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Mineralocorticoid Receptor Antagonism in Acutely Decompensated Heart Failure

Tese de Candidatura ao grau de Doutor em Ciências Médicas, submetida ao Instituto de Ciências Biomédicas Abel Salazar da Universidade do Porto.

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II. Ferreira JP, Santos M, Almeida S, Marques I, Bettencourt PM, Carvalho H. Tailoring diuretic therapy in acute heart failure: insight on early diuretic response predictors. Clin Res Cardiol 2013; 102: 745-753.

III. Ferreira JP, Santos M, Almeida S, Marques I, Bettencourt PM, Carvalho H. The role of albuminuria as a non-invasive marker for congestive acutely decompensated chronic heart failure and the spironolactone effect in elderly Portuguese: a non-randomized trial. Nephrology 2014; 19: 149–156.

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VII. Ferreira JP, Santos M, Oliveira JC, Marques I, Bettencourt PM, Carvalho H. The Influence of Spironolactone on Matrix Metalloproteinases in Acute Decompensated Heart Failure. Submitted.

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Contents

Introduction

Heart failure (HF) is the leading cause of hospitalization in patients older than 65 years of age and a major public health problem[1]. Hospital admissions for acutely decompensated heart failure (ADHF) are frequent and accompanied by high percentages of hospital complications and mortality[2]. There is a paucity of data derived from controlled clinical trials to define optimal treatment for patients with acute HF which is largely based on loop diuretics and vasodilators for congestion relief[3].

The pathophysiology of ADHF is characterized by abnormal neurohormonal activation, leading to an increase of sodium and water retention, arterial vasoconstriction, and activation of inflammatory cascades[4,5]. Excessive neurohormonal activation may lead to several harmful effects[6], including renal dysfunction and myocardial injury[7]. The recognition of neurohormonal activation in HF has led to the development of therapies able to inhibit inappropriate neurohormonal enhancement, particularly the renin-angiotensin-aldosterone system (RAAS). The advent of these therapies had high impact on morbidity and mortality of these patients[4].

Despite the use of angiotensin-converting enzyme inhibitors (ACEIs), angiotensin receptor blockers (ARBs), and beta-blockers (BBs), neurohormonal activation remains inappropriately elevated in patients hospitalized with ADHF[8-10]. Furthermore, there is growing evidence suggesting that aldosterone may only transiently be suppressed with ACEIs/ARBs. This phenomenon termed “aldosterone breakthrough” can have important clinical consequences given aldosterone sodium-retaining, profibrotic, and inflammatory properties[11-13]. The addition of mineralocorticoid receptor antagonists (MRAs) on top of ACEIs/ARBs and BBs has been shown to improve clinical outcomes[14]. The use of MRAs reduced morbidity and mortality in patients with chronic, severe systolic heart failure (HF)[14], in chronic HF with mild symptoms[15], and after myocardial infarction[16]. The improved morbidity and mortality observed in these trials is thought to be in part due to suppression of neurohormonal activation by MRAs, suggesting a potential interest in early initiation of MRAs in the ADHF setting.

Considering the anti-hypertensive and diuretic properties of MRAs, the ideal candidate for this therapy should be hyper- or normotensive and fluid overloaded, without evidence of cardiogenic shock. In addition, patients with systemic congestion, and/or with predominantly right-sided HF may have hepatic congestion and consequently have impaired clearance of neurohormonal factors[8], providing an attractive subset of patients likely to take the most benefit out of MRA therapy.

Mineralocorticoid Receptor Antagonists and Diuretic Resistance

Resistance to loop diuretics has been associated to a much higher plasma aldosterone concentration[17-19]. Chronic administration of loop diuretics has been shown

to cause hypertrophy of the distal nephron and increase expression of the sodium chloride cotransporter, which is an aldosterone-induced protein. These structural changes can lead to loop diuretic resistance and these effects are potentially reversed by a mineralocorticoid antagonist[20].

Congestion refractory to oral diuretics may be responsible for up to one-third of hospital admissions due to ADHF[1], and unresolved congestion may contribute to the high readmission rates observed in these patients[1]. Furthermore, approximately 30% of patients admitted for ADHF develop diuretic resistance, defined as reduced diuresis and natriuresis in response to a constant high dose of loop diuretics[21]. Strategies to overcome this clinical problem still have limited success. The use of diuretics to treat persistent congestion may lead to kidney injury and worsening renal function. Venovenous ultrafiltration is an option in these patients, however it is an expensive and invasive procedure that requires hospitalization. Most importantly, this technique did not reduce mortality or hospitalizations for HF compared to strategy of stepped diuretic-based therapy[22]. Therefore, the use of this technique does not seem justified for patients hospitalized for ADHF, worsened renal function, and persistent congestion. The use of natriuretic doses of MRAs in this setting may be an attractive alternative to reverse diuretic resistance and inappropriate RAAS activation[23].

Mineralocorticoid Receptor Antagonists May Prevent Myocardial Injury, Remodelling and Fibrosis

Myocardial injury, indicated by troponin release, is common in ADHF[7,24-27]. The ADHF episodes are associated with increased mechanical strain on the heart, activation of neurohormonal systems, and increased and oxidative stress[28]. These stimuli are known to mediate myocardial injury and accelerating myocyte loss[28]. Improvements in analytical sensitivity have transformed circulating troponin from a biomarker that was only detectable in a minority of patients to one that is detectable in the vast majority of patients with HF[25]. The high sensitivity troponin-T (hsTnT) test can detect very small changes in the circulating troponin levels[25,29]. Elevations in baseline troponin levels were demonstrated to be independent predictors of events during the acute hospitalization (worsening or persistent HF, death, and increased length of stay) and also independent predictors of post-discharge outcomes[24,27,30-33]. Accordingly, changes in troponin status during initial treatment for ADHF have been proposed as potentially important targets for drug development[34].

Mineralocorticoid receptor antagonists decrease myocardial fibrosis, inflammation, oxidative stress, apoptosis, neurohormonal activation, and remodelling in animal models and patients with HF[35,36]. The anti-fibrotic influence of MRAs in patients with ischaemic myocardial necrosis provides an additional therapeutic benefit as

demonstrated in post-myocardial infarction patients with left ventricular (LV) dysfunction in the EPHESUS trial[16]. Additionally, MRA therapy reduces biochemical markers of ventricular remodelling and improves LV function and structure[37]. Matrix metalloproteinases (MMPs) are a family of enzymes important for the resorption of extracellular matrices (ECM), control of vascular remodelling and repair. MMPs are important for proteolysis that can affect the composition of ECM and consequently myocardial remodelling. Additionally, increased ECM turnover may be associated with pathological myocardial remodelling, that is accelerated in ADHF[29,38]. Increased activity of matrix metalloproteinase-2 (MMP2) has been demonstrated in ADHF and a decrease of circulating MMPs has been demonstrated along with ADHF successful treatment[38,39]. Hence, a reduction in markers of ECM turnover may serve as a surrogate marker for deceleration of myocardial turnover and remodelling.

Cardiorenal Interactions and Mineralocorticoid Receptor Antagonists

Interactions between the heart and the kidneys are increasingly acknowledged by clinicians. The term cardiorenal syndrome (CRS) applies to the bidirectional nature of how disease in one organ system affects the function of the other organ system[40].

Despite the notable advance in the understanding of this area, management of patients presenting with ADHF and concomitant renal dysfunction remains challenging. Loop diuretics are the mainstay pharmacologic treatment for the management of ADHF patients presenting with volume overload[41]. Their use is likely to lead to improvement of symptoms and may even improve kidney function by decreasing renal venous pressure[42]. A recent study, showed an albuminuria decrease after ADHF treatment[43]. Unfortunately, the use of loop diuretics has several pitfalls. Loop diuretics can cause significant electrolyte abnormalities, hypotension, and increased neurohormonal activation[41]. Increased neurohormonal activation, particularly the overactivation of the RAAS, is one of the most important mechanism leading to diuretic resistance[44]. A possible approach to overcome diuretic resistance is administration of a second diuretic agent as an MRA in natriuretic doses.

Drug Selection: Is Spironolactone a Good Candidate to be Used In the Acutely Decompensated Heart Failure Setting?

In addition to patient and clinical setting selection, it is necessary to know which drug to use and in which dose.

Spironolactone is a non-selective MRA with moderate affinity for both progesterone and androgen receptors. The latter property increases the likelihood of endocrine side effects with spironolactone including loss of libido, menstrual irregularities, gynecomastia and impotence[45]. Spironolactone has extensive and complex metabolism in humans, its absorption can reach 80-90% and it is not affected

by food[46], however it undergoes extensive hepatic metabolism into the active metabolites - canrenone and sulfur-containing metabolites: 7 alpha-thiomethylspirolactone (IV) and 6 beta-hydroxy-7 alpha-thiomethylspirolactone (V), with prolonged half-lives ranging from 14 to 17 hours (h). The sulfur-containing metabolite IV rather than canrenone is the major metabolite in serum following single or repeated doses of spironolactone[47]. Spironolactone is poorly soluble in aqueous fluids and an intravenous formulation is not available for routine clinical use[45]. Following a single dose of oral spironolactone, peak serum concentrations of the drug and its metabolites occur at 1–2 and 2–4 h, respectively[48]. The peak response for spironolactone natriuretic effect can occur until 48 hours after the first administration[45]. This relatively slow peak of action has been related to the time active metabolites take to reach steady-state plasma levels[45]. Interestingly, a single intravenous dose of the spironolactone metabolite potassium canrenoate has demonstrated a rapid natriuretic effect (within 6 hours after administration), possibly by avoiding the first-pass metabolism, accelerating the obtainment of steady-state[49].

Overall, spironolactone is an attractive option to use in this setting, given the wide experience with the drug, the low incidence of side effects, and the rapid onset of action.

Blocking the Mineralocorticoid Receptor: Titration and Monitorization

Although the pharmacological blockade of RAAS is one of the mainstays of HF treatment, a suboptimal neurohormonal inhibition is still a critical issue in standard practice.

Measuring the activation and the effective blockade of RAAS would help us to enhance its inhibition and to tailor these therapies to the individual HF patients[50]. Thus reliable and practical biomarkers are warranted to establish the activity of the RAAS in clinical practice[51]. Taking advantage of the renal physiological action of aldosterone, the urinary sodium to potassium (uNa/K) ratio is an attractive candidate biomarker of RAAS modulation[51].

Aldosterone regulates sodium balance by increasing the expression of the epithelial Na^+ channel and the Na^+/K^+-ATPase pump found on the distal nephron epithelial cells[52], promoting reabsorption of Na^+ and the excretion of K^+[53]. Previous reports had indicated a measurable effect of MRA on uNa/K ratio consistent with effects of aldosterone on electrolyte balance[54,55]. Mineralocorticoid receptor blockade elicited an increase in uNa/K ratio in rats in a dose-dependent manner[51], supporting the use of uNa/K ratio as a translatable biomarker of MRA with the potential to enable dose selection for clinical trials[51].

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Setting and Methods

This project was designed to answer the questions elaborated in the *Introduction*.

a) Is high-dose spironolactone safe in ADHF?
b) Can spironolactone overcome diuretic resistance?
c) Can spironolactone mitigate myocardial and renal injury during the ADHF episode?
d) Can spironolactone reduce turnover and fibrosis biomarkers?
e) Does high-dose spironolactone affect the RAAS?
f) Is there an easily available surrogate marker for spironolactone use?

In order to answer these questions we designed a prospective, non-randomized, experimental, single-centre, and single-blinded trial conducted in Centro Hospitalar do Porto enrolling participants between February 2012 and February 2013.

Patients were eligible for enrollment if they presented with decompensation of chronic HF with symptoms leading to hospitalization. HF was diagnosed on the basis of the presence of history of chronic heart failure and at least one symptom (dyspnea, orthopnea, or edema) and one sign (rales, peripheral edema, ascites, or pulmonary vascular congestion on chest radiography). Exclusion criteria were: chronic use of MRAs, cardiac surgery within 60 days of enrollment, cardiac mechanical support, cardiac resynchronization-therapy within the last 60 days, comorbid conditions with an expected survival of less than 6 months, acute myocardial infarction at time of hospitalization, hemodynamically significant uncorrected primary cardiac valvular disease, patients requiring intravenous vasodilators or inotropic agents, supine systolic arterial blood pressure <90 mmHg, serum creatinine level >1.5 mg/dL, serum potassium level >5.0 mmol/L, hemoglobin level <9 g/dL, and sepsis.

Patients were non-randomly assigned in a sequential 1:1 ratio to the intervention or standard treatment. Chief investigator was responsible to assess the eligibility criteria and to allocate the intervention after being contacted by the patient assistant physician. Patients were blinded to the intervention allocation. Assistant physicians were not blinded to intervention allocation. Assistant physicians were Attending Physicians or Fellows of Internal Medicine or Cardiology depending on the ward where each patient was admitted. The assistant physicians evaluated the clinical signs and symptoms and registered their evaluation in the clinical diaries and then transcribed to our database by the authors.

Patients were assigned to either oral spironolactone (minimum and maximum initial dose of 50 - 100 mg/d, according to assistant physician) plus standard AHF

therapy or standard AHF therapy alone. Standard AHF therapy included intra-venous (i.v.) furosemide (bolus or continuous infusion), digoxin, ACEi, ARB, nitrates, and/or non-invasive ventilation (NIV), according to attending physician decision. At day 2, the attending physician had the option of adjusting spironolactone dose on the basis of the clinical judgment and laboratory results. At this time, the physician could decrease the dose by 50%, to a minimum of 50 mg/d, or maintain the same strategy.

Patient`s clinical status was prospectively recorded, by the assistant physicians, according to previous defined parameters.

An assessment of biomarkers, including plasma creatinine (pCr), ions, N-terminal pro-brain natriuretic peptide (NTproBNP), high sensitivity troponin T (hsTnT) and microalbuminuria was performed at a central core laboratory at admission day (day 1) and day 3. Clinical assessment and routine analyses were performed daily during hospital stay. All patients performed a transthoracic echocardiography within 72 hours upon admission. Left-ventricular ejection fraction was estimated by transthoracic echocardiography using the biplane Simpson method.

Patients characteristics are describe in Table 1.

Table 1. Baseline Characteristics of the Study Population

	Control Group	Spironolactone Group	p Value
Age (yrs)	78,8 ± 9,3	73,2 ± 11,7	0,01
Male sex – no. (%)	17 (34)	22 (44)	0,31**
Ejection Fraction (%)	45,5 ± 10,7	41,4 ± 12,4	0,08**
HFrEF – no. (%)	13 (26)	18 (36)	0,28**
HgB (g/dL)	12,2 ± 1,8	12,7 ± 2,3	0,22
Etiology of Heart Failure – no. (%)			
Ischemic	24 (48)	26 (52)	0,69**
Non-Ischemic	26 (52)	24 (48)	0,84**
Basal NYHA class – no. (%)			
II	41 (82)	44 (88)	0,4**
III	9 (18)	6 (12)	0,4**
History of Atrial Fibrillation or Flutter - no. (%)	34 (68)	25 (50)	0,07**
Outpatients Medications – no. (%)			
Furosemide	37 (74)	35 (70)	0,65**
ACE Inhibitors	24 (48)	18 (36)	0,22**
Beta-Blockers	26 (52)	10 (20)	<0,01**
Outpatients Oral Dose (mg)			
Furosemide	69,2 ± 37,8	68 ± 30	0,89
ACE Inhibitors	5 ± 3	4,3 ± 2,5	0,43
Beta-Blockers	4,4 ± 2,7	4,5 ± 1,1	0,90
BMI ≥ 30 (Kg/m^2) – no. (%)	14 (28)	19 (38)	0,29**
Diabetes Mellitus – no. (%)	25 (50)	20 (40)	0,31**
Glycated Hemoglobin (%)	6,9 ± 0,7	7,1 ± 1,2	0,43**
Obstructive Sleep Apnea Syndrome – no. (%)	5 (10)	13 (26)	0,32**
AHF precipitant (n)			
Undertreatment	34 (68)	29 (58)	0,30**
Dysrythmia	10 (20)	9 (18)	0,80**
Non-compliance	6 (12)	8 (16)	0,56**
NSAIDs	0	4 (8)	0,04**

Continuous variables are presented as mean value ± standard deviation [SD], p value. Categorical variables are presented as absolute number (%), p value. **Chi-square test.

HgB = hemoglobin; ACE = angiotensin-converting enzyme; BMI = Body Mass Index; NYHA = New York Heart Association; AHF = Acute Heart Failure; NSAID = Non-Steroidal Anti-Inflammatory Drug; HFrEF, heart failure with reduced ejection fraction.

Outcomes

The outcomes of our study were:

a) To test the influence of high-dose spironolactone in congestion assessment, evaluating the proportion of patients who were free of congestion at day 3 (defined as jugular venous pressure of < 8 cm, no orthopnea and no peripheral edema), weight change and also in congestion surrogate markers variation, such as N-terminal pro-brain natriuretic peptide (NT-pro BNP);
b) To test the safety of spironolactone use in ADHF, assessing creatinine and potassium change between day 1 and day 3;
c) To test the influence of spironolactone in surrogate markers such as albuminuria, hsTnT, uNa/K, MMP2, renin and aldosterone.

