were assigned to the congested group others were assigned to the non-congested group. Set-up and included variables of the linear congestion scale is shown in table 3.

Table 6 shows the characteristics of the (non-)congested study population at baseline and follow-up. At baseline, both groups had no statistically significant differences in clinical, echocardiography, renal ultrasonography and bio-impedance characteristics.

At follow-up, clinical characteristics of the non-congested versus the congested group were non-significant. However, orthopnea had a more frequent manifestation in the congested group (71.2%) compared to the non-congested group (0%). Edema was absent in 71.4% of the non-congested subjects, while only 28.6% of the congested subjects were free of edema. Laboratory analysis at follow-up of the two groups indicates a significant difference in plasma NT-proBNP (p=0.018).

Follow-up echocardiography measurements indicate a significant difference in E (p=0.035) and an almost statistical significance of A (p=0.051) between the two groups. Renal Doppler ultrasonography of non-congested subjects at follow-up, shows that 40% had a discontinuous flow, compared to 83.3% of the congested subjects. Excess fluid, measured by bio-impedance, at follow-up shows a median of 0 liters (0 – 2.08) excess fluid in the non-congested population and a median of 6.16 liters (5.07 -7.18) excess fluid in the congested population (p=0.038).

Table 6| Examination characteristics at baseline and follow-up for subjects with or without congestion at follow-up.

Variable	Baseline non-congested group (n=7)	Baseline congested group (n=7)	*p*-value	Follow-up non-congested group (n=7)	Follow-up congested group (n=7)	*p*-value
Clinical characteristics, n(%)						
Orthopnea	3 (42.9)	2 (28.6)	0.577	0 (0)	5 (71.2)	0.127
Bendopnea	2 (28.6)	3 (42.9)	0.577	0 (0)	1 (14.3)	0.299
Parox. noct. dysp.	1 (14.3)	0 (0)	0.299	0 (0)	1 (14.3)	0.299
Edema			0.321			0.132
Grade 0	3 (42.9)	1 (14.3)		5 (71.4)	2 (28.6)	
Grade 1	3 (42.9)	5 (71.4)		2 (28.6)	1 (14.3)	
Grade 2	1 (14.3)	0 (0)		0 (0)	2 (28.6)	
Grade 3	0 (0)	1 (14.3)		0 (0)	2 (28.6)	
Laboratory analysis						
Sodium, mmol/L	140 (135 -143)	139 (138 – 146)	0.520	138 ± 3.51	139 ± 2.77	0.565
Potassium, mmol/L	4.09 ± 0.64	3.92 ± 0.57	0.612	4.79 ± 0.39	4.62 ± 0.76	0.601
Hemoglobin, g/dl	13.49 ± 1.94	12.43 ± 1.58	0.286	13.69 ± 1.41	12. 64 ± 1.54	0.211
Estimated GFR, mL/min/1.73m²	49.14 ± 26.58	35.71 ± 7.02	0.221	43.71 ± 17.76	37.86 ± 8.28	0.444
NT-proBNP, ng/l	2356 ± 1850	3266 ± 1856	0.397	1469 (1267 – 1641)	2221 (1826 – 5318)	**0.018***
Echocardiography						
E, m/s	0.94 ± 0.26	1.26 ± 0.38	0.102	0.78 ± 0.28	1.17 ± 0.31	**0.035***
A, m/s	0.62 ± 17	0.55 ± 0.26	0.649	0.8 ± 0.17	0.39 ± 0.17	0.051
E/A	1.61 ± 0.81	2.27 ± 1.56	0.424	1.12 ± 0.67	3.26 ± 2.81	0.180
Deceleration time, ms	189 (154 – 211)	180 (166 – 226)	0.848	208.71 ± 59.11	193.5 ± 20.55	0.563

Table 6 continued| Examination characteristics at baseline and follow-up for subjects with or without congestion at follow-up.

Variable	Baseline non-congested subjects (n=7)	Baseline congested subjects (n=7)	*p*-value	Follow-up non-congested subjects (n=7)	Follow-up congested subjects (n=7)	*p*-value
Echocardiography						
Medial e', m/s	0.06 ± 0.009	0.07 ± 0.024	0.888	0.051 ± 0.011	0.054 ± 0.017	0.715
E/e'	14.65 ± 3.54	22.89 ± 13.97	0.156	15.22 ± 5.27	21.17 ± 8.42	0.147
RVSP, mmHg	39.27 ± 13.71	34.83 ± 6.31	0.486	24.98 (23.44 – 53.35)	30.23 (20.46 – 39.26)	1
RAP, mmHg			0.570			0.392
3	1 (16.7%)	0 (0%)		1 (25%)	0 (0%)	
8	3 (50%)	1 (33.3%)		1 (25%)	3 (50%)	
15	2 (33.3%)	2 (66.7%)		2 (50%)	3 (50%)	
Renal Doppler Ultrasonography, n(%)						
Discontinuous flow	3 (42.9)	4 (57.1)	0.593	2 (40)	5 (83.3)	0.137
Bio-impedance						
Excess fluid, L	1.33 ± 2.31	2.88 ± 2.77	0.278	0 (0 – 2.08)	6.16 (5.07 -7.18)	**0.038***

Data is presented as mean ± standard deviation (std) if normally distributed or by median and interquartile range (IQR). Discrete variables are expressed as sample size (n) and percent. A p-value of <0.05 was considered as statistical significant (*). A: late diastolic inflow velocity; E: early diastolic inflow velocity; e': mitral annulus early diastolic velocity; GFR: glomerular filtration rate; NT-proBNP: N-terminal pro-B-type natriuretic peptide; Parox. noct. dysp: paroxysmal nocturnal dyspnea; RAP: right arterial pressure; RVSP: right ventricle systolic pressure.

3.3 Urinary spot output

In total, 14 subjects collected urine spot samples twice a day over 14 days. Samples were taken at predisposed time points, i.e. first-morning void and 1 hour and 30 minutes after loop diuretic intake. Therefore, providing a daily pre- and post-diuretic urine spot sample. A total of 346 urine spot samples were analyzed and implemented in the analysis.

Subjects' diuresis efficiency induced by loop diuretics could be assessed by subtraction of the pre-diuretic sample from the post-diuretic urine spot sample. The four generated indexes, i.e. USAI, USAI_Osm, UCAI, UCAI_Osm, and their formulas are listed in subsection '2.3.1. Urine sample indexes'.

Figure 9, panel A demonstrates the daily mean USAI with corresponding 95% confidence interval of the entire study population. All days, except day 10, have a USAI greater than 0 mmol/dg. Throughout the study period of 14 days, USAI values remained stable as shown by the non-significant time-effect of p=0.787. Daily mean USAI_Osm, UCAI, UCAI_Osm are illustrated in figure 9, panel B, C and D, respectively. They show a similar pattern as USAI, with all daily values greater than 0 mmol/dg or 0 mol/Osm except for day 10. Non-significant time-effect of USAI_Osm (p=0.564), UCAI (p=0.744), and UCAI_Osm (p=0.458) shows that the urine output was stable over the study period.

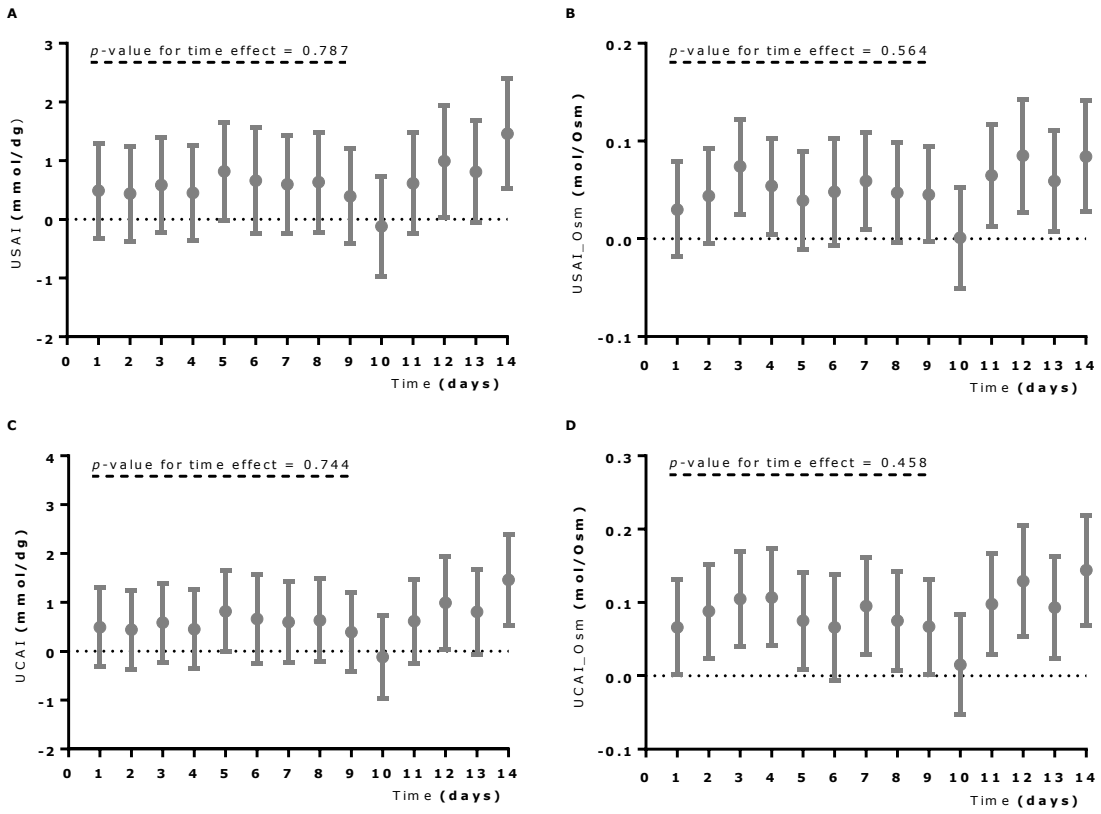

Figure 9| Longitudinal urinary spot output for the entire study population. **A, B, C, D**: Mean USAI, USAI_Osm, UCAI, UCAI_Osm profiles and 95% confidence interval expressed in mmol/dg or mol/Osm (y-axis) as appropriate.

3.3.1 Urinary spot output in relation to congestion status

The entire study population (n=14) was divided into two groups according to the congestion status at follow-up. An assessment on the degree of congestion was examined at baseline and follow-up through variant measurements. Variables obtained through these measurements allowed to set up a congestion scale. A detailed description of the congestion scale is provided in section '2.3.2 Congestion reoccurrence'. Two linear congestion scales were set up, i.e. the worsening congestion scale and the general congestion scale. According to the number on the congestion scale, subjects with a number < 5 were assigned to the non-congested group. Subjects with a number ≥ 5 on the congestion scale were assigned to the congested group.

- **Urinary spot output subdivided by the worsening congestion scale**

Figure 10 illustrates the longitudinal urine profiles of the study population subdivided according to the worsening congestion scale. Plotting of the urine profiles of these two groups resulted in two distinct profiles. Figure 10, panel A demonstrates the longitudinal mean USAI profiles and 95% confidence interval of the congested (n=7) and non-congested (n=7) group. Daily mean USAI values of the non-congested population had a minor fluctuating pattern (time-effect p=0.787) with a value greater than 0 mmol/dg except for day 10. The daily mean USAI of the non-congested group exceeds the daily mean USAI of the congested group, apart from days 4 and 10 (p=0.073). Figure 10, panel B illustrates the two distinct USAI_Osm profiles of the urine spot samples of the congested and the non-congested group with 95% confidence interval. All daily mean USAI_Osm values of the non-congested group are greater than 0 mol/Osm and exceed the mean USAI-Osm values of the non-congested group, aside for day 10. This difference is statistically significant with a p-value of 0.023.

Panel C and D of figure 9 shows the longitudinal mean UCAI, UCAI_Osm profiles, respectively, with 95% confidence interval of the two groups. It illustrates two distinct urine profiles for UCAI over time (p=0.091) and a statistically significant difference of the longitudinal mean UCAI_Osm values between the two groups (p=0.036).

To summarize, the longitudinal urine output of the two groups plotted against variant indexes, USAI, USAI_Osm, UCAI, UCAI_Osm, shows a distinct profile. The indexes USAI_Osm and UCAI_Osm display a statistically significant difference between the congested and the non-congested group, subdivided by the worsening congestion scale.