Papers

I. Mineralocorticoid Receptor Antagonism in Acutely Decompensated Chronic Heart Failure

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Abstract

Background/Objectives: Mineralocorticoid receptor antagonists (MRAs) use in acutely decompensated chronic heart failure (ADCHF) may improve congestion through diuretic effect and prevent neurohormonal activation. We aimed to evaluate the clinical effect and safety of spironolactone in ADCHF.

Methods: Prospective, experimental, single-centre, and single-blinded trial. Patients were treated with: standard ADCHF therapy or oral spironolactone 50 - 100 mg/d plus standard ADCHF therapy.

Results: During 1 year period, 100 patients were enrolled, 50 included in the treatment group. Mean (SD) spironolactone dose (mg) at day 1 was 94,5 ± 23,3 and at day 3 was 62,7 ± 24,3. Worsening renal function (increase in pCr ≥ 0,3 mg/dL from day 1 to day 3) was more likely to occur in control group (20% vs. 4%; p = 0,038), serum potassium did not differ between groups, and plasma NTproBNP had a significant decrease in spironolactone group at day 3 (median [IQR], 2488 [4579] vs. 1555 [1832]; p = 0,05). Furthermore, a greater proportion of patients in the treatment group were free of congestion at day 3: less edema, rales, jugular venous pressure (JVP) and orthopnea (all, p < 0,05). In addition, a significantly higher proportion of patients were on oral furosemide at day 3 (44% vs. 82%; p < 0,001).

Conclusions: Our study supports the safety of high dose spironolactone in ADCHF and suggests a positive impact in the resolution of congestion. The important findings of our pilot study need to be confirmed in larger trials.

Introduction

The recognition of the importance of chronic neurohormonal activation in heart failure (HF) pathophysiology was crucial to the development of new therapies beyond diuretics and digoxin. Pharmacological inhibition of renin-angiotensin-aldosterone system (RAAS) had a remarkable impact on morbidity and mortality of HF patients[1]. Likewise, mineralocorticoid receptor antagonists (MRAs) showed to be effective in reducing hospitalizations and mortality in systolic HF[2, 3].

The natural history of HF is characterized by recurrent episodes of acute HF (AHF). AHF defines a new onset HF or acutely decompensated chronic HF (ADCHF). Patients present with signs and symptoms needing urgent therapy[4]. Despite the prominent therapeutic advances in ambulatory HF patient, little progress has been made in the improvement of ADCHF patients treatment[5].

Aldosterone levels are elevated in patients with ADCHF despite the use of angiotensin-converting enzyme inhibitors (ACEi), angiotensin receptor blockers (ARB), and beta-blockers (BB)[6]. In this setting, aldosterone elevation may contribute to cardiorenal dysfunction, increasing the risk of death and ventricular arrhythmias[6-8]. Therefore, MRAs use in ADCHF treatment has two major putative advantages: improve congestion and hypervolemia through its diuretic effect and prevent the neurohormonal activation that characterizes ADCHF, and that is enhanced by loop diuretics[5, 9, 10].

The impact of MRAs in ADCHF patients has not been well-studied. We aimed to evaluate the short-term clinical effect and safety of the MRA antagonist spironolactone in worsening chronic HF patients.

Methods

Study Design

Prospective, experimental, single-centre, and single-blinded trial conducted in a Portuguese tertiary hospital enrolling participants between February 2012 and February 2013.

Study Participants

Patients were eligible for enrollment if they presented with decompensation of chronic HF with symptoms leading to hospitalization. HF was diagnosed on the basis of the presence of history of chronic heart failure and at least one symptom (dyspnea, orthopnea, or edema) and one sign (rales, peripheral edema, ascites, or pulmonary vascular congestion on chest radiography). Exclusion criteria were: chronic use of MRAs, cardiac surgery within 60 days of enrollment, cardiac mechanical support, cardiac resynchronization-therapy within the last 60 days, comorbid conditions with an

expected survival of less than 6 months, acute myocardial infarction at time of hospitalization, hemodynamically significant uncorrected primary cardiac valvular disease, patients requiring intravenous vasodilators or inotropic agents, supine systolic arterial blood pressure <90 mmHg, serum creatinine level >1.5 mg/dL, serum potassium level >5.0 mmol/L, hemoglobin level <9 g/dL, and sepsis.

Institutional review board or ethics committee approval was obtained. All patients provided written informed consent to participate in the study.

Treatment Assignments

Patients were non-randomly assigned in a sequential 1:1 ratio to the intervention or standard treatment. Chief investigator was responsible to assess the eligibility criteria and to allocate the intervention after being contacted by the patient assistant physician. Patients were blinded to the intervention allocation. Assistant physicians were not blinded to intervention allocation. Assistant physicians were Attending Physicians or Fellows of Internal Medicine or Cardiology depending on the ward where each patient was admitted. The assistant physicians evaluated the clinical signs and symptoms and registered their evaluation in the clinical diaries and then transcribed to our database by the authors.

Trial Intervention

Patients were assigned to either oral spironolactone (minimum and maximum initial dose of 50 - 100 mg/d, according to assistant physician) plus standard AHF therapy or standard AHF therapy alone. Standard AHF therapy included intra-venous (i.v.) furosemide (bolus or continuous infusion), digoxin, ACEi, ARB, nitrates, and/or non-invasive ventilation (NIV), according to attending physician. At day 2, the attending physician had the option of adjusting spironolactone dose on the basis of the clinical judgment and laboratory results. At this time, the physician could decrease the dose by 50%, to a minimum of 50 mg/d, or maintain the same strategy.

Study assessments

Patient`s clinical status was prospectively recorded, by the assistant physicians, according to previous defined parameters.

An assessment of biomarkers, including plasma creatinine (pCr), ions, N-terminal pro-brain natriuretic peptide (NTproBNP), high sensitivity troponin T (hsTnT) and microalbuminuria was performed at a central core laboratory at admission day (day 1) and day 3. Clinical assessment and routine analyses were performed daily during hospital stay. All patients performed a transthoracic echocardiography within 72 hours

upon admission. Ejection fraction was calculated according to biplane Simpson method.

End Points

The primary end point was the proportion of patients who were free of congestion at day 3 (defined as jugular venous pressure of < 8 cm, no orthopnea and no peripheral edema).

Two safety outcomes (pCr change between day 1 and day 3 plus potassium change between day 1 and day 3) were considered.

Secondary end points included changes in: body weight; NTproBNP levels; microalbuminuria; serum sodium; ionized calcium; serum magnesium; hsTnT; urinary sodium, potassium, urea; and the proportion of patients: taking oral furosemide at day 3; with increase in pCr ≥ 0,3 mg/dL from day 1 to day 3; and with hyper or hypokalemia during the study period.

Statistical Analysis

Comparison between groups was performed using parametric or non-parametric tests, as appropriate. Continuous variables are expressed as mean (standard deviation, SD) or median (inter-quartile range, IQR). Categorical variables are expressed in absolute numbers (no.) and proportions.

Association between different variables was tested by univariate analysis.

Significant association was defined by a p value ≤ 0,05.

Statistical analysis was performed using SPSS software (version 19, Chicago, IL, USA).

Results

The pre-specified duration of the enrolment period was one year and during that time we enrolled a total of 100 patients. Fifty patients were allocated to the treatment group. Despite the study protocol referred a range spironolactone dose of 50 to 100 mg/d, one patient had 200 mg at day 1. The mean ± (SD) spironolactone dose at day 1 was 94,5 ± 23,3 mg and at day 3 was 62,7 ± 24,3 mg.

Baseline characteristics of patients in each of the treatment groups are shown in *Table 1*. Patients in the control group were significantly older (mean ± (SD), 78,8 ± 9,3 vs. 73,2 ± 11,7 years; p = 0,01). The study groups were well balanced in most clinical characteristics, namely: gender, ejection fraction, baseline HF medications (except for beta-blockers, more common in control group – no. (%): 26 (52) vs. 10 (20);

p = 0,001), comorbidities[11], and risk stratification for in-hospital mortality [12, 13]. All patients were in New York Heart Association (NYHA) class IV upon admission. Analysed end-points are shown in *Table 2.* Patients in the treatment group had a significant respiratory rate (cycles/minute) reduction at day 3 (median [IQR], 20 [2] vs. 18 [3]; p < 0,001). No differences were observed in weight reduction, heart rate and systolic blood pressure (SBP). No patient developed hypotension (SBP < 90 mmHg). A greater proportion of patients in the treatment group was free of congestion at day 3: no edema (32% vs. 66%; p = 0,001), no rales (24% vs. 66%; p < 0,001), jugular venous pressure (JVP) ≤ 8 cm (90% vs. 100%; p = 0,02) and no orthopnea (76% vs. 96%; p = 0,004). In addition, a significantly higher proportion of patients were switched to oral furosemide at day 3 (44% vs. 82%; p < 0,001) – *Figure 1*. Furosemide dose was not significantly different in patients who remained on i.v. administration. ACEi and BB doses did not differ between study groups.

Worsening renal function (increase in pCr ≥ 0,3 mg/dL from day 1 to day 3) was more frequent in control group (20% vs. 4%; p = 0,038).

Indirect markers of glomerular damage were not significantly different between groups, but the treatment group appeared to have less glomerular damage after 3 days of treatment *i.e*. greater albuminuria reduction (median [IQR], – 7,3 [45,8] vs. -10,1 [71,2]; p = 0,32), and lower albuminuria ratio (median [IQR], 0,9 [0,8] vs. 0,7 [0,7]; p = 0,19). Fractional excretion of sodium (FENa) and urea (FEUr) did not differ between groups, however urine sodium to potassium (UNa/K) ratio significantly increased at day 3 in the spironolactone group (median [IQR], 2,1 [3,1] vs. 4,0 [3,9]; p = 0,007).

Serum potassium (K^+) levels did not differ significantly between groups – *Figure 2*. No patients developed hyperkalemia (serum potassium ≥ 5,5 mmol/L). More patients in the control group developed hypokalemia (serum potassium ≤ 3,5 mmol/L) but no significant differences were found (26% vs. 14%; p = 0,13).

Plasma NTproBNP had a significant decrease in spironolactone group at day 3 (median [IQR], 2488 [4579] vs. 1555 [1832]; p = 0,05) – *Figure 3*.

No significant differences were observed in hsTnT (median [IQR], - 0,0005 [0,01] vs. - 0,001 [0,01]; p = 0,57).

Hospital length of stay did not differ between the two groups [median [IQR], 9 [5] vs. 8 [5]; p = 0,8).

Discussion

Our study strongly suggests that the use of spironolactone in ADCHF patients is safe. Furthermore, spironolactone treatment was also associated with an earlier

resolution of symptoms and signs of congestion, as well as a more pronounced NTproBNP reduction.

Baseline clinical characteristics were well balanced between the two groups. Although control participants were older than those in the treatment group, that difference did not significantly change the mortality risk prediction scores. The control group had also higher proportion of baseline beta-blockers prescription. However, during hospitalization the beta-blocker treatment did not differ between groups.

The concern about hyperkalemia erroneously precludes the judicious prescription of MRAs in many clinical settings. Our study findings show that this therapy is safe in ADCHF as the use of spironolactone in this setting was not associated with renal dysfunction or hyperkalemia. The concomitant use of i.v. diuretics with kaliuretic properties could contributed to the absence of hyperkalemia[19]. In clinical trials enrolling chronic HF patients, MRAs were well tolerated when patients with pCr > 2,5 mg/dL and serum potassium > 5 mmol/L were excluded[2, 20]. Still regarding the potassium levels, we observed a trend to hypokalemia in the control group compared to the treatment group. These result is concordant with a previous study where the risk of hypokalemia was significantly reduced among patients receiving MRAs[3]. Reducing the risk of hypokalemia is a significant issue due to the fact that a potassium level below 4,0 mmol/L has been associated with an increased risk of death from any cause among patients with systolic heart failure[21].

Moreover, worsening renal function occurred more frequently in control group. Previous studies suggest that high i.v. diuretic dose is associated with worsening renal function in the short term[17, 18]. The frequent congestion status of ADHF and the diuretic therapy activate the RAAS and SNS, which induce renal arteriolar vasoconstriction, endothelial dysfunction and increased tubular reabsorption of sodium and urea[18]. The spironolactone diuretic and renoprotective potential can contribute to these clinical important findings.

We found that patients submitted to high dose spironolactone as add-on to standard AHF therapy had a faster resolution of congestive signs and an earlier switch to oral furosemide. The diuretic effect of spironolactone and possibly the attenuation of the pathological effects of mineralocorticoid receptor (MR) activation[14, 15] could explain these findings. In a previous study including 21 AHF patients with insufficient response to loop diuretics, 16 patients (76%) were submitted to 100 mg spironolactone once a day for 7 days in addition to high-dose loop diuretic (10 mg oral bumetanide) in combination with the maximum tolerable dose of an ACEi.
Spironolactone coadministration was highly effective in 13 of 16 patients (81%). Marked natriuresis and diuresis were achieved within the next week of treatment, and

HF symptoms decreased or disappeared[16]. Despite the earlier resolution of congestive signs found in the treatment group, no significant differences were observed in body weight change; this finding can be explained by the multiple patterns of congestion found *i.e*., many patients had *left heart* predominance with little weight change, and patients with *right heart* predominance presented with different levels of congestion. These differences (not discriminated in the study) may contribute for this apparent mismatch.

Consistent with the faster resolution of congestive signs and with the preservation of the renal function discussed above, we documented a significant reduction in NTproBNP within the spironolactone group. This is a notable finding since natriuretic peptides have shown to correlate with changes in ventricular wall stress, are inversely related to the severity of left ventricular dysfunction, and are robust prognostic predictors in HF[22-26].

Despite no significant differences were found, patients in the treatment group had greater albuminuria reduction during the treatment period. Albuminuria is an important predictor of adverse cardiovascular events in various populations and MRAs have been shown to attenuate vascular hypertrophy and reduce albuminuria[27, 28]. The improvement of this surrogate endpoint gives additional insight on possible benefits of MRA in ADCHF.

Mineralocorticoid receptor antagonists have been observed to stimulate natriuresis and improve diuretic responsiveness in patients with HF[29]. Although FENa did not differ between groups, urine sodium to potassium (UNa/K) ratio significantly increased at day 3 in the spironolactone group. Elevation of UNa/K ratio has been demonstrated to be a translatable biomarker of MRA effect[30]. These findings were reproducible in our study. Future research should be conducted to validate this promising neurohormonal biomarker in the ADHF setting.

Several limitations in this study should be noted. Firstly, given that no randomization or concealed allocation was performed, we cannot exclude a selection bias, which can impact on our conclusions' external validity. Secondly, the assistant physicians performed the congestive signs assessment, therefore, we cannot exclude an ascertainment bias. This can affect the internal validity of the comparison of subjective outcomes such as congestive signs or symptoms. Though, we had included an important internal control such as plasma NTproBNP that is unaffected by this bias and appear to be consistent with the earlier resolution of the congestive signs in the treatment group. Lastly, our study was underpowered to detect the differences of the expected low rate of adverse events between groups. Thus, our safety data should be viewed as exploratory.

Conclusion

Our study shows that treating ADHF patients with spironolactone was safe. It was also associated with an earlier resolution of the congestive signs and with a more pronounced NTproBNP reduction. Despite its exploratory nature, our study highlights the need to improve the treatment of ADHF and points out the direction of future investigation towards MRAs.

Acknowledgements

The authors acknowledge the lab technicians, specially Mr. Fernando Santos for technical assistance and to all physicians collaborating in the study.

Disclosures

The authors have no conflicts to disclose.

Tables

Table 1. Baseline Characteristics of the Study Population

	Control Group	Spironolactone Group	p value
Age (yrs)	78,8 ± 9,3	73,2 ± 11,7	0,01
Male sex – no. (%)	17 (34)	22 (44)	0,31**
Ejection Fraction (%)	45,5 ± 10,7	41,4 ± 12,4	0,08
Left Atrial Size (mm)	47,4 ± 5,3	46,3 ± 7,1	0,40
Charlson Index (pts)	6, 1 ± 1,1	5,9 ± 0,9	0,38
HgB (g/dL)	12,2 ± 1,8	12,7 ± 2,3	0,22
Albumin (mg/dL)	3,7 ± 0,4	3,6 ± 0,4	0,63
TSH (mUI/L)	2,8 ± 3,0	2,6 ± 2,8	0,79
Etiology of Heart Failure – no. (%) Ischemic Non-Ischemic	 24 (48) 26 (52)	 26 (52) 24 (48)	 0,69** 0,84**
Basal NYHA class – no. (%) II III	 41 (82) 9 (18)	 44 (88) 6 (12)	 0,40** 0,40**
History of Atrial Fibrillation or Flutter - no. (%)	34 (68)	25 (50)	0,07**
Outpatients Medications – no. (%) Furosemide ACE Inhibitors Beta-Blockers	 37 (74) 24 (48) 26 (52)	 35 (70) 18 (36) 10 (20)	 0,65** 0,22** 0,001**
Outpatients Oral Dose (mg) Furosemide ACE Inhibitors Beta-Blockers	 69,2 ± 37,8 5 ± 3 4,4 ± 2,7	 68 ± 30 4,3 ± 2,5 4,5 ± 1,1	 0,89 0,43 0,90
ADHERE: in-hospital mortality risk – no. (%) Low Intermediate 2 Intermediate 3	 44 (88) 2 (4) 4 (8)	 40 (80) 3 (6) 7 (14)	 0,27** 0,65** 0,34**
EFFECT: Heart Failure Mortality Risk Prediction – no. (%) Low Intermediate High	 4 (8) 27 (54) 18 (36)	 8 (16) 25 (50) 17 (34)	 0,22** 0,69** 0,83**
BMI ≥ 30 (Kg/m^2) – no. (%)	14 (28)	19 (38)	0,29**

Diabetes Mellitus – no. (%)	25 (50)	20 (40)	0,31**
Glycated Hemoglobin (%)	6,9 ± 0,7	7,1 ± 1,2	0,43
Obstructive Sleep Apnea Syndrome – no. (%)	5 (10)	13 (26)	0,32**
Non-Invasive Ventilation – no. (%)	7 (14)	10 (20)	0,42**
AHF precipitant (n)			
Undertreatment	34 (68)	29 (58)	0,30**
Dysrythmia	10 (20)	9 (18)	0,80**
Non-compliance	6 (12)	8 (16)	0,56**
NSAIDs	0	4 (8)	0,04**

Continuous variables are presented as mean value ± standard deviation [SD], p value. Categorical variables are presented as absolute number (%), p value. **Chi-square test.