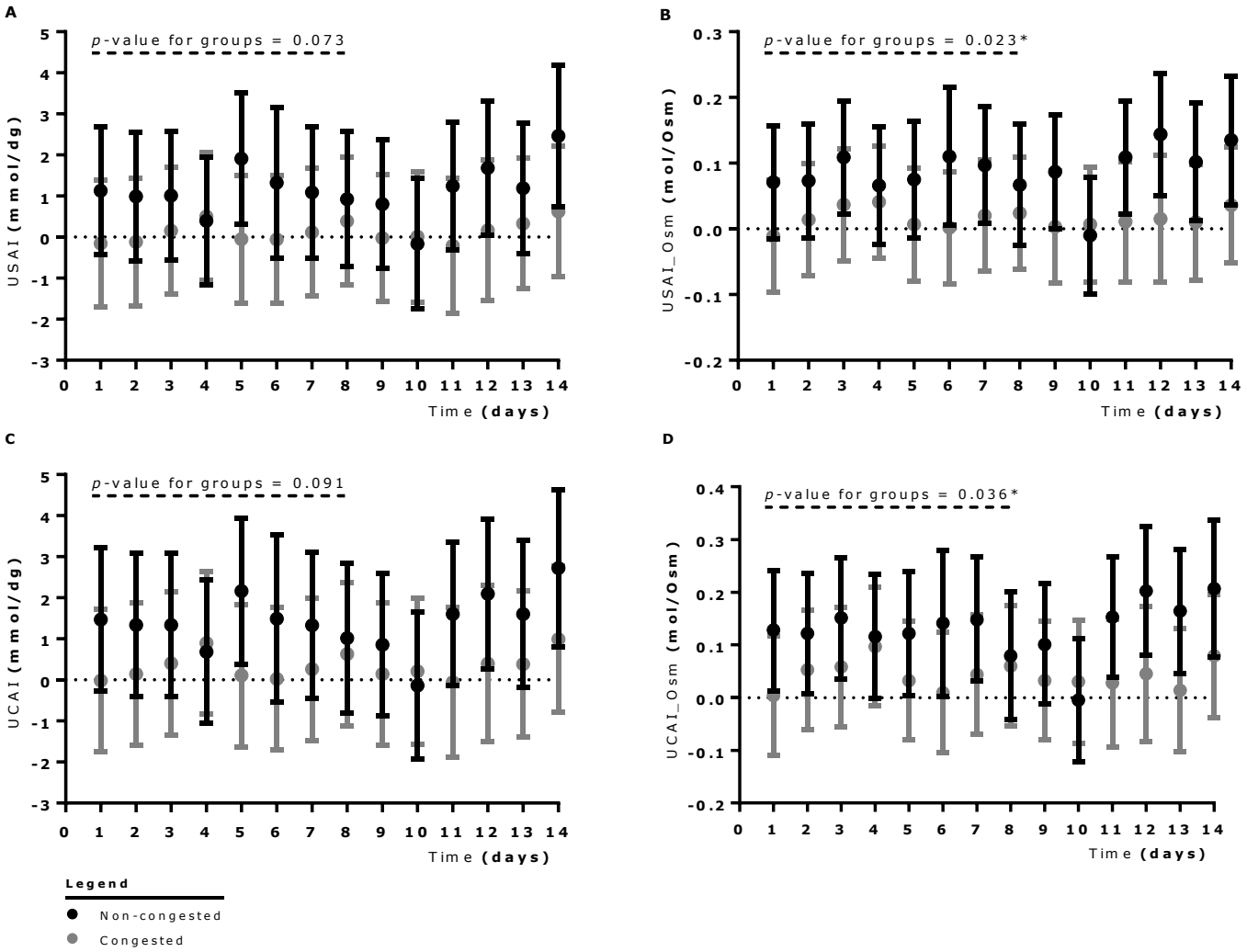

Figure 10| Longitudinal urinary spot output for the study population in subjects with or without signs of congestion at follow-up according to the worsening congestion scale. **A, B, C, D**: Mean USAI, USAI_Osm, UCAI, UCAI_Osm profiles and 95% confidence interval of the (non-)congested subjects expressed in mmol/dg or mol/Osm (y-axis) as appropriate.

- **Urinary spot output subdivided by the general congestion scale**

Usage of the general congestion scale also resulted in a subdivision of the study population in a congested group (n=3) and a non-congested group (n=11). Full demographics of these groups are shown in appendix table 7. Figure 11 panel A and B, shows the daily mean USAI and USAI_Osm with 95% confidence interval of the congested and non-congested group. The mean USAI and USAI-Osm of the non-congested group have a value greater than 0 mmol/dg and 0 mol/Osm, respectively. In contrast to the urine profiles of the non-congested subjects, the USAI and USAI_Osm profiles of the congested subjects have a more fluctuating pattern. Both appearing on the positive and negative range of the indexes. Nevertheless, the time-effect of p=0.934 and p=0.672 for USAI and USAI_Osm, respectively, indicates a stable urine profile over the study period of the two groups. No statistical significance was seen for the indexes USAI (p=0.133) and USAI_Osm (p=0.161).

Panel C and D of figure 11 indicates the mean UCAI and UCAI_Osm with 95% confidence interval of the non-congested and the congested group. Again, the non-congested group has a mainly positive UCAI and UCAI_Osm, while the urine profiles of the congested group have an oscillating pattern. Statistical difference between the two groups is p=0.121 and p=0.118 for UCAI and UCAI_Osm, respectively. Over time, the urine profiles have an overall steady course (time-effect UCAI p=0.881 and UCAI_Osm p=0.463).

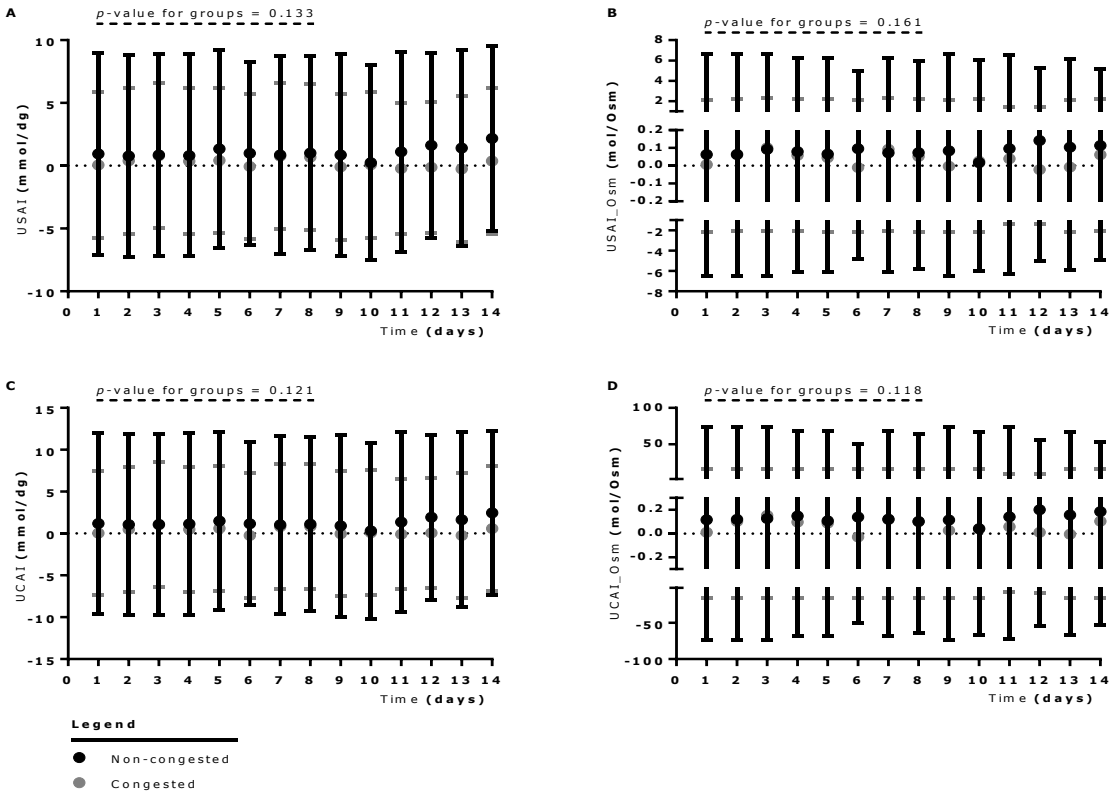

Figure 11| Longitudinal urinary spot output for the study population in subjects with or without signs of congestion at follow-up according to the general congestion scale. **A, B, C, D**: Mean USAI, USAI_Osm, UCAI, UCAI_Osm profiles and 95% confidence interval of the (non-)congested subjects expressed in mmol/dg or mol/Osm (y-axis) as appropriate.

3.3.2 Urinary spot output in relation to acute heart failure hospitalization

In total, three subjects of the entire study population (n=14) were hospitalized due to an AHF episode in the period of January 2019 to July 2019. The median time to this first AHF episode after study enrollment was 21 (20.5 – 58) days. AHF hospitalized subjects were all male (3 (100%)), had a median age of 71 (67 – 71) years and were predominantly mild symptomatic (NYHA class II (66.7%)). No statistical significant differences were present between two groups, i.e. AHF hospitalization (n=3) and no AHF hospitalization (n=11), except for COPD (p=0.031). Full demographical data is displayed in appendix table 8.