HgB = hemoglobin; ACE = angiotensin-converting enzyme; BMI = Body Mass Index; NYHA = New York Heart Association; AHF = Acute Heart Failure; NSAID = Non-Steroidal Anti-Inflammatory Drug.

Table 2. Study End-Points.

	Control Group	Spironolactone Group	p Value
Heart Rate (beats/min) Day 1 Day 3	 91,2 ± 24,7 74,9 ± 12,4	 96,1 ± 23,9 77,9 ± 11,4	 0,30 0,20
SBP (mmHg) Day 1 Day 3	 140,5 ± 23,9 122 ± 15,6	 139 ± 27,9 121,9 ± 16,8	 0,80 0,97
RR (cycles/min) Day 1 Day 3	 35 [5] 20 [2]	 33,5 [6] 18 [3]	 0,90* < 0,001*
BMI (Kg/m^2) Day 1 Day 3	 29,3 ± 5,7 28,1 ± 5,4	 29,5 ± 6,6 27,7 ± 6,6	 0,90 0,76
Peripheral Edema – no. (%) Day 1 Day 3	 50 (100) 34 (68)	 50 (100) 17 (34)	 0,001**
Rales – no. (%) Day 1 Day 3	 50 (100) 38 (76)	 50 (100) 17 (34)	 <0,001**
JVP ≥ 8 cm – no. (%) Day 1 Day 3	 32 (64) 5 (10)	 28 (56) 0	 0,41** 0,02**
Orthopnea – no. (%) Day 1 Day 3	 50 (100) 12 (24)	 50 (100) 2 (4)	 - 0,004**
pCr (mg/dL) Day 1 Day 3	 1,15 ± 0,27 1,23 ± 0,43	 1,03 ± 0,29 1,06 ± 0,33	 0,026 0,035
pCr Change(mg/dL) Day 3 – Day 1	 0,075 ± 0,3	 0,038 ± 0,17	 0,47
Increase in pCr ≥ 0,3 mg/dL from Day 1 to Day 3 – no. (%)	10 (20)	2 (4)	0,038**
pUrea Day 1 Day 3	 59,32 ± 22,27 67,08 ± 27,09	 51,10 ± 18,63 57,54 ± 23,03	 0,048 0,061
pUrea Change (mg/dL) Day 3 – Day 1	 7,7 ± 22,9	 6,4 ± 17,9	 0,75
Albuminuria (mg/g) Day 1 Day 3	 73,50 [196,5] 60,16 [203]	 54,05 [203,7] 27,9 [80,2]	 0,521* 0,118*
Albuminuria Change (mg/g) Day 3 – Day 1	 - 7,3 [45,8]	 - 10,1 [71,2]	 0,32*
Albuminuria ratio Day 3 / Day 1	 0,9 [0,8]	 0,7 [0,7]	 0,19*
FENa (%) Day 1 Day 3	 1,4 [2] 1,2 [2]	 2,4 [3,5] 1,6 [2,5]	 0,24* 0,27*
FEUr (%) Day 1	 38,9 ± 12,7	 38,2 ± 11,1	 0,47

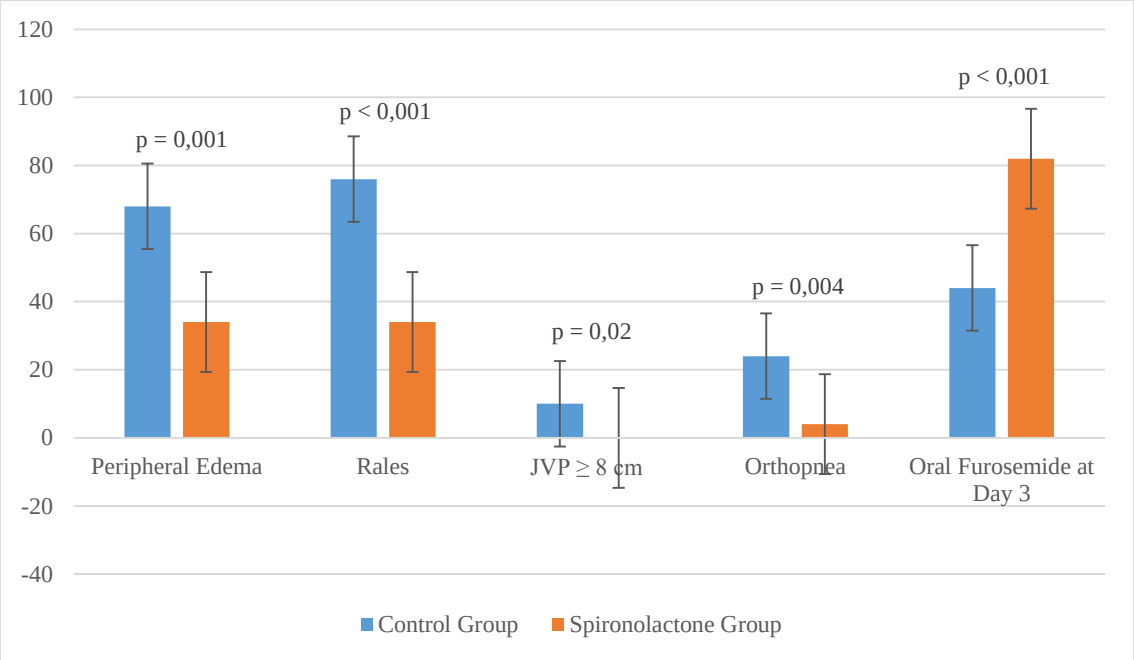
Day 3	39,5 ± 13,2	37,1 ± 10,5	0,31
UNa/K Ratio			
Day 1	2,7 [3,7]	3,6 [4,7]	0,18*
Day 3	2,1 [3,1]	4,0 [3,9]	0,007*
Serum Potassium (mmol/L)			
Day 1	4,1 ± 0,4	4,0 ± 0,6	0,33
Day 3	3,9 ± 0,5	4,1 ± 0,5	0,15
Hypokalemia (mmol/L) at Day 3 – no. (%)	13 (26)	7 (14)	0,13**
Serum Sodium (mmol/L)			
Day 1	140 [7]	141 [4]	0,9*
Day 3	141,2 ± 4,3	140,2 ± 3,5	0,2
Serum Ionized Calcium (mmol/L)			
Day 1	1,2 [0,1]	1,2 [0,1]	0,6*
Day 3	1,2 [0,1]	1,2 [0,1]	0,1*
Serum Magnesium (mmol/L)			
Day 1	0,84 [0,1]	0,8 [0,1]	0,2*
Day 3	0,87 [0,1]	0,85 [0,1]	0,6*
ProBNP (pg/ml)			
Day 1	3102 [6408]	2701 [3541]	0,17*
Day 3	2488 [4579]	1555 [1832]	0,05*
TnT (ng/mL)			
Day 1	0,034 [0,035]	0,03 [0,032]	0,5*
Day 3	0,032 [0,036]	0,029 [0,028]	0,3*
TnT Reduction (ng/mL) Day 3 – Day 1	- 0,0005 [0,01]	- 0,001 [0,01]	0,57*
CkMB (UI/L)			
Day 1	15,5 [9]	14 [9]	0,14*
Day 3	14 [8]	12 [8]	0,13*
CkMB Reduction (UI/mL) Day 3 – Day 1	- 1,7 ± 6,2	- 2,3 ± 4,3	0,63
IV Furosemide Dose (mg)			
Day 1	80 [20]	80 [30]	0,86*
Day 3	60 [20]	60 [30]	0,22*
Oral Furosemide at Day 3 – no. (%)	22 (44)	41 (82)	< 0,001**
ACE Inhibitors Dose (mg)			
Day 1	2,5 [3,8]	2,5 [2,5]	0,75*
Day 3	2,5 [3,8]	2,5 [3,8]	0,72*
ACE Inhibitors – no. (%)			
Day 1	19 (38)	25 (50)	0,20**
Day 3	30 (60)	31 (62)	0,80**
Beta-Blockers Dose (mg)			
Day 1	2,5 [0]	2,5 [2,5]	0,95*
Day 3	2,5 [2,5]	2,5 [1,25]	0,46*
Beta-Blockers - no. (%)			
Day 1	21 (42)	16 (32)	0,30**
Day 3	27 (54)	30 (60)	0,50**
Length of Stay (days)	9 [5]	8 [5]	0,8*

Continuous variables are presented as mean value ± standard deviation [SD], p value or median [inter-quartile range, IQR], p value. Categorical variables are presented as absolute number (%), p value. *Non-parametric test; **Chi-square test.

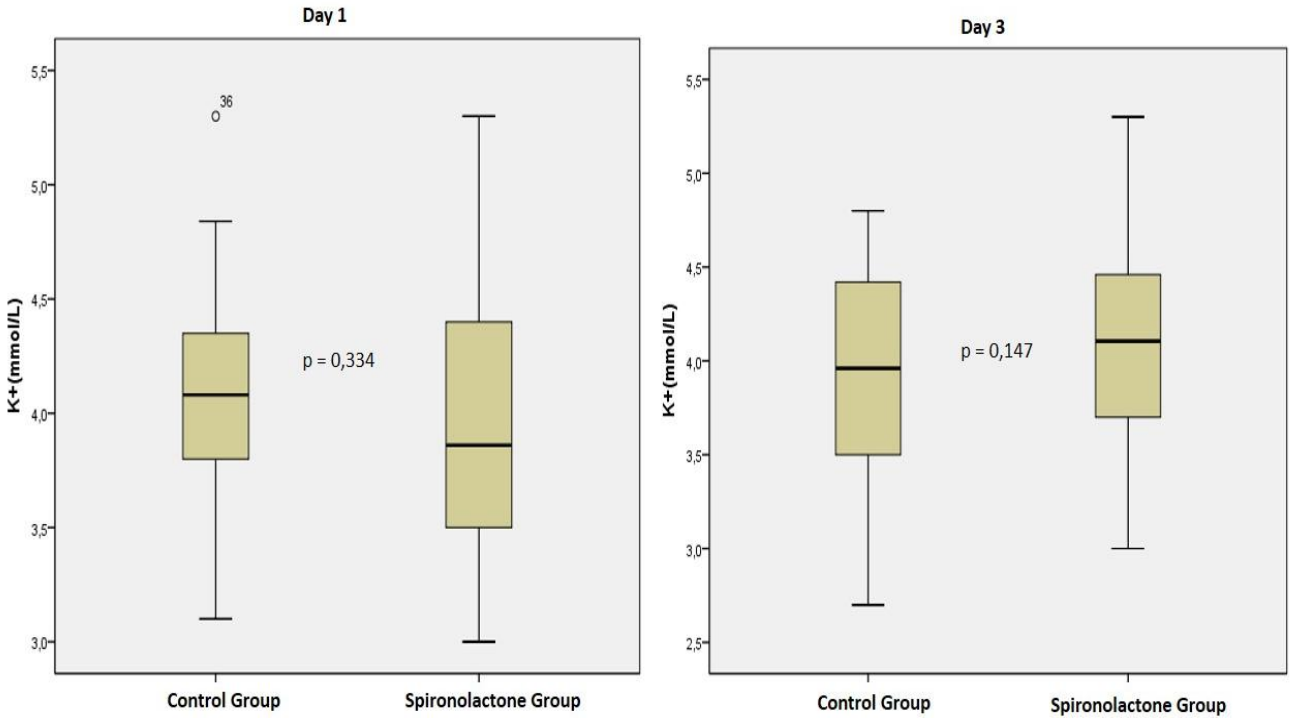
SBP = systolic blood pressure; RR = respiratory rate; JVP = jugular venous pressure; BMI = Body Mass Index; PaO_2/FiO_2 = partial pressure arterial oxygen/fraction inspired oxygen; pCr = plasma creatinine; FENa = spot urine fractional excretion of sodium; FEUr = spot urine fractional excretion of urea; UNa/K = urinary sodium to potassium ratio; proBNP = N-terminal pro brain natriuretic peptide; TnT = high-sensitivity troponin T; CkMB = creatine kinase-MB; ACE = angiotensin-converting enzyme.

Figures

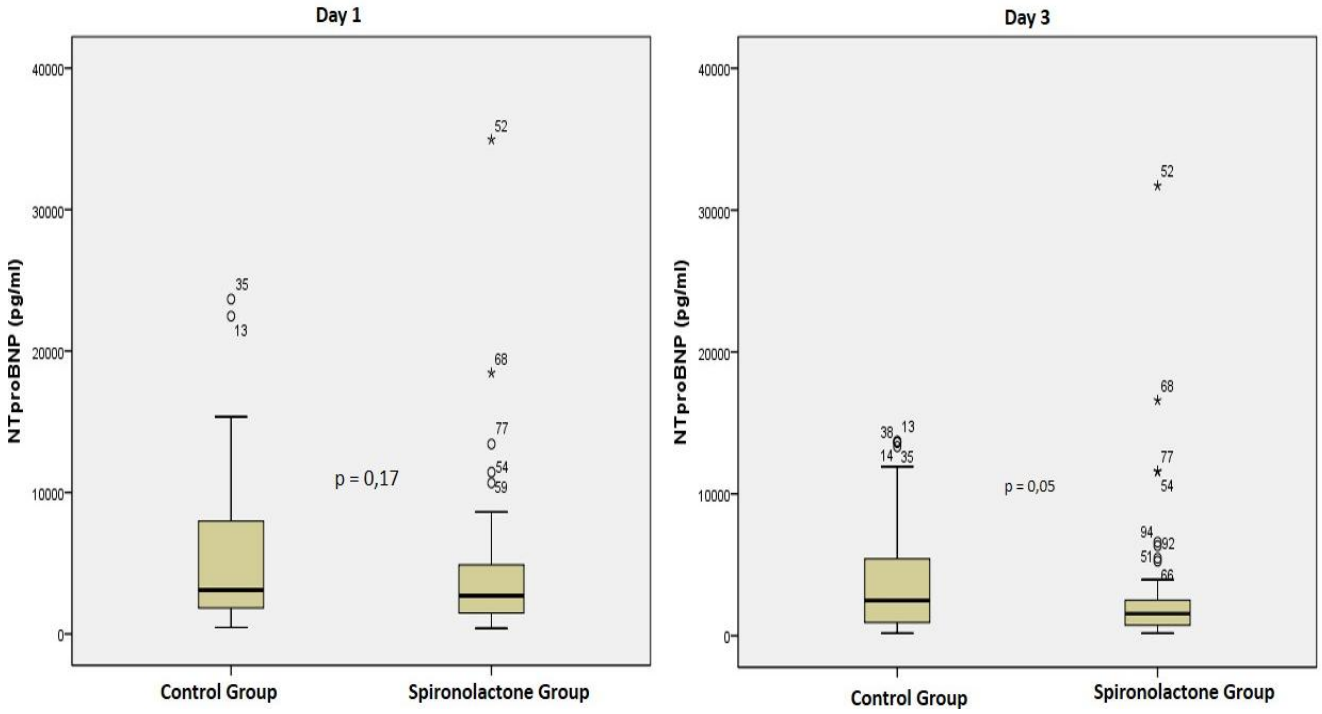
Graph 1. Changes in Congestive Signs and Patients Taking Oral Furosemide at Day 3 (%) in the Control and Spironolactone Groups.



Graph 2. Changes in Serum Potassium (K^+) from Day 1 to Day 3 in the Control and Spironolactone Groups.



Graph 3. Changes in Mean Plasma Levels of NT Pro-Brain Natriuretic Peptide (NTproBNP) from Day 1 to Day 3 in the Control and Spironolactone Groups.



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II. Tailoring diuretic therapy in acute heart failure: insight on early diuretic response predictors

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Abstract

Background/Objectives: Few data exist to help physicians how to use diuretics to provide the greatest symptomatic benefit with the least adverse effect and to select which subset of patients require a more aggressive diuretic strategy and monitorization. The aim of this study is to identify early predictors of diuretic response in a selected group of patients with acutely decompensated chronic heart failure (ADCHF).

Methods: Observational, retrospective secondary analysis of a study including 100 patients with ADCHF.

Results: Mean ± standard deviation (SD) age was 76,0 ± 10,9 years. Sixty-one patients were female. Due to the persistence of congestive signs after three days of inpatient treatment, 16 (16%) patients maintained or increased i.v. furosemide dose (slow diuretic response, SDR) during the study period. The other 84 patients had greater congestion relief and decreased i.v. furosemide dose or switched furosemide to oral route (fast diuretic response, FDR). Admission day factors predicting SDR were: higher levels of pUr (mean ± SD, 69,6 ± 20,9 vs 52,5 ± 19,8, p = 0,002); higher levels of pUr / pCr ratio (mean ± SD, 58,3 ± 15,2 vs 49,6 ± 15,1, p = 0,036); higher levels of albuminuria (median [IQR], 131,5 [396,9] vs 47,1 [143,6], p = 0,011); higher levels of RDW (median [IQR], 16,0 [1,9] vs 15,1 [1,5], p = 0,039); lower levels of HgB (mean ± SD, 11,5 ± 1,8 vs 12,6 ± 2,1, p = 0,04); and higher levels of hsTnT (median [IQR], 0,05 [0,05] vs 0,03 [0,03], p = 0,026). By multivariate analysis the strongest independent early predictors of SDR were: pUr (OR [$_{95\%}$CI], 1,04 [1,01 – 1,07], p = 0,006), and red-cell distribution width (RDW) (OR [$_{95\%}$CI], 1,47 [1,07 – 2,02], p = 0,018). During the first three days of hospitalisation the strongest independent factor associated with SDR was NTproBNP increase or decrease by less than 30% from day 1 to day 3 (OR [$_{95\%}$CI], 4,84 [1,14 – 20,55], p = 0,032). No use of spironolactone increases the risk of SDR (OR [$_{95\%}$CI], 5,98 [1,17 – 30,42], p = 0,031).

Conclusions: High RDW and high levels of pUr at admission are strong predictors of slower diuretic response. No change or increase in NTproBNP in the first three days of treatment is associated with slower diuretic response. On the other hand, the use of high dose spironolactone is associated with faster diuretic response.

Introduction

Acutely decompensated chronic heart failure (ADCHF) is a common cause of hospitalization, in particular among patients over 65 years old[1, 2]. Most patients present with normal or high blood pressure and with fluid overload[3]. In this common clinical scenario, the relief of congestion is a critical goal and intravenous loop diuretics are used for that goal in approximately 90% of patients[4]. Monitorization of therapy response is mainly performed through assessment of congestive signs (rales, edema, jugular venous pressure, weight), symptoms (dyspnea, orthopnea, nocturnal paroxysmal dyspnea) and net fluid loss[5].

Despite the wide clinical experience, few data exist to help physicians how to use diuretics to provide the greatest symptomatic benefit with the least adverse effect and to select the subset of patients who require a more aggressive diuretic strategy[2, 6, 7]. In addition, high doses of loop diuretics may have deleterious effects, including activation of the renin-angiotensin-aldosterone system (RAAS) and sympathetic nervous system, electrolyte disturbances, and worsening of renal function[4, 8]. Given that the majority of therapeutic advances in heart failure have been centered on the concept of neurohormonal antagonism, it is not surprising that there has been a great interest regarding the influence of diuretics on outcomes in patients with heart failure[9].

The aim of this study is to identify early predictors of slower diuretic response in a selected group of patients with ADCHF in order to help the tailoring of diuretic therapy in this common clinical scenario.

Methods

Study Design

We analysed a database from a previous conducted prospective, interventional trial that we performed. In that study we enrolled a 100 consecutive patients who presented in a Portuguese tertiary hospital with ADCHF, between February 2012 and February 2013. They were assigned in a sequential 1:1 ratio to spironolactone plus standard ADCHF therapy or standard ADCHF therapy alone. Patients were eligible for enrollment if they presented with decompensation of chronic heart failure (HF) with symptoms leading to hospitalization. ADCHF was diagnosed on the basis of the presence of history of chronic HF and at least one symptom (dyspnea, orthopnea, or edema) and one sign (rales, peripheral edema, ascites, or pulmonary vascular congestion on chest radiography). Exclusion criteria were: chronic use of mineralocorticoid receptor antagonists (MRAs), cardiac surgery within 60 days of enrollment, cardiac mechanical support, cardiac resynchronization-therapy within the last 60 days, comorbid conditions with an expected survival of less than 6 months,

acute myocardial infarction at time of hospitalization, hemodynamically significant uncorrected primary cardiac valvular disease, patients requiring intravenous vasodilators or inotropic agents, supine systolic arterial blood pressure <90 mmHg, plasma creatinine (pCr) level >1,5 mg/dL, serum potassium level >5,0 mmol/L, hemoglobin (HgB) level <9 g/dL, and sepsis.

During the study period 50 patients were non-randomly assigned to take oral spironolactone (minimum and maximum initial doses of 50 - 100 mg/d).

Institutional review board or ethics committee approval was obtained. All patients provided written informed consent to participate in the study.

Study assessments

Patient`s clinical status including physical examination and was prospectively recorded by the same assistant physician at day 1 and day 3.

Medications and respective dosages were prospectively recorded by the investigators according to the assistant physician prescriptions.

An assessment of biomarkers, including pCr, electrolytes, N-terminal pro-brain natriuretic peptide (NTproBNP), high-sensitivity troponin T (hsTnT) and albuminuria was performed at a central core laboratory at admission day (day 1) and day 3. Clinical assessment and routine analyses were performed daily during hospital stay. All patients performed a transthoracic echocardiography within 72 hours upon admission. Ejection fraction was calculated according to biplane Simpson method.

Variable definitions

We classified patients according to their response to diuretic therapy. Patients were considered to be fast diuretic responders (FDR) if they had a decrease in intravenous (i.v.) furosemide or a switch to oral furosemide in the first three days of in-hospital treatment. On the other hand, we defined a slower diuretic response (SDR) if the assistant physician increased or maintained i.v. furosemide dosage after three days of in-hospital treatment.

We compared these two different groups regarding the following covariates: comorbidities such as diabetes mellitus, chronic obstructive pulmonary disease (COPD), and sleep apnea; indirect signs of congestion including body mass index (BMI); heart failure etiology; echocardiographic parameters such as ejection fraction and left atrial size; furosemide ambulatory dose, proportion of outpatients on angiotensin converting enzyme inhibitors (ACEi) and beta-blockers (BB); i.v. furosemide at day 1; pCr, plasma urea (pUr), NTproBNP levels, hsTnT, sodium, potassium and albuminuria at day 1 and day 3; HgB, serum albumin, renin, aldosterone

and red cell distribution width (RDW) at day 1; proportion of inpatients on spironolactone, ACEi, and BB.

Statistical Analysis

Comparison between groups (versus [vs]) was performed using parametric, non-parametric tests, or chi-square tests, as appropriate.

Continuous variables are expressed as mean (standard deviation, SD) or median (inter-quartile range, IQR). Categorical variables are expressed in absolute numbers (no.) and proportions (%).

Association between different variables was tested by univariate analysis. Variables with significant association were tested by multivariate analysis in a stepwise manner. Predictors of outcome were identified by logistic regression analyses.

Significant association was defined by a p value < 0,05.

Statistical analysis was performed using SPSS software (version 19, Chicago, IL, USA).

Results

Mean ± SD age was 76,0 ± 10,9 years. Sixty-one patients were female.

All patients had congestive signs at admission. After three days of inpatient treatment, 16 (16%) patients maintained or increased i.v. furosemide dose (SDR). This group of patients had more indirect signs of fluid overload (rales: 87,5% vs 48,8%, p = 0,004; peripheral edema: 81,3% vs 45,2%, p = 0,008; orthopnea: 37,5% vs 9,5%, p = 0,008; JVP ≥ 8 cm: 18,8% vs 2,4%, p = 0,006). Body mass index also increased in SDR group (mean ± SD, 31,3 ± 5,4 vs 27,8 ± 6,2, p = 0,038). The other 84 patients had FDR, greater congestion relief and a decrease in i.v. furosemide dose or oral route furosemide switch (dose reduction – no. [%] = 21 [25]; oral route – no. [%] = 63 [75]) – *table 1*.

We did not find any significant differences between both groups (SDR vs FDR) regarding baseline characteristics, comorbidities, and BMI - *table 1*.

All patients received i.v. bolus furosemide. Combination therapy with thiazide diuretics was not used. Intravenous bolus furosemide dose (mg) on admission day did not differ between groups (mean ± SD, 72,5 ± 20,5 vs 76,4 ± 21,8).

Univariate analysis of the variables potentially linked to SDR is shown in *table 2*.

Admission day covariates predicting SDR were: higher levels of pUr (mean ± SD, 69,6 ± 20,9 vs 52,5 ± 19,8, p = 0,002); higher levels of pUr / pCr ratio (mean ± SD, 58,3 ± 15,2 vs 49,6 ± 15,1, p = 0,036); higher levels of albuminuria (median [IQR], 131,5 [396,9] vs 47,1 [143,6], p = 0,011) – *figure 1*.; higher levels of RDW (median

[IQR], 16,0 [1,9] vs 15,1 [1,5], p = 0,039); lower levels of HgB (mean ± SD, 11,5 ± 1,8 vs 12,6 ± 2,1, p = 0,04); and higher levels of hsTnT (median [IQR], 0,05 [0,05] vs 0,03 [0,03], p = 0,026). Higher levels of pCr at day 1 are also likely to predict SDR (mean ± SD, 1,21 ± 0,28 vs 1,06 ± 0,28, p = 0,06) and a trend to higher levels of renin on admission day was found in SDR patients (8,1 [14,3] vs 4,1 [6,7], p = 0,098).

During the first three days of hospitalisation the covariates associated with SDR were: higher levels of pCr at day 3 (mean ± SD, 1,40 ± 0,46 vs 1,1 ± 0,36, p = 0,004); increment in pCr ≥ 0,3 mg/dL from day 1 to day 3 (37,5% vs 7,1%, p = 0,001) – *figure 2*.; higher levels of pUr at day 3 (mean ± SD, 79,7 ± 24,9 vs 59,0 ± 24,3, p = 0,002); higher levels of albuminuria at day 3 (median [IQR], 123,9 [358,4] vs 26,4 [85,2], p = 0,001) – *figure 1*.; higher levels of NTproBNP at day 3 (median [IQR], 3013 [4116] vs 1701 [2563], p = 0,009); NTproBNP maintenance or increment (37,5% vs 13,1%, p = 0,017) – *figure 3*.; higher levels of hsTnT at day 3 (median [IQR], 0,06 [0,04] vs 0,03 [0,03], p = 0,004).

Patients treated with spironolactone had FDR (12,5% vs 57,1%, p = 0,001) – *figure 4*. Such differences were not found in patients taking ACEi and BB.

Multivariate analysis results are shown in *table 3*. The strongest independent early predictors of SDR were: pUr (OR [$_{95\%}$CI], 1,04 [1,01 – 1,07], p = 0,006), and red-cell distribution width (RDW) (OR [$_{95\%}$CI], 1,47 [1,07 – 2,02], p = 0,018). NTproBNP increase or decrease by less than 30% from day 1 to day 3 was the strongest independent factor associated with SDR (OR [$_{95\%}$CI], 4,84 [1,14 – 20,55], p = 0,032). Noteworthy, no use of spironolactone increases the risk of SDR (OR [$_{95\%}$CI], 5,98 [1,17 – 30,42], p = 0,031).

Time to oral furosemide (in days) was longer in patients with SDR (mean ± SD, 5,9 ± 1,7 vs 3,6 ± 2,2, p < 0,0001) and hospital length of stay (in days) was also longer in SDR group (mean ± SD, 10,9 ± 3,2 vs 8,5 ± 3,3, p = 0,008). No deaths or complete treatment failure occurred.

Discussion

Our study suggests that higher levels of pUr and RDW at admission can predict a subset of ADCHF patients with a slow diuretic response. Moreover, patients with little reduction or increase in NTProBNP levels during the first few days of hospitalisation needed to maintain high doses of i.v. diuretics. On the contrary, patients taking spironolactone had faster diuretic response.

Urea is freely filtered through the glomerulus and undergoes substantial tubular reabsorption[9]. This reabsorption of pUr is flow dependent so that more urea is reabsorbed at lower urine flow rates[10]. The neurohormonal response in HF involves the

nonosmotic secretion of arginine vasopressin (AVP), stimulation of the RAAS and sympathetic nervous system[10], which cause decrease of renal blood flow by renal vasoconstriction, and increased proximal tubular reabsorption of sodium and water. These changes will result in increased urea reabsorption caused by slow tubular flow in the collecting duct and higher AVP plasma concentration[11, 12]. This neurohormonal activation is exacerbated by loop diuretics because they block sodium chloride absorption at the macula densa and consequently increase renin secretion by the juxtaglomerular apparatus [13]. In HF patients with higher pUr, neurohormonal activation with high-dose loop diuretics can be greater than the withdrawal of neurohormonal activation resulting from decongestion, leading to a poorer survival[9, 14]. Components of the neurohormonal axis are not routinely measured in HF patients. Therefore, the rise in pUr may serve as an indirect marker of neurohormonal activation[10]. Higher plasma concentrations of plasma renin activity are associated with increased risk of death in HF, as occurred with the higher admission pUr values and changes in pUr values during hospitalization[10]. Volume management strategies based on pUr concentration or other markers of renal neurohormonal activation is a potential field for clinical investigation. In this regard, the use of natriuretic doses of potassium-sparing diuretics may allow both the minimization of loop diuretic doses and the maintenance of euvolemia[9]. Furthermore, an elevated admission pUr / Cr ratio has been associated with an increased incidence of post-discharge WRF, independent of the discharge glomerular filtration rate (GFR)[15]. In our study, high pUr and pUr / pCr ratio at admission predicted SDR. High renin also seemed to predict SDR. These results at day 1 are independent of the use of i.v. diuretics. These findings can lead to an early identification of patients who should have tighter follow-up and individualized diuretic approach, particularly the use of high dose spironolactone which showed to increase diuretic response in this pool of patients without severe renal dysfunction.

Red blood cell distribution width (RDW) is a percentual measure of the variability in the size of circulating erythrocytes, recorded during a standard complete blood count[16]. Disorders related to ineffective erythropoiesis or increased destruction cause greater heterogeneity in size and a higher RDW[17]. High RDW may reflect nutritional deficiencies, bone marrow dysfunction, or systemic inflammation, representing an integrative measure of the pathological processes occurring in HF[18]. Higher RDW was shown to be a strong independent predictor of greater morbidity and mortality in patients with chronic HF[17, 19], but the mechanism underlying the association between RDW and death in patients with HF is unclear[18]. Higher RDW level at discharge as also associated with a worse long-term outcome in patients hospitalized with acute heart failure (AHF), regardless of anemia status[18]. In our population higher

RDW predicted SDR, to the best of our knowledge this was the first report of this association.

Excessive activation of the RAAS causes glomerular hypertension and proteinuria[20]. A close correlation between increased excretion of urinary albumin and mortality in chronic HF has been reported[21, 22]. Our results suggests that increased albuminuria may also predict a subset of SDR in ADCHF patients.

An elevation in cardiac troponin indicates the presence of myocyte injury or death[23]. Myocyte loss is recognized to be a prominent pathophysiological mechanism in the evolution of cardiac dysfunction[24]. The pathophysiological factors that that are thought to be responsible for ongoing myocyte injury include neurohormonal activation and abnormalities in inflammatory cytokines, oxidative and mechanical stress[25]. An association between higher mortality and positive troponin in patients with ADCHF has been demonstrated[26, 27]. In our study higher levels of hsTnT at admission also predict SDR, adding an additional marker for early identification of patients who will require early implementation of aggressive therapy and monitorization.

Worsening renal function with persistence of congestive signs is associated with SDR. This observation is consistent with recent findings suggesting that the link between high-dose diuretics and poor outcomes may reflect the severity of the illness rather than an independent harmful effect of diuretics[6]. We also found an association between maintenance or increase in NTProBNP and SDR, suggesting that NTProBNP is a reliable biomarker for the evaluation of treatment efficacy and might help clinicians to reconsider treatment strategies in the first days of hospitalization. This is another potential role for this biomarker, since variations in NTproBNP levels during hospitalization are also associated with higher hospital readmission rates and 6-month mortality[28].

Our study has several limitations that need to be considered. First, it was a single-centre investigation of a small sample size. Second, the decision to withdraw diuretic therapy was based on subjective assessment of congestive signs and symptoms so we cannot rule out the inter-observer variability. However, in real-life patients, the decision to step down diuretic therapy is also based on subjective clinical evaluation. Finally, the external validity of our conclusions is limited to patients with mild renal failure HF patients since pCr level ≤ 1,5 mg/dl was an inclusion criteria.

Conclusion

High RDW and high levels of pUr at admission are strong predictors of slower diuretic response. No change or increase in NTproBNP and worsening renal function in the first three days of treatment are associated with slower diuretic response. On the

other hand, the use of high dose spironolactone is associated with faster diuretic response.

Our findings warrant further investigation to establish the value of these predictors in guiding diuretic strategies in hospitalised ADCHF patients.