Figure 12 panel A, B, C and D shows the 14 days mean urine profiles and 95% confidence intervals of USAI, USAI_Osm, UCAI and UCAI_Osm, respectively, of the (non-)AHF hospitalized subjects. None of the indexes shows statistical significance (USAI p=0.144; USAI_Osm p=0.158; UCAI p=0.125; UCAI_Osm p=0.085), however, an evident pattern is visualized. Non-hospitalized subjects had a clear positive value on all the indexes, aside from day 10. While the urine profiles of the AHF hospitalized subjects had a frequently negative value and is exceeded by the mean urine output of the non-hospitalized subjects on nearly all days.

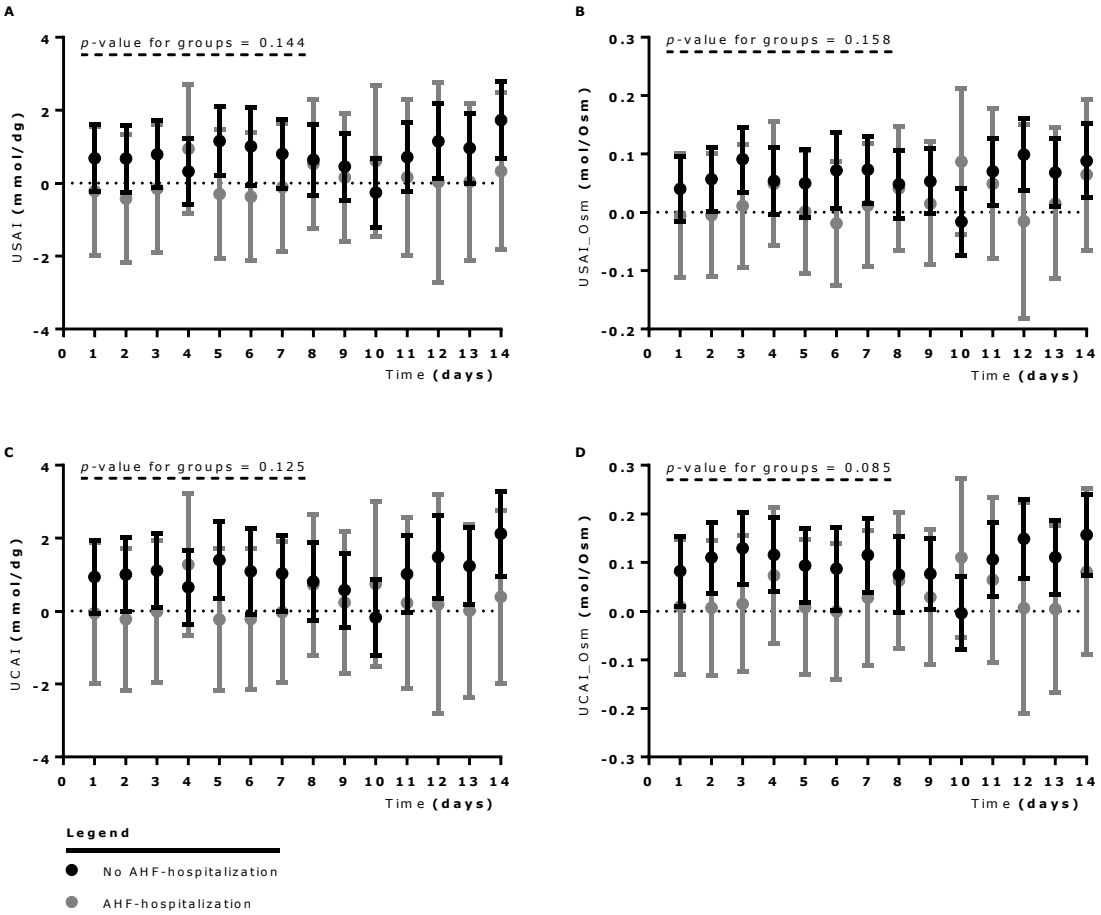

Figure 12| Longitudinal urinary spot output for the study population in subjects with or without acute heart failure (AHF) hospitalization. **A, B, C, D:** Mean USAI, USAI_Osm, UCAI, UCAI_Osm profiles and 95% confidence interval of the (non-)congested subjects expressed in mmol/dg or mol/Osm (y-axis) as appropriate.

4 Discussion

To our knowledge, this study is the first to provide insights into the urinary sodium and chloride profiles of HF patients' transition from ADHF to CHF within the vulnerable phase. Main findings are: 1| Urinary sodium and chloride concentration of ADHF patients enabled to predict congestion reoccurrence. 2| ADHF subjects have an overall stable USAI and UCAI output in the weeks following hospital discharge. 3| The worsening congestion scale enabled to subdivide the study population in a congested and a non-congested group, thereby identifying two significant distinct urine profiles. 4| Subjects hospitalized for ADHF after study enrollment have a lower USAI and UCAI compared to non-hospitalized subjects. 5| These hospitalized subjects are all identified by the worsening congestion scale as congested at follow-up.

There is an increasing interest in the usage of urinary sodium as a new metric for the assessment of congestion. Several studies have demonstrated the association of a poor urinary sodium output after decongestive therapy with a poor clinical outcome, i.e. ADHF hospitalizations and cardiovascular mortality (49, 51-55). However, as previously mentioned, no data is available on the urinary sodium and chloride profiles of ADHF patients within the vulnerable phase after ADHF hospitalization. Hence, this study offers insights on the urinary sodium and chloride profiles of ADHF patients in relation to congestion status.

4.1 Congestion status

This study gathered data on subjects' congestion status through variant techniques, i.e. general clinical examination, echocardiography, renal Doppler ultrasonography and bio-impedance. Congestion parameters of the entire study population were not statistically different at follow-up compared to baseline, except for blood potassium levels and medial e' velocity.

The clinical congestion variables orthopnea and edema were less frequently present at follow-up, indicating a less congested profile at follow-up compared to baseline. This manifestation might be explained by the additional decongestion that occurred at home. Overall, the entire study population was able to decongest according to these parameters.

Blood potassium levels were significantly increased at follow-up in the total study population. However, this increase was still within the optimal reference range of 3.5 – 5.1 mmol/L and therefore displays no abnormalities. Equally interesting is the level plasma NTproBNP, which lowered at follow-up. Plasma NTproBNP is a cleavage product of the natriuretic peptide BNP, which is released as a response on increased cardiac wall stress and is, therefore, the primary marker for (severity of) HF. A blood value greater than 900 ng/L corresponds to a state of AHF (1, 58). Thus, a decrease in plasma NTproBNP levels at follow-up illustrates a decrease in the severity of HF. However, both at baseline and follow-up plasma NTproBNP levels were >900 ng/L, indicating that subjects suffered from AHF.

Medial e' velocity was also statistically different. According to literature, a medial e' velocity of <0.1 m/s is a prognostic marker for left ventricle diastolic dysfunction (59). Since both mean medial

e' values were <0.1 m/s at baseline and follow-up it can be assumed that diastolic dysfunction was present in the study population as would be expected since subjects suffered from HF.

Finally, bio-impedance data shows that overall the entire study population had an increase in excess fluid. Indicating a more congested profile of the subjects at follow-up.

To conclude, this data showed that the entire study population suffered from diastolic dysfunction and that they were recovering from ADHF. However, both arguments for congestion and decongestion at circa one month follow-up are present.