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Disclosures

The authors have no conflicts to disclose.

Tables

Table 1. Baseline Characteristics and Differences in Congestive Signs at Day 1 and Day 3

	Furosemide Maintenance or Increase (n=16)	Furosemide Decrease or Oral Administration (n=84)	p Value
Age (yrs)	78,8 ± 6,9	75,5 ± 11,4	0,13
Male Sex – no. (%)	5 (31,3)	34 (40,5)	0,49**
Diabetes Mellitus – no. (%)	10 (62,5)	35 (41,7)	0,13**
COPD – no. (%)	2 (12,5)	15 (17,9)	0,46**
Dementia – no. (%)	3 (18,8)	9 (10,7)	0,40**
Sleep Apnea – no. (%)	3 (42,9)	15 (40,5)	0,61**
BMI (%)	29,7 ± 5,5	29,4 ± 6,3	0,83
Charlson Index (pts)	6,38 ± 0,6	5,9 ± 1,1	0,29
Ischemic Etiology for Heart Failure – no. (%)	10 (62,5)	40 (47,6)	0,28**
Furosemide Ambulatory Dose (mg)	68,3 ± 30,1	68,8 ± 33,6	0,98
Outpatients on ACEi – no. (%)	8 (50)	34 (40,5)	0,48**
Outpatients on Beta-blockers – no. (%)	7 (43,8)	50 (59,5)	0,24
Left Atrial Size (mm)	48,6 ± 5,3	46,6 ± 6,4	0,42
Ejection Fraction (%)	43,0 ± 11,2	43,6 ± 11,9	0,86
Rales – no. (%) Day 1 Day 3	 16 (100) 14 (87,5)	 84 (100) 41 (48,8)	 - 0,004**
Peripheral Edema – no. (%) Day 1 Day 3	 16 (100) 13 (81,3)	 84 (100) 38 (45,2)	 - 0,008**
Orthopnea – no. (%) Day 1 Day 3	 16 (100) 6 (37,5)	 84 (100) 8 (9,5)	 - 0,003**
JVP ≥ 8 cm – no. (%) Day 1 Day 3	 12 (75) 3 (18,8)	 48 (57,1) 2 (2,4)	 0,18 0,006**
BMI (Kg/m^2) Day 1 Day 3	 29,7 ± 5,5 31,3 ± 5,4	 29,4 ± 6,3 27,8 ± 6,2	 0,83 0,038

Continuous variables are presented as mean value ± standard deviation [SD], p value. Categorical variables are presented as absolute number (%), p value. **Chi-square test.

COPD = chronic obstructive pulmonary disease; BMI = Body Mass Index; ACEi = angiotensin-converting enzyme inhibitors; JVP = jugular venous pressure.

Table 2. Univariate Analysis for Furosemide Response Predictors, Furosemide Response Associations, Time to Oral Furosemide and Hospital Length of Stay

	Furosemide Maintenance or Increase	Furosemide Decrease or Oral Administration	p Value
IV Furosemide at Day 1 (mg)	72,5 ± 20,5	76,4 ± 21,8	0,51
pCr (mg/dL) Day 1 Day 3	 1,21 ± 0,28 1,40 ± 0,46	 1,06 ± 0,28 1,1 ± 0,36	 0,06 0,004
Increase in pCr ≥ 0,3 mg/dL from Day 1 to Day 3 – no. (%)	6 (37,5)	6 (7,1)	0,001**
pUrea (mg/dL) Day 1 Day 3	 69,6 ± 20,9 79,7 ± 24,9	 52,5 ± 19,8 59,0 ± 24,3	 0,002 0,002
pUrea Change from Day 1 to Day 3	10,1 ± 30,0	6,5 ± 19,4	0,52
pUrea to pCr ratio Day 1 Day 3	 58,3 ± 15,2 58,8 ± 12,4	 49,6 ± 15,1 54,3 ± 16,7	 0,036 0,32
Albuminuria (mg/g) Day 1 Day 3	 131,5 [396,9] 123,9 [358,4]	 47,1 [143,6] 26,4 [85,2]	 0,011* 0,001*
Albuminuria Change from Day 1 to Day 3	-13,3 [67,8]	-7,8 [56,1]	0,84*
Serum Potassium (mmol/L) Day 1 Day 3	 3,9 ± 0,5 3,8 ± 0,6	 4,1 ± 0,5 4,1 ± 0,5	 0,28 0,08
Serum Sodium (mmol/L) Day 1 Day 3	 140,6 ± 4,1 140,8 ± 4,7	 140,5 ± 4,5 140,7 ± 3,8	 0,98 0,89
HgB at Day 1 (g/dL)	11,5 ± 1,8	12,6 ± 2,1	0,04
RDW at Day 1	16,0 [1,9]	15,1 [1,5]	0,039*
Albumin at Day 1 (mg/dL)	3,8 ± 0,4	3,6 ± 0,4	0,08
NTproBNP (pg/mL) Day 1 Day 3	 3390 [4511] 3013 [4116]	 2698 [4577] 1701 [2563]	 0,26 0,009*
NTproBNP Decrease by Less than 30% or Increase from Day 1 to Day 3 – no. (%)	6 (37,5)	11 (13,1)	0,017**
hsTnT (ng/mL) Day 1 Day 3	 0,05 [0,05] 0,06 [0,04]	 0,03 [0,03] 0,03 [0,03]	 0,026* 0,004*
hsTnT Change from Day 1 to Day 3	-0,0005 [0,01]	-0,001 [0,01]	0,51*
Aldosterone at Day 1 (ng/dL)	34 [98,3]	35,5 [75,7]	0,84*
Renin at Day 1 (pg/mL)	8,1 [14,3]	4,1 [6,7]	0,098*
Inpatients on Spironolactone – no. (%)	2 (12,5)	48 (57,1)	0,001**
Inpatients on ACEi – no (%)	7 (43,8)	37 (44)	0,98**

Inpatients on Beta-Blockers – no (%)	7 (43,8)	30 (35,7)	0,54**
Time to Oral Furosemide (days)	5,9 ± 1,7	3,6 ± 2,2	<0,0001
Hospital Length of Stay (days)	10,9 ± 3,2	8,5 ± 3,3	0,008

Continuous variables are presented as mean value ± standard deviation [SD], p value or median [inter-quartile range, IQR], p value. Categorical variables are presented as absolute number (%), p value. *Non-parametric test; **Chi-square test.
pCr = plasma creatinine; pUrea = plasma urea; HgB = hemoglobin; RDW = red cell distribution width; NTProBNP = N-terminal pro-brain natriuretic peptide; hsTnT = high-sensitivity troponin T; ACEi = angiotensin-converting enzyme inhibitors.

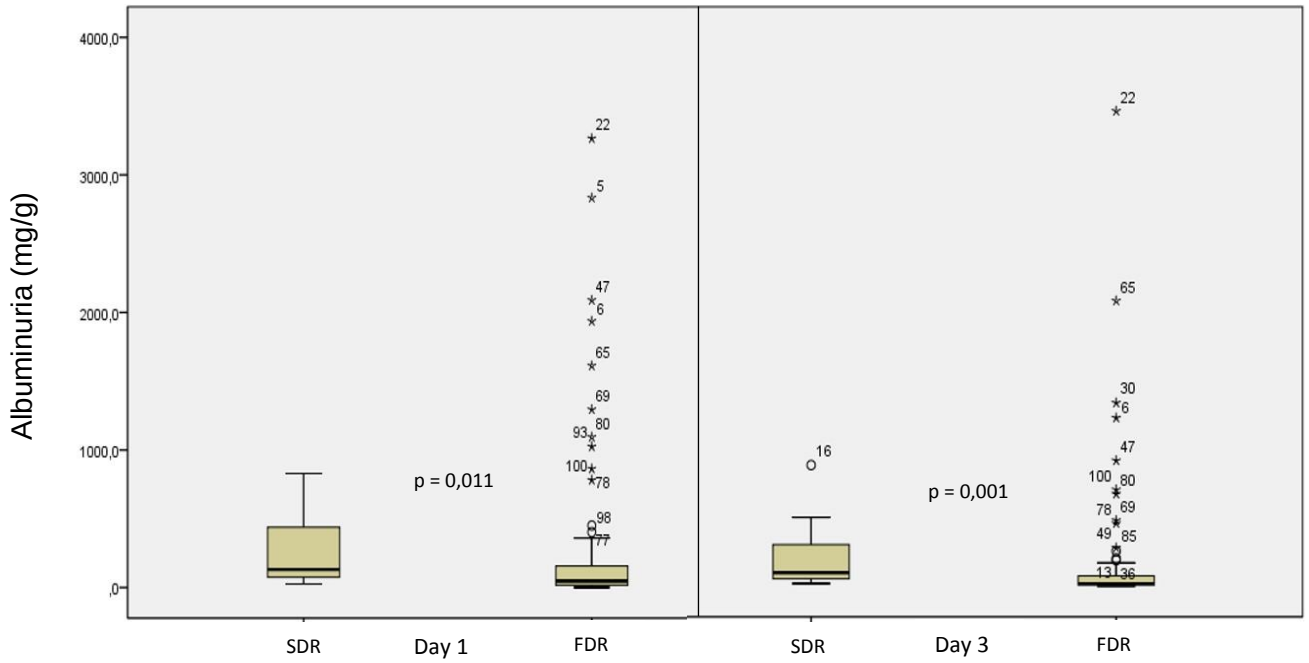
Table 3. Multivariate Analysis: Odds Ratios (OR) and 95% Confidence Intervals ($_{95\%}$CI) for Predictors and Factors Associated with Slow Diuretic Response.

	Furosemide Maintenance or Increase, OR ($_{95\%}$CI)	p Value
RDW at Day 1	1,47 (1,07 – 2,02)	0,018
pUrea at Day 1	1,04 (1,01 – 1,07)	0,006
No Spironolactone Use	5,98 (1,17 – 30,42)	0,031
NTProBNP Increase or Decrease by Less than 30% from Day 1 to Day 3	4,84 (1,14 – 20,55)	0,032
Increase in pCr ≥ 0,3 mg/dL from Day 1 to Day 3	4,07 (0,85 – 19,45)	0,079
pUrea at Day 3	1,02 (1,00 – 1,05)	0,087

RDW = red cell distribution width; pUrea = plasma urea; NTproBNP = N-terminal pro-brain natriuretic peptide; pCr = plasma creatinine; pUrea = plasma urea.

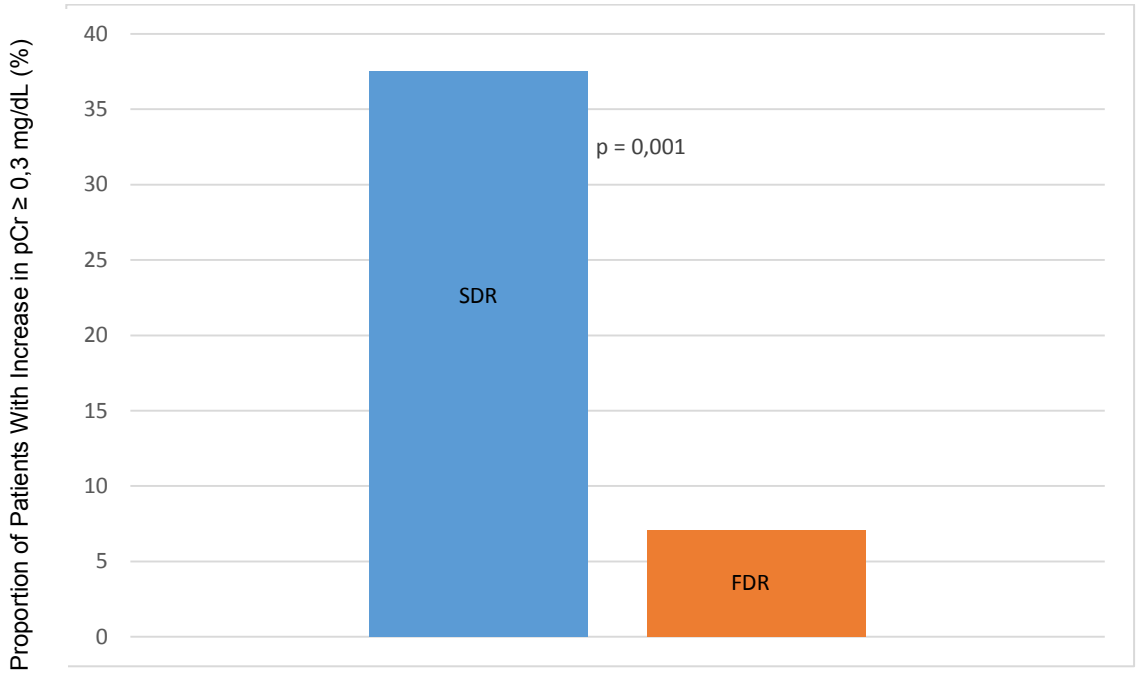
Figures

Figure 1. Comparison of Albuminuria (mg/g) in Slow versus Fast Diuretic Responders at Day 1 and Day 3 of Hospitalization.



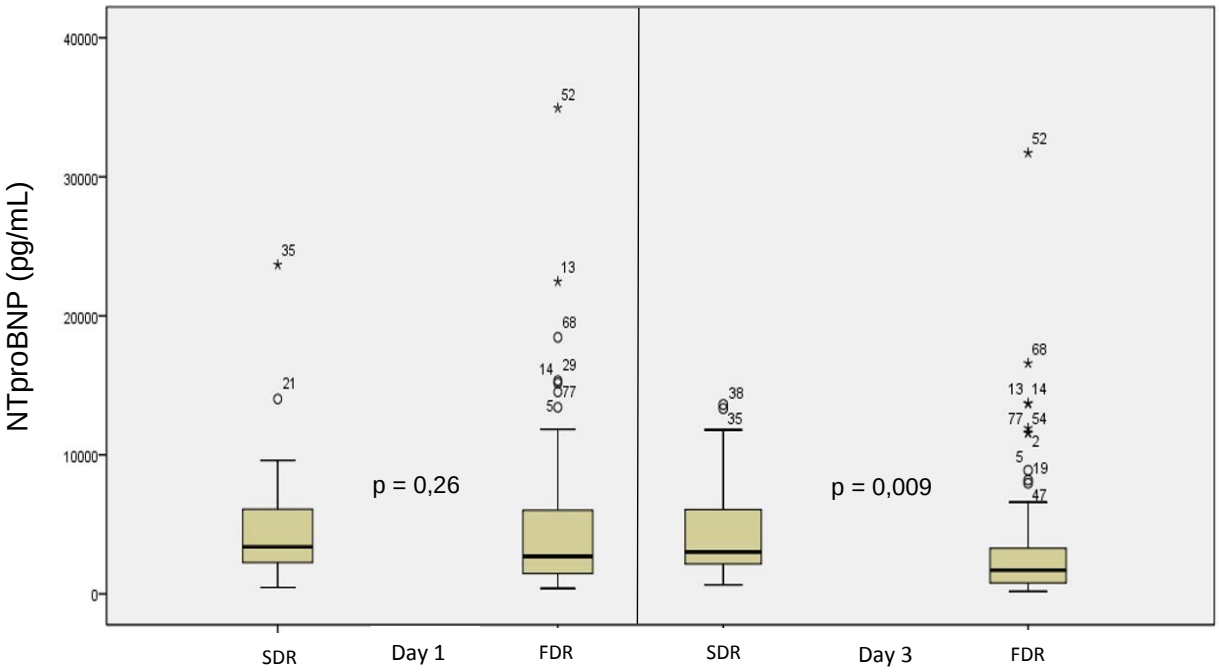
Legend: SDR, Slow Diuretic Responders; FDR, Fast Diuretic Responders

Figure 2. Proportion (%) of Patients with Worsening Renal Function From Day 1 to Day 3. Comparison Between Slow and Fast Diuretic Responders.



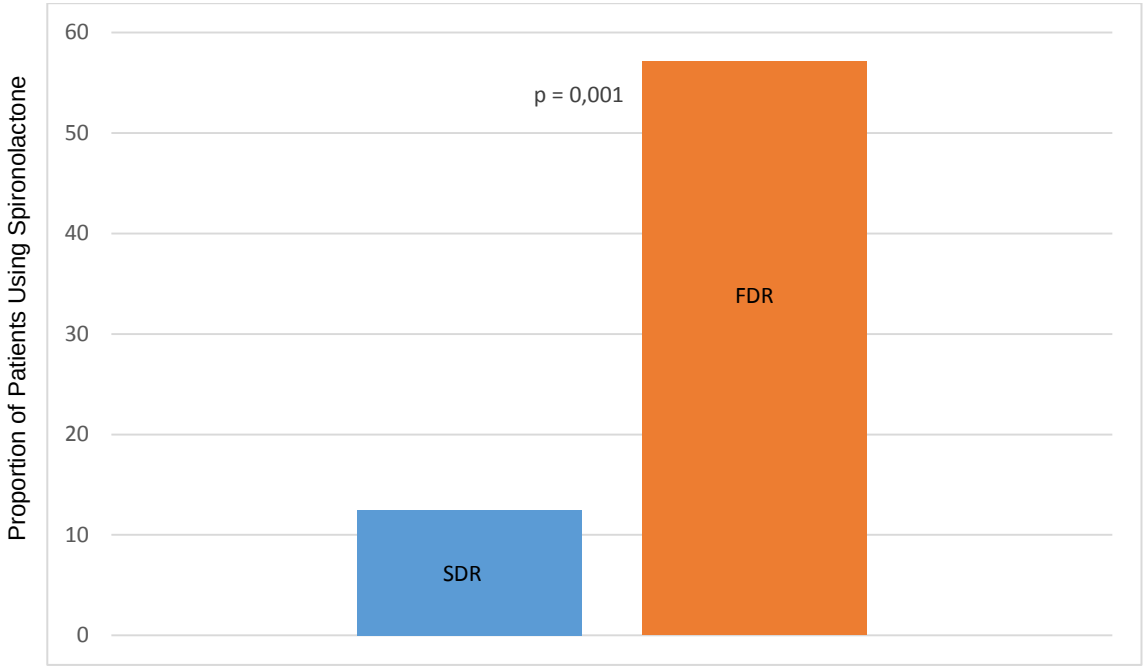
Legend: SDR, Slow Diuretic Responders; FDR, Fast Diuretic Responders

Figure 3. Comparison of N-Terminal Pro-Brain Natriuretic Peptide (NTproBNP) in Slow versus Fast Diuretic Responders at Day 1 and Day 3 of Hospitalization.



Legend: SDR, Slow Diuretic Responders; FDR, Fast Diuretic Responders; NTproBNP, N-Terminal Pro-Brain Natriuretic Peptide.

Figure 4. Proportion (%) of Patients Using Spironolactone (dose range: 50 -100 mg/day) in the First Three Days of Hospitalization. Comparison Between Slow and Fast Diuretic Responders.



Legend: SDR, Slow Diuretic Responders; FDR, Fast Diuretic Responders

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III. The Role of Albuminuria as a Non-Invasive Marker for Congestive Acutely Decompensated Chronic Heart Failure and the Spironolactone Effect in Elderly Portuguese: a Non-Randomized Trial

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Abstract

Background/Objectives: Albuminuria is a robust, validated cardiovascular risk factor. It is a simple and widely available test that was shown to be a powerful and independent predictor of prognosis in chronic heart failure. Mineralocorticoid receptor antagonists may reduce the acute and chronic harmful effects of mineralocorticoid receptor activation on the kidney. The objectives of the trial were to compare the effect of spironolactone versus standard acutely decompensated heart failure (ADHF) therapy on albuminuria and to investigate the role of albuminuria as a prognostic marker in patients with ADHF.

Methods: Secondary analysis of a prospective, interventional study including 100 patients with ADHF. Fifty patients were non-randomly assigned to spironolactone 100 mg/day plus standard ADHF therapy (intervention group) or standard ADHF therapy alone (control group).

Results: Patients in control group were older, had higher creatinine and urea levels, and had higher proportion of microalbuminuria (all, $p < 0,05$). Paired comparison of baseline and day 3 log albuminuria within each group, showed a more pronounced decrease in the intervention group (1,79 ± 0,75 to 1,59 ± 0,67, $p = 0,003$ vs. 1,89 ± 0,70 to 1,79 ± 0,74, $p = 0,096$). In addition, the proportion of patients with normoalbuminuria increased from baseline to day 3 in spironolactone group (20 (40%) to 27 (54%), $p < 001$), accordingly the number of patients in the micro and macroalbuminuria groups was reduced. Day 1 albuminuria was positively correlated with day 1 N-terminal pro-brain natriuretic peptide (0,260 [0,105 to 0,758], $p = 0,009$).

Conclusions: High-dose spironolactone added to standard ADHF therapy is likely to induce a more pronounced albuminuria decrease and a significant reduction in the proportion of micro and macroalbuminuria.

Key-words: Acute heart failure. Albuminuria. Acute kidney injury.

Introduction

Albuminuria is a robust, validated cardiovascular (CV) risk factor. It is independently associated with major adverse events such as stroke, myocardial infarction (MI) and CV death[1].

Screening for increased albuminuria is recommended for risk stratification of patients with diabetes and hypertension in order to adequate the treatment aggressiveness[2,3].

Albuminuria determination is a simple and widely available test that was shown to be important for the risk stratification of patients with heart failure (HF), and a powerful and independent predictor of prognosis in HF[4]. In these patients, increased albuminuria could be a consequence of the increased neurohormonal activation, endothelial injury, systemic inflammation, and renal dysfunction[5,6]. These mechanisms are also involved in the pathophysiology of acute decompensated heart failure (ADHF) [4,7, 8] and have a multitude of effects in the kidney, including arteriolar vasoconstriction and increased tubular reabsorption of sodium and urea[9,10]. However, the prognostic value of albuminuria is not yet established in this clinical setting.

Mineralocorticoid receptor antagonists (MRAs) have been shown to attenuate ventricular and vascular hypertrophy, potassium and magnesium loss, glomerulosclerosis, renal interstitial fibrosis and proteinuria in patients with chronic kidney disease (CKD)[11,12]. Thus, MRAs may reduce the acute and chronic harmful effects of mineralocorticoid receptor activation on the kidney, effectively augment diuresis in diuretic-resistant patients, and attenuate the development of the vasomotor nephropathy in ADHF patients[13].

Our aims were to compare the effect of spironolactone versus standard acutely decompensated heart failure (ADHF) therapy on albuminuria and to investigate the role of albuminuria as a prognostic marker in patients with ADHF.

Methods

Study Design

We analysed data from a previous prospective, interventional, clinical trial that we performed. In that study we enrolled 100 consecutive patients who presented in a Portuguese tertiary hospital with ADCHF, between February 2012 and February 2013. Patients were eligible for enrollment if they presented with decompensation of chronic HF with symptoms leading to hospitalization. ADCHF was diagnosed on the basis of the presence of history of chronic HF and at least one symptom (dyspnea, orthopnea, or edema) and one sign (rales, peripheral edema, ascites, or pulmonary vascular congestion on chest radiography). Patients were non-randomly assigned in a

sequential 1:1 ratio to spironolactone plus standard ADCHF therapy or standard ADCHF therapy alone, 50 patients within each arm (*i.e*. patients were alternatively assigned to spironolactone arm or standard ADCHF therapy arm in a sequential manner - the first patient to one arm and the next to the other arm. This sequence was repeated until we reach 100 patients, 50 patients within spironolactone group and 50 patients within control group). Patients were blinded to the allocation, and the clinicians were not blinded to the allocation. The recommended spironolactone dose was 100 mg/day, however the assistant physician could decrease the spironolactone dose to 50 mg/day after 48h upon admission. After 72h the study was open label. Furosemide dose and route of administration was adjusted clinically according to the hydration status of the patients – Figure 1.

Exclusion criteria were: chronic use of mineralocorticoid receptor antagonists (MRAs), cardiac surgery within 60 days of enrollment, cardiac mechanical support, cardiac resynchronization-therapy within the last 60 days, comorbid conditions with an expected survival of less than 6 months, acute MI at time of hospitalization, hemodynamically significant uncorrected primary cardiac valvular disease, patients requiring intravenous vasodilators or inotropic agents, supine systolic arterial blood pressure <90 mmHg, plasma creatinine (pCr) level >1,5 mg/dL, serum potassium level >5,0 mmol/L, hemoglobin (HgB) level <9 g/dL, and sepsis.

Institutional review board or ethics committee approval was obtained. All patients provided written informed consent to participate in the study.

Clinical assessment of participants

Patient`s clinical status including physical examination and was prospectively recorded by the same assistant physician at day 1 and day 3. Medications and respective dosages were prospectively recorded by the investigators according to the assistant physician prescriptions.

Blood and spot urine samples were collected in the first 24 hours (h) after admission (day 1) of the patient to the hospital, and the day 3 samples were collected between 72 and 96 h of hospitalization. Samples were analysed at a central core laboratory, and included pCr, plasma urea (pUr), electrolytes, N-terminal pro-brain natriuretic peptide (NTproBNP), high-sensitivity troponin T (hsTnT) and albuminuria. Clinical assessment and routine analyses were performed daily during hospital stay. Estimated glomerular filtration rate (eGFR) was determined using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation[14]. All patients performed a transthoracic echocardiography within 72 hours upon admission. Left ventricular ejection fraction (LVEF) was calculated according to biplane Simpson method.

Albuminuria and urine creatinine were measured using the COBAS INTEGRA Tina-quant Albumin Gen.2 urine application (Roche Diagnostics) and expressed as mg/g of Cr.

We categorized albuminuria using standard cut-off points: normoalbuminuria (<30 mg/gCr), microalbuminuria (30-299 mg/gCr), and macroalbuminuria (≥300mg/gCr).

Variable definitions

We studied albuminuria regarding the following covariates: comorbidities such as diabetes mellitus (DM), chronic obstructive pulmonary disease (COPD), and sleep apnea; body mass index (BMI); heart rate (HR); systolic blood pressure (SBP); atrial fibrillation (AFib); HF etiology; echocardiographic parameters such as EF; furosemide dose, proportion of patients on angiotensin converting enzyme inhibitors (ACEi), beta-blockers (BB), and spironolactone; pCr, pUr, NTproBNP, hsTnT, sodium, potassium; HgB and serum albumin.

Statistical Analysis

Normally distributed continuous variables are expressed as mean ± standard deviation (SD), and skewed distributions are presented as median [inter-quartile range, IQR].

Because of the positively skewed distributions of BMI, HR, SBP, pCr, eGFR, NTproBNP, hsTnT, and albuminuria, these variables were log transformed for analysis.

Categorical variables are expressed in absolute numbers (no.) and proportions (%).

Comparison between groups was performed using parametric, non-parametric tests, or chi-square tests, as appropriate. A p value < 0,05 was considered statistically significant.

The relationships between baseline characteristics, day 3 and changes (Δ) in albuminuria during the first 3 days and baseline, day 3 or Δ in other variables were tested by univariate analysis linear regression. Factors with a probability value of ≤0.10 by single variable linear regression analyses were included in a multivariable linear regression analyses together with age and sex. In other words, simple linear regression analyses were performed to examine the relationship between baseline characteristics, day 3 and Δ in other variables and albuminuria. Thereafter, a multivariable linear regression analysis was performed, which included age, sex, and the factors with a probability value of ≤0.10 in the bivariate analyses. Simple linear regression values are presented as non-adjusted coefficient (NAC) plus 95% confidence interval ($_{95\%}$CI). Multiple regression values are presented as adjusted coefficient (AC) plus ($_{95\%}$CI).

Statistical analysis was performed using SPSS software (version 19, Chicago, IL, USA).

Results

Baseline Patients Characteristics in Treatment and Control Groups

Patients in control group were older (78,8 ± 9,3 versus [vs.] 73,2 ± 11,7 years, p = 0,01), had higher creatinine and urea levels (1,15 ± 0,27 vs. 1,03 ± 0,30, p = 0,026 and 59,32 ± 22,27 vs. 51,10 ± 18,63, p = 0,048), and had higher proportion of microalbuminuria (58% vs. 38%, p = 0,045). No differences between groups were found regarding sex, DM, COPD, dementia, sleep apnea, NIV, IHD, AFib, LVEF, BMI, HR, SBP, potassium, sodium, HgB, albumin, NTproBNP, hsTnT, albuminuria, furosemide dose and in the proportion of patients on ACEi and BB – Table 1.

Spironolactone Influence on Albuminuria Dynamic Changes

Change in albuminuria levels from baseline to day 3 was not significantly different between groups, despite the reduction was more pronounced in spironolactone group (-0,09 ± 0,40 vs. -0,20 ± 0,44, p = 0,226) – Table 2. However, paired comparison of baseline and day 3 log albuminuria within each group, showed a more pronounced decrease in the intervention group (1,79 ± 0,75 to 1,59 ± 0,67, p = 0,003 - within spironolactone group - and 1,89 ± 0,70 to 1,79 ± 0,74, p = 0,096 - within control group) – Figure 2. In addition, the proportion of patients with normoalbuminuria increased from baseline to day 3 in spironolactone group (20 (40%) to 27 (54%), p < 001), accordingly the number of patients in the micro and macroalbuminuria groups was reduced (19 (38%) to 16 (32%), p = 0,014 and 11 (22%) to 7 (14), p < 0,001, respectively) – Figure 3.

Albuminuria Correlations

By bivariate analysis, day 1 albuminuria was positively correlated with day 1 NTproBNP (0,260 [0,105 to 0,758], p = 0,009). Day 3 albuminuria was positively correlated with day 3 BMI (0,254 [0,025 to 0,047], p = 0,011), SBP (0,447 [0,006 to 0,027], p < 0,001), log hsTnT (0,220 [0,037 to 0,849], p = 0,028), and log pCr (0,248 [0,266 to 2,185), p = 0,013), and negatively correlated with log eGFR (-0,251 [-1,915 to -0,246], p = 0,012). Albuminuria decrease was negatively correlated with beta-blocker use during hospitalization (-0,243 [-0,355 to – 0,036], p = 0,015). By multivariate analysis, day 1 albuminuria correlated with NTproBNP (0,298 [0,167 to 0,830], p = 0,004), and day 3 albuminuria correlated with SBP (0,394 [0,009 to 0,025], p < 0,001) and log hsTnT (0,198 [0,043 to 0,759], p = 0,028) – Table 3.

Discussion

Our study showed a more pronounced albuminuria decrease and a significant reduction in the proportion of micro and macroalbuminuria in ADHF patients submitted to spironolactone treatment.

Increased albuminuria in patients with HF was found to be associated with increased risk of adverse clinical outcomes, including death[4,15]. Most patients in the Studies of Left Ventricular Dysfunction (SOLVD) had a urine dipstick test for protein at baseline[16]. Of 5487 (81% of total) tested, 177 (3%) had proteinuria. This subset of patients was more symptomatic, had higher blood pressure, higher prevalence of DM, and also greater left ventricular systolic dysfunction. Urinary albumin to creatinine ratio (UACR) was measured at baseline and during follow-up in the Candesartan in Heart failure: Assessment of Reduction in Mortality and morbidity (CHARM) Programme[4]. Of 2310 patients, 1349 (58%) had a normal UACR, 704 (30%) had microalbuminuria, and 257 (11%) had macroalbuminuria. Patients with an increased UACR were older, had more cardiovascular comorbidity, worse renal function, and a higher prevalence of DM than did those with normoalbuminuria. Elevated UACR was associated with increased risk of the composite outcome and death even after adjustment for other prognostic variables including renal function, DM, and HgBA1c. Interestingly, albuminuria *per se* has been associated with subsequent heart failure, even in individuals with few cardiovascular risk factors and UACR within the normal range[17].

Despite the strong body of evidence in chronic HF, few studies approach albuminuria in the acute HF setting. In a recently published study[8], albuminuria was assessed at day 1 and 7 of hospitalization in 115 patients presenting with acute HF. Nearly 70% of patients had elevated UACR at admission. UACR decreased significantly after 7 days of treatment (from 83 to 22 mg/gCr, p <0,0001). The decrease was correlated with serum NTproBNP and bilirubin, but not with changes in renal function. Despite the evidence that effective HF treatment may reduce albuminuria during the acute episode, our study points towards an additional and interesting potential effect of MRAs in this setting. Neurohormonal activation causes vasoconstriction of both the afferent and efferent renal arterioles, and stimulates mesangial contraction in glomeruli, thus diminishing the glomerular filtration surface[18]. Excessive activation of the renin–angiotensin–aldosterone system (RAAS) causes glomerular hypertension and proteinuria, whereas its inhibition reduces proteinuria[19,20]. Excessive activation of the sympathetic nervous system also causes proteinuria[21,22]. Therefore, inhibition of neurohormonal activation is determinant to improve outcomes in patients with HF[23-28]. In our study, the increased albuminuria at admission might also reflect an excessive neurohormonal activation. This hypothesis is reinforced by the

greater decrease in albuminuria observed in patients submitted to spironolactone treatment. The MRAs have shown to reduce proteinuria in CKD patients already on ACEis and ARBs, however with an increased risk for hyperkalemia[29], and a reduction of albuminuria was also observed with MRAs in the setting of chronic heart failure[30]. However, to the best of our knowledge our results are the first to demonstrate a greater reduction in albuminuria in ADHF patients submitted to spironolactone treatment. The rationale for this effect may rely on effective neurohormonal blockade, particularly on the activated the RAAS[9], suggesting a potential renal protective effect of this treatment.

Studies in animals have shown that increasing renal venous pressure leads to a reduction in glomerular filtration, which was probably mediated by a decreased renal perfusion[31]. Increased renal vein pressure in heart failure patients showed a marked reduction in renal blood flow as well as water and salt excretion[32,33]. Acute HF was correlated with higher albuminuria in patients with acute myocardial infarction[34]. Thus one can hypothesize that elevation of albuminuria can be a surrogate marker of severe volume overload. This hypothesis is reinforced by the positive correlation of albuminuria with NTproBNP at admission. Increased albuminuria probably has a hemodynamic basis in HF, particularly when renal venous congestion is associated with reduced renal blood flow, since urinary albumin excretion was inversely related to renal blood flow in patients with heart failure[35,36]. In addition, renal venous congestion caused proteinuria in dogs[37]. These findings support albuminuria as a surrogate marker of volume overload. High-dose spironolactone has shown to potentiate NTproBNP reduction and probably greater congestion relief in ADHF patients[38], these findings provide additional clues to explain the observed reduction of albuminuria in patients submitted to spironolactone treatment – these patients may have greater congestion relief in addition to the effective neurohormonal blockade.