4.1.1 Congestion status according to the worsening congestion scale

Analysis of the congestion-related parameters enabled to subdivide the study population in a non-congested and a congested group. Baseline characteristics of the non-congested and the congested group show that both groups are matched at baseline, as indicated by the non-significant differences. Illustrating that all subjects, whether showings signs of congestion at follow-up or not, were discharged from the hospital in a stable and generally equal health status. Nevertheless, changes in health status were present at follow-up.

Although no significant differences were present in clinical characteristics at follow-up, a higher frequency of orthopnea, bendopnea and paroxysmal nocturnal dyspnea in the congested group indicates that the subjects of this group suffered more from shortness of breath.

Blood sample analysis demonstrated a significant difference in plasma NTproBNP in the congested-group compared to the non-congested group at follow-up. In relation to baseline values, both groups were able to lower the amount of plasma NTproBNP. As previously mentioned is plasma NTproBNP a marker for (severity of) HF (1, 58). Hence, the high plasma NTproBNP levels of the congested group at follow-up, indicates that the subjects had a more advanced stage of HF. Even though both groups lowered the amount of plasma NTproBNP at follow-up, which proves that subjects were recovering from AHF, subjects of the congested group had a more advanced stage of HF.

There was a significant difference in early diastolic inflow velocity, i.e. E, and statistical significance was almost reached in late diastolic inflow velocity, i.e. A, at follow-up. E velocity is a reliable tool to assess left ventricle filling pressure, with an E velocity >0.5 m/s corresponding to an increased left ventricle filling pressure (60). It would be expected that the subjects of both groups have an E velocity >0.5 m/s as they suffered from HF. The E velocity of the non-congested and congested group lowers at follow-up and is greater than 0.5 m/s, illustrating a decrease in left ventricle filling pressure but still with the presence of abnormal filling pressure. As for the E velocity of the congested subject, this parameter showed only a minor decrease with a significant difference compared to the E velocity of the non-congested subjects at follow-up. An A velocity of >0.3 m/s is an indicator of elevated left ventricle end-diastolic pressure, a characteristic typical for HF (60). This variable is greater than 0.3 m/s in both groups. Interestingly, the A velocity is elevated in the non-congested group at follow-up compared to baseline while in the congested group, A velocity diminished. This indicates that subjects of the non-congested group had a more severe elevation of the left ventricle end-diastolic pressure.

Renal Doppler ultrasonography shows that a vast majority of the subjects in the congested group had a discontinuous venal flow. Due to volume overload present in patients with HF, the renal arterial vascular resistance elevates causing a decrease in the diastolic renal blood outflow. This decrease in venal flow is visualized by renal Doppler ultrasonography as a discontinuous flow (57). Since discontinuous renal flow was most present in the congested group, it shows that these subjects probably had a greater amount of volume overload. This is also substantiated by the significantly higher amount of excess fluid in the congested group compared to the non-congested subjects. However, the presence of missing data should be kept in consideration while interpreting echocardiography and renal Doppler ultrasonography data. There is a great inter-individual difference in image quality which leads to the inability to measure certain parameters or which could not assure correct analysis.

4.2 Urinary spot output

One of the study objectives was to investigate the diuresis efficiency of ADHF patients after hospital discharge. Moreover, the goal was to investigate the induced urinary sodium and chloride excretion by loop diuretics. This could be assessed by four indexes, i.e. USAI, USAI_Osm, UCAI, and UCAI_Osm which are the result of subtracting the pre-diuretic urine sample from the post-diuretic sample. The pre-diuretic sample shows the diuretic capacity without diuretics, while the post-diuretic sample demonstrates the maximal diuretic capacity induced by loop diuretics. As the second urinary sample was taken at approximately 1 hour and 30 minutes after loop diuretic intake, i.e. the time point at which loop diuretics are fully absorbed by the body and can exert its maximal effect (61).

On a population level, urinary data of the four indexes showed that the subjects' urine profiles had a stable course throughout the study period. Illustrating that subjects' urinary response to loop diuretics is steady over time after ADHF hospitalization.

4.2.1 Urinary spot output in relation to congestion status

Subdivision of the study population in a congested and a non-congested group via the worsening or general congestion scale demonstrated two distinct urine profiles. Those differences were not influenced by a difference in age, HF type, comorbidities, NYHA class or loop diuretic medication use as these variables were not statistically significant between both groups. However, the subdivision of the population by the worsening congestion scale showed that systolic blood pressure and heart rate were significantly different between the two groups. The general congestion scale showed a statistical difference in weight and aldosterone antagonist use in both groups. Hence, the statistic model was adjusted for these four variables. The rationale for utilizing two congestion scales was to investigate which scale would best subdivide the study population in a congested and non-congested group and could best predict the primary endpoint of congestion reoccurrence, leading to hospital readmission.

- **Urinary spot output subdivided by the worsening congestion scale**

All four indexes illustrate a distinct urine profile of the study population, subdivided by the worsening congestion scale. In general, the urinary sodium and chloride excretion of the non-congested group exceeded the sodium and chloride excretion of the congested group. Only day 10 shows an adverse

profile. This can be explained by two data outliers present in the non-congested group. These outliers might be present due to a change in the urinary tubes. Subjects were well informed on the performance of urine spot sampling, however, it could be possible that the urine tubes of the pre- and post-diuretic sample were exchanged leading to this variant outcome at day 10. Nonetheless, mean USAI and UCAI tend to be variant in both groups, while USAI_Osm and UCAI_Osm show a statistical difference between the non-congested and congested group.

These results show that there is a relationship between a low USAI_Osm or UCAI_Osm and a worsening clinical outcome, i.e. congestion reoccurrence. Several aspects might lay at the foundation of this relationship. First, differences in the ingested amount of chloride and sodium could not be monitored during the study period. However, none of the subjects indicated a change in their food pattern on the biometrical questionnaire. Nonetheless, it could be possible that subjects of the congested group, who show symptoms of fluid overload, ingested a lower amount of chloride and sodium leading to a diminished excretion of these two ions via the urine. HF patients are recommended to restrict their salt intake to 2 – 3 g/day, if subjects of the congested group were more adherent to these guidelines it could display differences in urine output (39). As such, literature has shown that a too stringent salt restriction causes the activation of compensatory neurohumoral mechanisms leading to fluid overload (46). Second, a reduced diuresis due to the inability to excrete a sufficient amount of chloride and/or sodium might also explain the difference in urine output. Subjects with a difficulty to excrete a sufficient amount of chloride and sodium have difficulty to maintain euvolemia (62). This incomplete diuresis can be caused by diuretic resistance, which is a phenomenon that can alter the renal distal tubule cells, resulting in sodium retention. Therefore, causing a lower output of sodium in the urine accompanied by an increased risk of decompensation (37). Thirdly, a diminished urinary sodium and chloride output in the congested group might be caused by an inadequate diuretic dosing. The loop diuretic dose should be adjusted to the subject's need, a too high diuretic dose can cause diuretic resistance as described previously, while a too low diuretic dose might lead to an insufficient diuretic response. According to the ESC guidelines, a recommended daily loop diuretic dose is 1 – 5 mg of bumetanide (2). Subjects of both the congested and the non-congested group were dosed according to these guidelines and no statistical differences in loop diuretic dose was present between the two groups. The data obtained during this study argues against the fact that inadequate diuretic dosing might reason for a diminished chloride and sodium output in the congested group.

A goal of this study is to set up a urinary sodium/chloride range associated with the lowest rate of recurrence of congestion and hospital readmission or which directly correlates to an increased risk for hospital readmission. Gathered data of 14 subjects indicates that a USAI_Osm value <0.05 mol/Osm and a UCAI_Osm value <0.08 mol/Osm are correlated with an increased risk for congestion reoccurrence. With additional research and data, this range can be optimized and tested.

Equally interesting is the comparison of the USAI values to the UCAI values and the comparison of USAI_Osm values to the UCAI_Osm. Overall, USAI and USAI_Osm values are exceeded by the UCAI and UCAI_Osm, respectively. This event can be explained by the mechanism of action of loop diuretics. Loop diuretics inhibit the sodium-potassium-chloride symporter in the thick ascending loop of Henle. This symporter normally re-absorbs two chloride ions for each sodium that passes through

the symporter (36). Therefore, once this symporter is inhibited the urine output can contain an increased amount of chloride ions compared to the sodium ions.

- **Urinary spot output subdivided by the general congestion scale**

The general congestion scale was the second scale utilized to subdivide the study population. The four indexes which assessed the diuretic efficacy after loop diuretic intake show no statistical difference between both groups. Demonstrating that no difference in urinary sodium or chloride output was present between the congested and non-congested group, while the worsening congestion scale clearly showed that a distinct urine output is present.

The absence of a significant difference in urinary output between the two groups, whilst using the general congestion scale, could be explained by the considerable small sample size of the congested group compared to the non-congested group. Consequently, limiting the event of observing a significantly different effect between both groups. A greater study population might, therefore, show different results. However, the predictive capacity of the general congestion scale to identify subjects at risk for recurrence of congestion and ADHF hospital readmission is disputed, since not all subjects readmitted with ADHF were identified in the congested group. Therefore, the data obtained during this study favors the use of worsening congestion scale instead of the general congestion scale as a correct scale to identify subjects at risk for hospital readmission.

4.2.2 Urinary spot output in relation to acute heart failure hospitalization

AHF hospitalization occurred in a minority of the study population. Subjects with or without AHF hospitalization were demographically matched. Therefore, differences in urinary profiles were not due to these variables independently. Only COPD was significantly different between the two groups, this effect was corrected in the statistical model.

Subjects who were hospitalized for ADHF after study participation showed an overall lower urinary USAI(_Osm) and UCAI(_Osm) profile compared to the non-hospitalized subjects. This difference in urine output is however not statistically significant between the two groups, relating to the small sample size. Data of both groups illustrates that subjects who were re-hospitalized with ADHF in the vulnerable phase tend to excrete a lower amount of sodium and chloride. The relationship between a low response to diuretics and a poor clinical outcome was previously demonstrated by other researchers (53, 54). During this study, it was shown that a tendency of this relationship is also present during the vulnerable phase and not only in subjects with CHF.

The tendency of a low urinary chloride and sodium and an increased risk for ADHF re-hospitalization might be explained by the same reasons as described in section '4.2.1 Urinary sodium output in relation to congestion status'. First being that subjects of the AHF hospitalization group might have ingested a lower amount of sodium (39). Yet, the absence of a change in the subjects' daily food pattern argues against this idea. Second, diuretic resistance could be present in the population of the AHF hospitalized group causing a diminished diuresis (37). Thirdly, the argument that inadequate or unequal diuretic dosing would be present between both groups is contradicted by the non-statistical difference in furosemide dose between groups and prescription of drug dose in accordance with the guidelines (2).

According to the predictive capacity of the two congestion scales to identify patients at risk for ADHF readmission, it would be supposed that hospitalized ADHF patients were included in the congested group. The worsening congestion scale identified all subjects with a hospital readmission in the congested group, while the general congestion scale only identified two subjects with readmission. This current data favors, therefore, the use of the worsening congestion scale to identify patients at risk for hospital readmission.

4.3 Study limitations

First, this is a small single-centered study. Hence, the study aimed at generating a new hypothesis on the urinary sodium concentration of ADHF patients in the vulnerable phase. Therefore, future research is required to confirm our findings. Second, the rather small sample size of the study should be interpreted according to the intensive study protocol. Subjects needed to perform repeated urinary spot samples according to a strict schedule. Since, in total 28 urine samples needed to be taken, the chosen collecting method was via urine spot sampling. This is not the golden standard for urine collections, however, this method has been proven to be of equal quality. Third, subjects' compliance with the study protocol cannot be completely assured. Subjects were well informed and received the necessary documentation of the study protocol. Together with direct questioning at follow-up, subjects' compliance was estimated assuring reliable study data. Fourth, the total drop-out rate in this study was 71.4%, relating to the intensive study protocol. Sixth, subjects were subdivided in a congested and non-congested group at follow-up by a general/worsening linear congestion scale. This is not a standardized scale, nevertheless, the scale was obtained after thorough literature search including main aspects which indicate the state of congestion, assuring reliable results. Finally, the difference in sodium intake by the subjects cannot be accounted for.

5 Conclusion

To conclude, urinary sodium and chloride concentration of ADHF patients enabled to predict congestion reoccurrence. Demonstrating that urinary spot output can be a metric to identify patients at risk for congestion reoccurrence.

Investigation of the urinary sodium and chloride output of ADHF patients, treated with loop-diuretics, showed that they had a stable profile during the first two weeks after hospital discharge. However, two congestion scales, i.e. worsening and general congestion scale, enabled to subdivide the study population in a non-congested and a congested group. This allowed to observe two distinct urine profiles.

The worsening congestion scale identified seven subjects in the congested group and seven subjects in the non-congested group. No differences in age, NYHA class, HF type or medication use was present in both groups. Indicating that differences observed in urine output were not individually caused by these demographics. USAI and UCAI output of the subjects of the non-congested group exceeded the USAI and UCAI output of the congested group. This difference was not significantly different but shows that subjects of the congested group tend to excrete a lower amount of sodium and chloride. USAI_Osm and UCAI_Osm output was significantly different between the two groups. Illustrating that a difference in diuresis efficiency, i.e. sodium and chloride excretion, is present in subjects exhibiting (no) signs of congestion. Namely, subjects presenting signs of congestion at approximately one month after ADHF hospitalization excrete a significantly lower amount of sodium and chloride. This scale could, therefore, be used to identify patients at risk for congestion reoccurrence in the future.

The congested group (n=3) and non-congested group (n=11) subdivided by the general congestion scale showed no significant difference in diuresis efficiency. However, the absence of a different urinary output can be explained by the incorrect subdivision of the study population in a congested and non-congested group. Therefore, the predictive capacity of the general congestion scale to identify subjects at risk for congestion reoccurrence is disputed.

Additionally, subjects hospitalized for an AHF episode (n=3) after study completion showed a urinary sodium and chloride output inferior to the subjects who were not hospitalized. This difference was not statistically significant, however, a trend can be observed showing that a difference in diuresis is present in subjects with or without AHF hospitalization. The subjects of the AHF hospitalized group did not have a more severe disease state, therefore the observed differences are not caused by disease severity.

Finally, all three subjects with a recurrent AHF hospitalization were identified by the worsening congestion scale and assigned to the congested group, i.e. the group at risk for hospital readmission. This finding substantiates the use of the worsening congestion scale instead of the general congestion stale to identify patients at risk for AHF hospitalization since the general congestion scale did not identify all subjects.