Our study has several limitations that need to be considered. First, it was a single-centre investigation of a small sample size. Second, we used a single spot urine sample to determine albuminuria, which can be insufficient, as it might fluctuate. Finally, the external validity of our conclusions is limited to normo-hypertensive and fluid overloaded HF patients with mild renal failure, since all these factors were considered inclusion criteria. On the other hand, our conclusions can be reproducible in this set of patients widely common in clinical practice.

Conclusions

High-dose spironolactone added to standard ADHF therapy is likely to induce a more pronounced albuminuria decrease and a significant reduction in the proportion of

micro and macroalbuminuria. Further studies are required to explore the role of albuminuria as a prognostic marker in ADHF.

Acknowledgements

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Disclosures

The authors have no conflicts of interest to disclose.

Tables

Table 1. Baseline Population Characteristics, Laboratory Results and Medications in Treatment and Control Groups

	Control Group (n = 50)	Spironolactone Group (n = 50)	p Value
Age (yrs)	78,8 ± 9,3	73,2 ± 11,7	0,010
Male Sex – %	34	44	0,31**
Diabetes Mellitus - %	50	40	0,31**
Glycated HgB (%)	6,9 ± 0,7	7,1 ± 1,2	0,43
COPD - %	10	26	0,32**
Dementia - %	16	8	0,22**
Sleep Apnea - %	10	26	0,32**
Non-Invasive Ventilation - %	14	20	0,42**
Ischemic Heart Disease -%	48	52	0,69**
Atrial Fibrillation - %	68	50	0,07**
LV Ejection Fraction (%)	45,5 ± 10,7	41,4 ± 12,4	0,08
LV Ejection Fraction ≥ 40% - %	70	64	0,52**
Body Mass Index (Kg/m^2)	29,3 ± 5,7	29,5 ± 6,6	0,90
Heart Rate (bpm)	91,2 ± 24,7	96,1 ± 23,9	0,30
SBP (mmHg)	140,5 ± 23,9	139 ± 27,9	0,80
Plasma Creatinine (mg/dL)	1,15 ± 0,27	1,03 ± 0,30	0,026
eGFR (mL/min/1,73 m^2)	54,48 ± 16,45	68,28 ± 23,55	0,001
Plasma Urea (mg/dL)	59,32 ± 22,27	51,10 ± 18,63	0,048
Serum Potassium (mmol/L)	4,1 ± 0,4	4,0 ± 0,6	0,33
Serum Sodium (mmol/L)	140,52 ± 5,04	140,56 ± 3,65	0,964
Hemoglobin (g/dL)	12,2 ± 1,8	12,7 ± 2,3	0,22
Albumin (mg/dL)	3,7 ± 0,4	3,6 ± 0,4	0,63
NTproBNP (pg/mL)	3102 [1797 – 8204]	2701 [1463 – 5004]	0,167*
hsTnT (ng/mL)	0,034 [0,023 – 0,059]	0,030 [0,018 – 0,049]	0,490*
Albuminuria (mg/g)	73,50 [28,83 – 225,45]	54,05 [17,43 – 221,10]	0,521*
Normoalbuminuria - %	26	40	0,137**
Microalbuminuria - %	58	38	0,045**
Macrolbuminuria - %	16	22	0,444**
IV Furosemide Dose (mg/d)	75,60 ± 20,72	76,00 ± 25,50	0,927
ACEi – no. (%)	19 (38)	25 (50)	0,201**
Ramipril Eq. Dose (mg/d)	3,16 ± 2,21	3,15 ± 1,94	0,990
Beta-Blocker - %	21 (42)	16 (32)	0,302**
Bisoprolol Eq. Dose (mg/d)	2,98 ± 1,01	3,05 ± 1,20	0,847
Spironolactone Dose (mg/d)	-	94,5 ± 23,3	-

Continuous variables are presented as mean value ± standard deviation [SD], p value or median [inter-quartile range, IQR], p value. Categorical variables are presented as absolute number (%), p value.

*Non-parametric paired sample test; ** Chi-square test.

Legend: COPD = chronic obstructive pulmonary disease; LV = left ventricular; eGFR = estimated glomerular filtration rate; NTproBNP = N-terminal pro brain natriuretic peptide; hsTnT = high sensitivity troponin T; IV = intra-venous; ACEi = angiotensin converting enzyme inhibitors.

Normoalbuminuria < 30 mg/gCr; Microalbuminuria 30 – 299 mg/gCr; Macroalbuminuria ≥ 300 mg/gCr.

Table 2. Change (Δ) in Albuminuria Between the Study Groups

	Control Group	Spironolactone Group	p Value
Log Albuminuria Baseline Day 3 Δ (day 3 – Baseline)	 1,89 ± 0,70 1,79 ± 0,74 -0,09 ± 0,40	 1,79 ± 0,75 1,59 ± 0,67 -0,20 ± 0,44	 0,499 0,159 0,226

Legend: Comparison between groups was performed using independent samples t test.

Table 3. Crude and Adjusted β Coefficients (95%CI) for the Change (Δ) in Log Albuminuria as Prognostic indicator in Acutely Decompensated Heart Failure

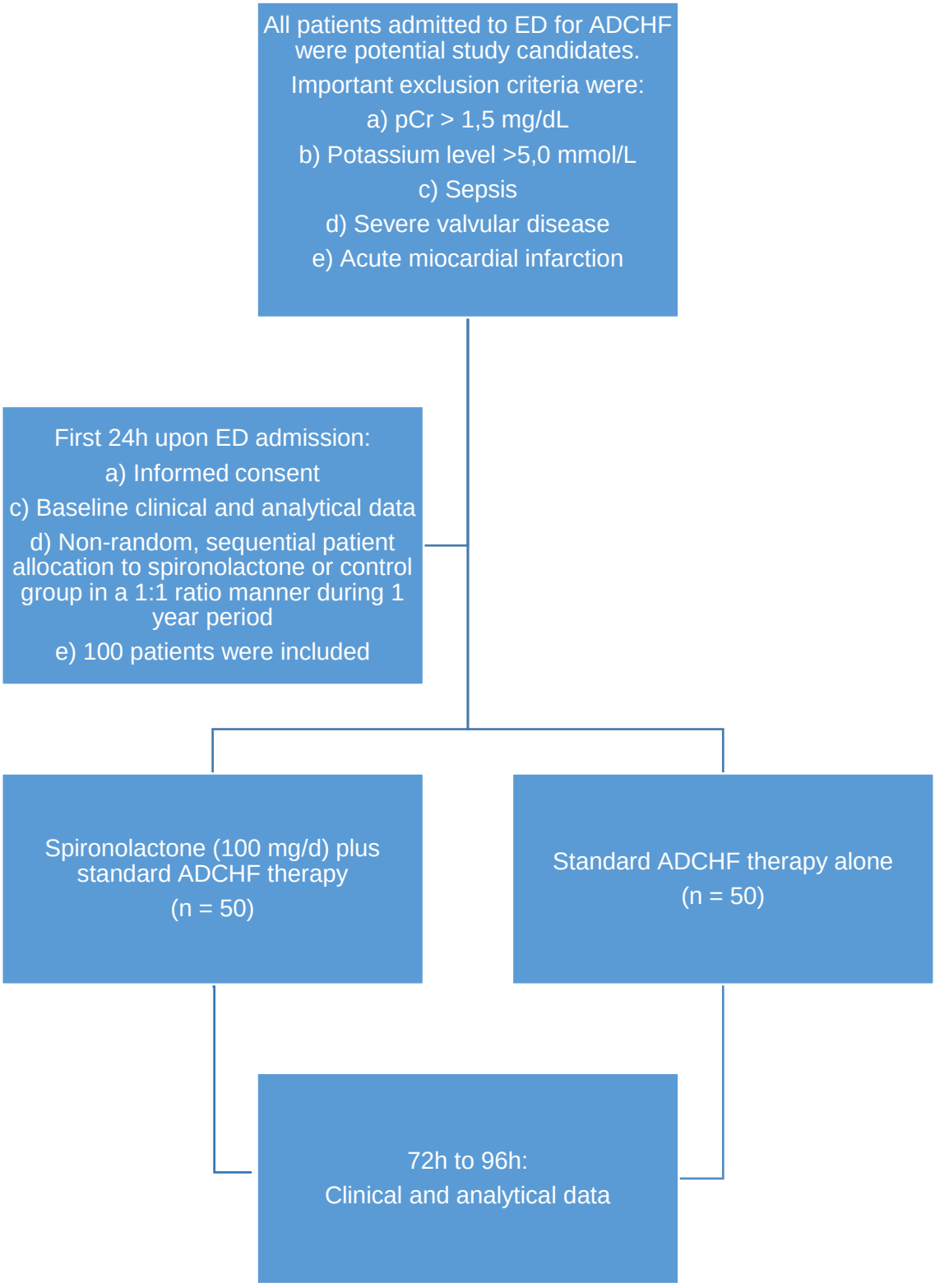
	Nonadjusted Coefficient for Log Albuminuria	95%CI	p Value	Adjusted Coefficient for Log Albuminuria	95%CI	p Value
Age	0,192	-0,001 to 0,017	0,056			
Male Sex	-0,102	-0,257 to 0,076	0,313			
DM	0,109	-0,077 to 0,258	0,278			
HgBA1c	-0,038	-0,138 to 0,108	0,805			
LVEF	0,084	-0,002 to 0,006	0,408			
Ischemic HF	0,128	-0,057 to 0,215	0,206			
Beta Blocker	-0,162	-0,310 to 0,031	0,108			
ACEi	-0,099	-0,250 to 0,084	0,329			
Spironolactone	-0,122	-0,265 to 0,058	0,226			
BMI						
Baseline	0,167	-0,003 to 0,036	0,099			
Day 3	0,254	0,025 to 0,047	0,011			
Δ BMI	0,167	-0,008 to 0,089	0,099			
HR						
Baseline	-0,58	-0,006 to 0,00	0,565			
Day 3	-0,075	-0,017 to 0,006	0,457			
Δ HR	0,040	-0,007 to 0,006	0,696			
SBP						
Baseline	0,176	-0,001 to 0,025	0,080			
Day 3	0,447	0,006 to 0,027	<0,001	0,394	0,009 to 0,025	<0,001
Δ SBP	0,057	-0,007 to 0,025	0,576			
Log NTproBNP						
Baseline	0,260	0,105 to 0,758	0,009	0,298	0,167 to 0,830	0,004
Day 3	0,160	-0,058 to 0,509	0,114			
Δ Log NTproBNP	0,060	-0,215 to 0,408	0,551			
Log hsTnT						

Baseline	0,131	-0,125 to 0,626	0,193			
Day 3	0,220	0,037 to 0,849	0,028	0,198	0,043 to 0,759	0,028
Δ Log hsTnT	0,099	-0,265 to 0,791	0,325			
Log eGFR						
Baseline	-0,098	-1,486 to 0,507	0,332			
Day 3	-0,251	-1,915 to -0,246	0,012			
Δ Log eGFR	-0,025	-0,915 to 0,706	0,802			
Log pCr						
Baseline	0,149	-0,278 to 2,125	0,140			
Day 3	0,248	0,266 to 2,185	0,013			
Δ Log pCr	-0,009	-1,025 to 0,936	0,926			
pUrea						
Day 1	0,134	-0,001 to 0,007	0,184			
Day 3	0,132	-0,003 to 0,005	0,190			
Δ pUrea	-0,097	-0,007 to 0,004	0,338			
Albumin at Day 1	-0,153	-0,636 to 0,075	0,129			
Hemoglobin at Day 1	0,114	-0,029 to 0,108	0,258			

Day 1 values are compared with day 1 Albuminuria and hsTnT; day 3 values are compared with day 3 albuminuria and hsTnT; Δ, age, sex, DM, HgBA1c, LVEF, ischemic HF, and medications are compared with changes (Δ) in albuminuria and hsTnT between day 1 and day 3 (day 3 – day 1).
Legend: DM = diabetes mellitus; HgBA1c = glycated hemoglobin; LVEF = left ventricular ejection fraction; HF = heart failure; ACEi = angiotensin converting enzyme inhibitors; BMI = body mass index; HR = heart rate; SBP = systolic blood pressure; NTproBNP = N-terminal pro brain natriuretic peptide; hsTnT = high sensitivity troponin T; eGFR = estimated glomerular filtration rate; pCr = plasma creatinine; pUrea = plasma urea; Δ = changes between day 3 and day 1 (day 3 – day 1).

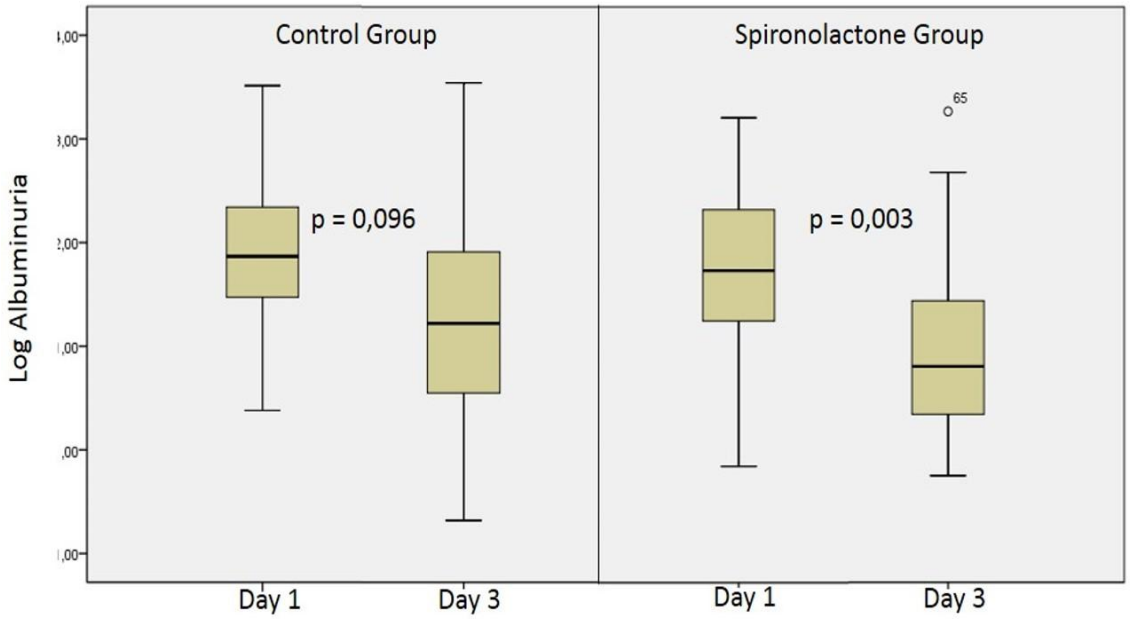
Figures

Figure 1. Study Consort Diagram



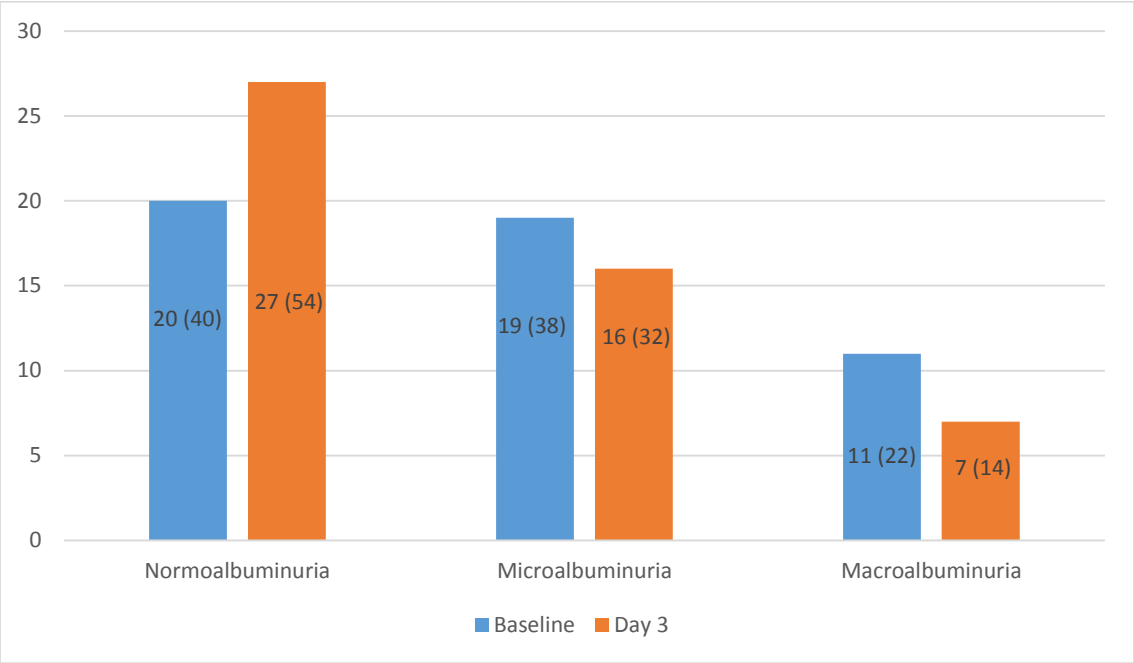
Legend: ADCHF, acutely decompensated chronic heart failure; ED, emergency department; pCr, plasma creatinine.
All patients included in the study completed the study, we had no dropouts, therefore all patients counted to the final results in the per-protocol analysis.