5.1 Future perspectives

This study aimed at generating new insights into the urinary profile of ADHF patients within the days following hospital discharge. During this phase, also known as the vulnerable phase, HF patients are highly susceptible to HF-related hospital readmission. The study demonstrated that a significant difference of urinary sodium and chloride output was present in recently discharged ADHF patients after subdividing the study population in a non-congested and a congested group.

To substantiate these findings the main goal is to include a greater amount of study participants. Currently, a Ph. D. Fellow is executing the MORE-RESPONSE study and is including patients until a study population of circa 35 patients is reached. With this increase in study participants, a greater amount of urine spot samples will be collected and analyzed. This can be used to validate the conclusions of the MORE-RESPONSE study written in this thesis and to investigate new objectives.

A novel objective would be to investigate whether the indexes showing a statistical difference, i.e. USAI_Osm and UCAI_Osm, have an equal relevance or if one index has a higher significant predictive value on the primary endpoint of congestion reoccurrence, leading to hospital readmission. Moreover, it can be investigated if the two other indexes, USAI and UCAI, might also show a statistical difference in urinary profiles of the congested and non-congested group. Furthermore, the urinary sample protocol can be optimized. It can be questioned if specific days are crucial predictors for an increased risk of hospital readmission or if a shorter period than 14 days would be an (equal) accurate predictor for this endpoint. Additionally, with the analyzed urine profiles, a urinary sodium/chloride range can be set up, which directly correlates to an increased risk for hospital readmission. At the moment, data suggest that a USAI_Osm value <0.05 mol/Osm and a UCAI_Osm value <0.08 mol/Osm correlates with an increased risk for congestion reoccurrence. With additional data this range can be optimized and can eventually be implemented by the cardiologist and help them to identify the patients at risk for congestion reoccurrence possibly leading to ADHF related hospitalization.

The ultimate goal is to implement this method in a home environment. Recently discharged ADHF patients would analyze their urinary sodium and chloride output in a self-measured method by using urinary dipsticks. These sticks, together with urinary sodium/chloride range, will indicate whether the patient has an increased risk for congestion reoccurrence and ADHF related hospital readmission.

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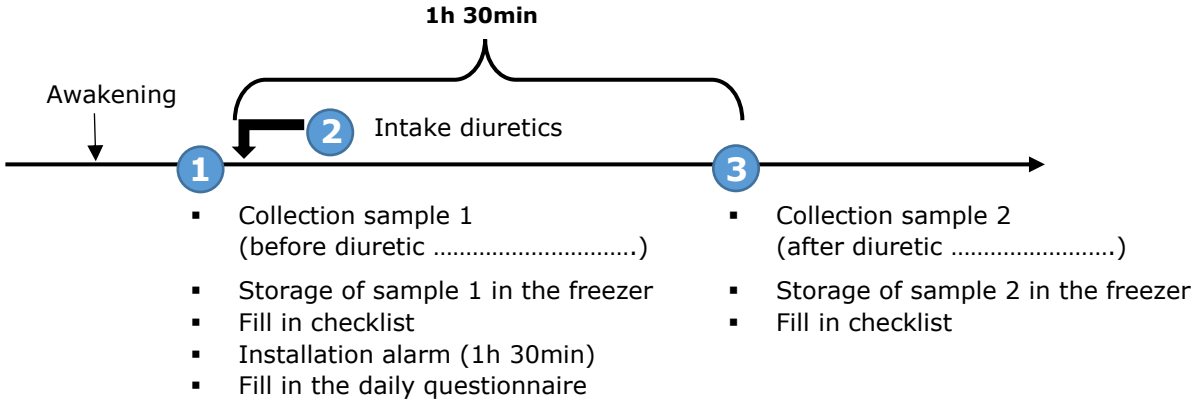
Appendix

Protocol urine sampling at home (MORE-RESPONSE study)

During the next 14 days, you will collect urine spot samples. The following steps indicate the actions that need to be performed every day. These actions are graphically shown in the below-mentioned scheme.



Step 1: Collection sample 1

- After awakening in the morning, the first urine sample will be collected.
  - Remove the blue cap of the plastic disposable cup and urinate in the cup.
  - Next, place the blue cap again on the plastic disposable cup.
  - Remove the white tag of the blue cap.
  - Take the urinary test tube indicated with 'Sample 1 and the matching day'.
  - Place the urinary test tube in the aspiration port of the blue cap.
  - Fill the urinary test tube up to the indicated line and remove the urinary test tube of the disposable collecting cup.
- Storage of urine sample.
  - Place the urine sample in the freezer.
- Filling in the checklist.
  - Indicate if you have collected the first urinary sample on the checklist.
- Installation of alarm.
  - Installation of alarm over 1 hour and 30 minutes.
- Daily questionnaire
  - Fill in the daily questionnaire.

Step 2: Intake diuretic

- After collection of the first urinary sample, you may take you prescribed diuretics.

Step 3: Collection sample 2

- After 1 hour and 30 minutes the alarm, set in **step 1**, will go off.
- Collect the second urinary sample.
  - Urinate in the disposable collecting cup as described in **step 1**.
  - Take the urinary test tube indicated with 'Sample 2 and the matching day'.
- Storage of urine sample.
  - Place the urine sample in the freezer.
- Filling in the checklist
  - Indicate if you have collected the second urinary sample on the checklist.

Control list urine samples	**(MORE-RESPONSE study)**	
Name and surname: **Date of birth:**		
Days	**Sample 1 (before diuretic)**	**Sample 2 (after diuretic)**
Day 1	❑ Sample 1 collected ❑ Sample 1 **not** collected ……	❑ Sample 2 collected ❑ Sample 2 **not** collected ……
Day 2	❑ Sample 1 collected ❑ Sample 1 **not** collected ……	❑ Sample 2 collected ❑ Sample 2 **not** collected ……
Day 3	❑ Sample 1 collected ❑ Sample 1 **not** collected ……	❑ Sample 2 collected ❑ Sample 2 **not** collected ……
Day 4	❑ Sample 1 collected ❑ Sample 1 **not** collected ……	❑ Sample 2 collected ❑ Sample 2 **not** collected ……
Day 5	❑ Sample 1 collected ❑ Sample 1 **not** collected ……	❑ Sample 2 collected ❑ Sample 2 **not** collected ……
Day 6	❑ Sample 1 ❑ Sample 1 **not** collected ……	❑ Sample 2 ❑ Sample 2 **not** collected ……
Day 7	❑ Sample 1 collected ❑ Sample 1 **not** collected ……	❑ Sample 2 collected ❑ Sample 2 **not** collected ……

Days	Sample 1 (before diuretic)	Sample 2 (after diuretic)
Day 8	❑ Sample 1 collected ❑ Sample 1 **not** collected ……	❑ Sample 2 collected ❑ Sample 2 **not** collected ……
Day 9	❑ Sample 1 collected ❑ Sample 1 **not** collected ……	❑ Sample 2 collected ❑ Sample 2 **not** collected ……
Day 10	❑ Sample 1 collected ❑ Sample 1 **not** collected ……	❑ Sample 2 collected ❑ Sample 2 **not** collected ……
Day 11	❑ Sample 1 collected ❑ Sample 1 **not** collected ……	❑ Sample 2 collected ❑ Sample 2 **not** collected ……
Day 12	❑ Sample 1 collected ❑ Sample 1 **not** collected ……	❑ Sample 2 collected ❑ Sample 2 **not** collected ……
Day 13	❑ Sample 1 collected ❑ Sample 1 **not** collected ……	❑ Sample 2 collected ❑ Sample 2 **not** collected ……
Day 14	❑ Sample 1 collected ❑ Sample 1 **not** collected ……	❑ Sample 2 collected ❑ Sample 2 **not** collected ……

Daily questionnaire

Study sticker

MORE-RESPONSE

Blood pressure ☐☐☐☐☐☐ mmHg	Body weight ☐☐☐☐☐☐ kg

VAS score dyspnea	**Medication dose**
Score shortness of breath 0 – 100. *0: No complaints – no shortness of breath.* *100: Seriously short out of breath.* ☐☐☐	*(Lasix/Burinex)* Diuretic dose …….. mg

Edema– fluid accumulation in legs

- ❑ 0 (No fluid accumulation in legs)
- ❑ 1+ (Pit in leg after push in leg with finger, disappears quickly)
- ❑ 2+ (Pit in leg after push in leg with finger, stays present)
- ❑ 3+ (Clear fluid accumulation in lower leg)
- ❑ 4+ (Fluid accumulation above the knee)

Diet

Were there any changes in your diet today?

❑ No

❑ Yes, explain

……

……

……

Table 7| Baseline characteristics of subjects subdivided according to the general congestion scale**.**

Variable	Non-congested group (n=11)	Congested group (n=3)	*p*-value
Demographics			
Age, years	71 ± 5	81 ± 9	0.270
Male, n(%)	9 (81.8)	2 (66)	0.571
Active smokers, n(%)	3 (27.3)	0 (0)	0.382
Heart failure type, n(%)			0.051
HFpEF	4 (36.6)	3 (100)	
HFrEF	7 (63.6)	0 (0)	
Heart failure etiology if HFrEF, n(%)			/
Ischemic	5 (83.3)	/	
Non-ischemic	1 (16.7)	/	
Physical features			
SBP, mmHg	115.5 ± 20.1	119 ± 23.9	0.796
DBP, mmHg	69 (62 – 78)	62 (61.5 – 70.5)	0.659
Weight, kg	96.4 ± 22.4	73 ± 4.5	**0.007***
BMI, kg/m²	33.5 ± 7.4	24.6 ± 2	0.066
Heart rate, beats/min	65.3 ± 13.3	73.3 ± 8.5	0.346
Comorbidities, n(%)			
Atrial fibrillation	7 (63.6)	3 (100)	0.217
COPD	2 (18.2)	1 (33.3)	0.571
Hypertension	7 (63.6)	1 (33.3)	0.347
Diabetes	7 (63.6)	0 (0)	0.051
NYHA class, n(%)			0.249
Class I	2 (18.2)	2 (66.7)	
Class II	8 (72.7)	1 (33;3)	
Class III	1 (9.1)	0 (0)	
Guideline directed HF-therapy, n(%)			
ACE-I (if HFrEF)	1 (14.3)	/	/
ARB (if HFrEF)	1 (14.3)	/	/
Beta-blocker (if HFrEF)	6 (85.7)	/	/
Aldosterone antagonist	10 (90.9)	1 (33.3)	**0.031***

Table 7 continued| Baseline characteristics of subjects subdivided according to the general congestion scale**.**

Variable	Non-congested group (n=11)	Congested group (n=3)	*p*-value
Guideline directed heart failure-therapy, n(%)			
Furosemide equivalent			0.346
40 mg	6 (54.5)	3 (100)	
60 mg	1 (9.1)	0 (0)	
80 mg	4 (36.4)	0 (0)	

Data is presented as mean ± standard deviation (std) if normally distributed or by median and interquartile range (IQR). Discrete variables are expressed as sample size (n) and percent. A p-value of <0.05 was considered as statistical significant (*). ACE-I: angiotensin converting enzyme inhibitor; ARB: angiotensin receptor blocker; COPD: chronic obstructive pulmonary disease; DBP: diastolic blood pressure; HFpEF: heart failure with preserved ejection fraction; HFrEF: heart failure with reduced ejection fraction; NYHA: New York heart association; SBP: systolic blood pressure.

Table 8| Baseline characteristics of subjects with or without an AHF hospitalization.

Variable	AHF hospitalization (n=3)	No AHF hospitalization (n=11)	*p*-value
Demographics			
Age, years	71 (67 – 71)	75 (66 – 87)	0.555
Male, n(%)	3 (100%)	8 (72.3%)	0.308
Active smokers, n(%)	0 (0%)	3 (27.3%)	0.078
Heart failure type, n(%)			0.515
HFpEF	2 (66.7%)	5 (45.5%)	
HFrEF	1 (33.3%)	6 (54.5%)	
Heart failure etiology if HFrEF, n(%)			0.659
Ischemic	1 (100%)	5 (83.3%)	
Non-ischemic	0 (0%)	1 (26.7%)	
Physical features			
SBP, mmHg	123 ± 33.5	114.4 ± 16.4	0.703
DBP, mmHg	72.7 ± 12.9	71.9 ± 18.1	0.948
Weight, kg	85.7 ± 26.3	92.8 ± 22	0.639
BMI, kg/m²	30 ± 9.4	31.8 ± 6.8	0.869
Heart rate, beats/min	72 ± 5.6	65.6 ± 13.8	0.461
Comorbidities, n(%)			
Atrial fibrillation	2 (66.7%)	8 (72.7%)	0.837
COPD	2 (66.7%)	1 (9.1%)	**0.031***
Hypertension	2 (66.7%)	6 (54.4%)	0.707
Diabetes	2 (66.7%)	5 (45.5%)	0.515
NYHA class, n(%)			0.858
Class I	1 (33.3%)	3 (27.3%)	
Class II	2 (66.7%)	7 (63.6%)	
Class III	0 (0%)	1 (9.1%)	
Guideline directed heart failure-therapy, n(%)			
ACE-I (if HFrEF)	0 (0%)	1 (16.7%)	0.659
ARB (if HFrEF)	0 (0%)	1 (16.7%)	0.659
Beta-blocker (if HFrEF)	1 (100%)	5 (83.3%)	0.659
Aldosterone antagonist	3 (100%)	8 (72.7%)	0.308

Table 8 continued| Baseline characteristics of subjects with or without an AHF hospitalization**.**

Variable	AHF hospitalization (n=3)	No AHF hospitalization (n=11)	*p*-value
Guideline directed HF-therapy, n(%)			
Furosemide equivalent			0.346
40 mg	3 (100%)	6 (54.5%)	
60 mg	0 (0%)	1 (9.1%)	
80 mg	0 (0%)	4 (36.4%)	

Data is presented as mean ± standard deviation (std) if normally distributed or by median and interquartile range (IQR). Discrete variables are expressed as sample size (n) and percent. A p-value of <0.05 was considered as statistical significant (*). ACE-I: angiotensin converting enzyme inhibitor; AHF: acute heart failure; ARB: angiotensin receptor blocker; COPD: chronic obstructive pulmonary disease; DBP: diastolic blood pressure; HFpEF: heart failure with preserved ejection fraction; HFrEF: heart failure with reduced ejection fraction; NYHA: New York heart association; SBP: systolic blood pressure.