Figure 2. Albuminuria at Day 1 and Day 3 Within Control and Treatment Groups.



Legend: Log albuminuria had a more pronounced decrease in the intervention group than in control group. Comparison was performed comparing baseline and day 3 log albuminuria values using paired sample t tests within each group (1,79 ± 0,75 to 1,59 ± 0,67, p = 0,003 and 1,89 ± 0,70 to 1,79 ± 0,74, p = 0,096, respectively).

Figure 3. Comparison of the Prevalence of Normo, Micro, and Macroalbuminuria at Baseline and Day 3 within Spironolactone Group.



Legend: Normoalbuminuria: 20 (40%) to 27 (54%), p < 001; Microalbuminuria: 19 (38%) to 16 (32%), p = 0,014; Macroalbuminuria: 11 (22%) to 7 (14), p < 0,001. Data are presented in absolute number (%). Comparison between groups was performed using chi-square test.

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IV. High-Dose Spironolactone Changes Renin and Aldosterone Levels in Acutely Decompensated Heart Failure

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Abstract

Background: In acutely decompensated heart failure (ADHF) patients higher aldosterone levels correlate with worse post-discharge outcomes, suggesting that further modulation of the mineralocorticoid system during or immediately after hospitalization might favourably improve outcomes.

Methods and Results: This was an observational, retrospective secondary analysis of a study including 100 patients with ADHF. In that study 50 patients were submitted to spironolactone treatment (50 – 100 mg/day). A higher proportion of patients with renin levels above 16,5 pg/mL and aldosterone levels above 100 ng/dL was observed in subjects submitted to spironolactone treatment (44,7% vs. 66,7% and 56% vs. 64,7%, respectively, both $p < 0,05$). In the group of patients submitted to spironolactone treatment the proportion of patients with renin and aldosterone levels above the cutoff had a significant increase from baseline to day 3 (24% to 32% and 16% to 44%, respectively, both $p < 0,05$). Log renin and aldosterone were higher in patients with renin and aldosterone levels above the cutoff point (both $p < 0,05$).

Conclusions: High-dose spironolactone added to standard ADHF therapy induces an additional increase in renin and aldosterone levels. Whether higher levels of renin and aldosterone due to the reactive response to full MRA still have prognostic value requires further investigation.

Key-words: mineralocorticoid receptor antagonism; renin; aldosterone; acute heart failure.

Introduction

The use of mineralocorticoid receptor antagonists (MRAs) has demonstrated to improve outcomes and reduce mortality in chronic heart failure (HF) and post-myocardial infarction[1-3]. The benefit observed with MRAs is probably due to excessive neurohormonal activation blockade.

Particularly, aldosterone is probably essential for the progression of HF. Higher aldosterone levels were found in patients with chronic HF when compared with controls, and were found to be associated with poor outcome[4-7]. A rise in aldosterone levels was also observed in the acute myocardial infarction setting[8,9], and likewise associated with worse outcomes in this setting[10].

In acutely decompensated heart failure (ADHF) patients with ejection fraction (EF) < 40%, higher aldosterone levels correlate with worse post-discharge outcomes[11], suggesting that further modulation of the mineralocorticoid system during or immediately after hospitalization might favourably improve outcomes. Regarding this matter, high dose spironolactone as add-on therapy in the acutely decompensated heart failure (ADHF) setting has demonstrated to be safe and likely to provide greater symptomatic relief translated into a more pronounced decrease in natriuretic peptides[12].

We used an ADHF model to study the influence of the MRA spironolactone on renin and aldosterone. The aim of this study is to demonstrate the renin and aldosterone associations and changes before and after spironolactone introduction.

Methods

Study Design

This study is based on analysed data from a previous prospective, interventional, clinical trial that we performed[12]. In that study we enrolled 100 consecutive patients who presented in a Portuguese tertiary hospital with ADHF, between February 2012 and February 2013. They were non-randomly assigned in a sequential 1:1 ratio to spironolactone plus standard ADHF therapy or standard ADHF therapy alone, 50 patients within each arm (*i.e*. patients were alternatively assigned to spironolactone arm or standard ADHF therapy arm in a sequential manner - the first patient to one arm and the next to the other arm. This sequence was repeated until we reach 100 patients, 50 patients within spironolactone group and 50 patients within control group). Patients were blinded to the allocation, and the clinicians were not blinded to the allocation. The recommended spironolactone dose was 100 mg/day, however the assistant physician could decrease the spironolactone dose to 50 mg/day

after 48h upon admission. After 72h the study was open label. Furosemide dose and form of administration was performed according to the treating physician.

Patients were eligible for enrollment if they presented with decompensation of chronic HF with symptoms leading to hospitalization. All patients presented at the emergency department severely symptomatic in NYHA class IV. ADHF was diagnosed on the basis of the presence of history of chronic HF, at least one symptom (dyspnea, orthopnea, or edema), one sign (rales, peripheral edema, ascites, or pulmonary vascular congestion on chest radiography) and elevated natriuretic peptides. Exclusion criteria were: chronic use of MRAs, cardiac surgery within 60 days of enrollment, cardiac mechanical support, cardiac resynchronization-therapy within the last 60 days, comorbid conditions with an expected survival of less than 6 months, acute MI at time of hospitalization, hemodynamically significant uncorrected primary cardiac valvular disease, patients requiring intravenous vasodilators or inotropic agents, supine systolic arterial blood pressure <90 mmHg, plasma creatinine (pCr) level >1,5 mg/dL, serum potassium level >5,0 mmol/L, hemoglobin (HgB) level <9 g/dL, and sepsis.

Institutional review board or ethics committee approval was obtained. All patients provided written informed consent to participate in the study.

Study assessments

Patient`s clinical status including physical examination and was prospectively recorded by the same assistant physician at day 1 and day 3.

Medications and respective dosages were prospectively recorded by the investigators according to the assistant physician prescriptions.

Blood and spot urine samples were collected in the first 24 hours (h) after admission (day 1) of the patient to the hospital. The first dose of spironolactone was only administered after the first sample was collected. Fifty patients had daily oral spironolactone according to the study protocol described above. The day 3 samples were collected between 72 and 96 h of hospitalization. All samples were collected in the morning with the patient in supine position, and first-morning spot urine was used. All patients had low-salt, low-calorie hospital diet. Extra fruit and vegetables administration was not allowed. An assessment of biomarkers (including pCr, plasma urea [pUr], electrolytes, N-terminal pro-brain natriuretic peptide [NTproBNP], high-sensitivity troponin T [hsTnT] and proteinuria) was performed at a central core laboratory at day 1 and day 3. Clinical assessment and routine analyses were performed daily during hospital stay. All patients performed a transthoracic echocardiography within 72 hours upon admission. Left ventricle ejection fraction (LVEF) was calculated according to biplane Simpson method.

Aldosterone was measured using radioimmunoassay (RIA) Coat-a-Count® (Siemens) and renin with RIA (DiaSource®).

Variable definitions

We defined high renin levels when values were above the 16,5 pg/mL cutoff, and high aldosterone levels when values were above the 100 ng/dL cutoff. The manufacturer suggested a cutoff of 16,5 pg/mL for renin and a cutoff of 160 ng/dL for aldosterone. We lowered aldosterone cutoff to 100 ng/dL to increase test sensitivity, although levels above 160 ng/dL are more specific, we might miss important information, since *p.e*. in the EVEREST trial only 33,2% of patients had aldosterone levels above 160 ng/dL[11], and increased mortality was also observed in lower aldosterone quartiles.

We studied aldosterone (ng/dL) and renin (pg/mL) regarding the following covariates: age; sex; diabetes mellitus (DM); ischemic HF; EF (%); atrial fibrillation (AF); systolic blood pressure (SBP); intravenous (IV) furosemide dose; proportion of patients with IV furosemide at day 3; proportion of patients on angiotensin converting enzyme inhibitors (ACEi), angiotensin receptor blockers (ARB), beta-blockers (BB), and spironolactone; pCr (mg/dL), pUr (mg/dL), NTproBNP (pg/mL), hsTnT (ng/mL), sodium (mmol/L), potassium (mmol/L), uNa/K ratio, proteinuria (g/g), red-cell distribution width (RDW), HgB (g/dL), and length of stay.

Statistical Analysis

Normally distributed continuous variables are expressed as mean ± standard deviation (SD), and skewed distributions are presented as median [inter-quartile range, IQR].

Because of the positively skewed distributions of aldosterone, renin, pCr, proteinuria, RDW, NTproBNP, hsTnT and uNa/K ratio, these variables were log transformed for analysis.

Categorical variables are expressed in proportions (%).

Comparison between groups was performed using parametric, non-parametric tests, or chi-square tests, as appropriate. Significant association was defined by a probability (p) value ≤0,05.

Statistical analysis was performed using SPSS software (version 19, Chicago, IL, USA).

Results

Baseline Characteristics, Medications and Lab Results

Mean ± SD age of the 100 patients admitted due to ADHF was 76,0 ± 10,9 years. Thirty-nine patients were male; 50 patients had documented ischemic heart disease (IHD); 45 had DM; 59 had AF; 32 patients had EF < 40%.

Furosemide dose, SBP, proteinuria, NTproBNP, renin, aldosterone and hsTnT decreased from admission to day 3 (all, p <0,05). The proportion of patients taking ACEi/ARB and BB did not differ between admission and day 3.

Patient characteristics, lab results at admission day and hospital length of stay are shown in Table 1.

Comparison Between Normal and High Renin Values at Admission

A higher proportion of patients with renin levels above 16,5 pg/mL had AF (52,5% vs. 85%, p = 0,008). Log RDW, log renin, and hospital length of stay were higher in patients with renin levels above the cutoff (1,18 ± 0,03 vs. 1,21 ± 0,06, p = 0,004 and 8,36 ± 3,21 vs. 10,85 ± 3,25, p = 0,006, respectively) – Table 2. On the other hand, U Na/K ratio was lower in patients with higher renin levels (0,50 ± 0,38 vs. 0,22 ± 0,31, p = 0,003) – Table 2. and Figure 1.

Comparison Between Normal and High Aldosterone Values at Admission

Patients with lower aldosterone levels were more often male (45,5% vs. 17,4%, p = 0,015). Higher levels of serum potassium, log proteinuria, log RDW, log NTproBNP, log aldosterone, and plasma urea were observed in patients with aldosterone levels above the 100 ng/dL cutoff (all p < 0,05) – Table 2. Similarly to renin findings, a lower uNa/K ratio was observed in patients with higher aldosterone levels (0,49 ± 0,37 vs. 0,29 ± 0,37, p = 0,026) – Table 2. and Figure 1.

Comparison Between Normal and High Renin Values at Day 3

A higher proportion of patients with renin levels above 16,5 pg/mL was observed in subjects submitted to spironolactone treatment (44,7% vs. 66,7%, p = 0,050). Log renin and aldosterone levels were higher in patients with renin levels above the cutoff point (both, p < 0,05). Contrary to the findings at admission, uNa/K ratio did not differ between groups – Table 3.

Comparison Between Normal and High Aldosterone Values at Day 3

Patients with lower aldosterone levels were older (78,02 ± 9,28 vs. 72,09 ± 12,70, p = 0,009). Similarly to the findings described for renin at day 3, a higher proportion of patients with aldosterone levels above 100 ng/dL was observed in subjects submitted to spironolactone treatment (56% vs. 64,7%, p = 0,035). Log renin and aldosterone were also higher in this group of patients (both, p < 0,05), and uNa/K ratio did not differ between groups – Table 3.

Spironolactone Influence in Renin and Aldosterone Levels

After three days of spironolactone administration the proportion of patients with renin and aldosterone levels above the cutoff had a significant increase (from 24% to 32%, p = 0,003, and from 16% to 44%, p < 0,001, respectively) – Table 4. and Figure 2.

Discussion

The main results of our pilot study suggest that in patients submitted to spironolactone treatment the levels of renin and aldosterone increase. Higher levels of renin and aldosterone were not observed with the use of drugs other than spironolactone (p.e. ACEi/ARBs, BB or loop diuretics).

At admission higher renin and aldosterone levels were associated with lower urinary Na/K ratio levels, these findings were not reproduced when patients were submitted to MRA. In addition, higher levels of renin and aldosterone at admission were associated with higher RDW. Higher renin was associated with longer length of stay, and higher aldosterone with higher NTproBNP.

Baseline aldosterone blood levels measured in chronic HF patients with LVEF < 40% during the first days after admission for AHF were significantly correlated with higher mortality and re-hospitalization for HF[11]. Aldosterone levels were found to increase substantially during hospitalization and remain elevated after discharge, despite excellent background RAAS inhibitor therapy. The association between higher aldosterone levels and worse outcome remains positive, although weaker, when considering discharge aldosterone measurement[11]. Based on these findings the authors of this trial suggest that further neurohormonal modulation may be required in order to improve outcomes. In our interventional trial[12] we demonstrated that additional MR blockade during the acute decompensation is not associated with electrolyte disorders or kidney dysfunction and probably portends greater congestion relief, translated into a steep natriuretic peptide decrease. In this secondary analysis we demonstrated that renin and aldosterone levels are increased by spironolactone and not by ACEi/ARB, beta blockers or diuretics, as shown by the significant higher proportion of patients with levels of renin and aldosterone above the cutoff within spironolactone group, and not in patients under ACEi/ARB, beta blockers or diuretics. Furthermore, blood pressure and electrolytes are not likely to influence the observed variation in these hormones. These findings are concordant with previous reports in which the physiological elevation in plasma renin activity (PRA) and aldosterone were demonstrated in response to eplerenone and spironolactone treatment[13-17]. A previous study reported an increase in both PRA and aldosterone after eplerenone treatment for

10 days in dogs[18]. Similarly, in humans, eplerenone resulted in elevations in serum aldosterone and PRA when administered for 8-weeks in addition to a fixed-dose of an ACEi in mildly hypertensive patients[16]. A larger study, reported an increase in serum aldosterone with eplerenone treatment that was dose-responsive[19]. Furthermore, in patients with resistant hypertension an inverse correlation between blood pressure and serum aldosterone was demonstrated after treatment with eplerenone for 12 weeks[20], and another study demonstrated a time-dependent aldosterone increased in response to eplerenone[13]. The most robust explanation to this finding results from the demonstration that the main physiologic regulators of aldosterone synthase are angiotensin II and potassium[21]. Thus, increases in serum potassium and angiotensin II resulting from a decreased sodium and increased potassium reabsorption in the proximal tubules, would lead to an up-regulation of aldosterone synthase, consequently increasing aldosterone levels. Another potential explanation for the increase in aldosterone is a direct regulation of aldosterone synthase by mineralocorticoid receptor[13]. Our results can be very interesting and innovative, since MRA can probably improve outcomes in ADHF and also can increase renin and aldosterone levels, therefore higher levels of aldosterone may not held place for prognostic purposes when full MRA is provided. However, further studies are warranted to fully understand the mechanisms underlying the rise in aldosterone following MRA and the prognostic significance of MR blockade in the decompensated HF setting.

The median aldosterone levels at admission appear higher than the median values found in previous reports of patients with acute and chronic heart failure after the widespread use of ACEi/ARB[4-7,11]. This finding may be explained by the lower proportion (only 44%) of the patients on baseline ACEi/ARB, potentially leading to higher levels of renin and aldosterone[7,22], similar to the levels found before the widespread use of the RAAS inhibitors[6,23].

In the present study, higher levels of renin and aldosterone at admission were associated with higher RDW and lower urinary Na/K ratio levels. Additionally, higher aldosterone levels alone were associated with higher NTproBNP and higher levels of renin alone were associated with longer length of stay. Despite these side findings do not compose the bulk of our study, we think they deserve a brief discussion.

Higher levels of renin and aldosterone at admission were associated with higher RDW. RDW is a percentual measure of the variability in the size of circulating erythrocytes[24]. Disorders related to ineffective erythropoiesis or increased destruction cause greater heterogeneity in size and a higher RDW[25]. In patients with ADHF higher RDW has been associated with slower diuretic response[26] and increased long-term mortality[27]. The relationship between elevated hormone levels and poor outcome can

reflect the possibility that hormonal activation is not only serving as a marker for the severity of the disease, but is also contributing to progression of HF[5].

Higher aldosterone levels were associated with higher NTproBNP. To the best of our knowledge this is the first study to demonstrate a significant association between increased aldosterone levels and increased natriuretic peptides. However, previous studies demonstrated that aldosterone receptor antagonism with spironolactone induces a more noticeable decreases in plasma BNP levels than placebo or no spironolactone[12,28], suggesting that aldosterone may have an important role in the process of left ventricular remodeling[28].

As described above, increased neurohormonal activation is associated is worse outcome[11]. Our study was underpowered to detect major events, but length of stay could serve as a potential indirect severity measure. However length of stay may be affected by co-morbidities other than HF and in-hospital complications. Despite the association of higher levels of renin at admission and longer length of stay may appear to be an attractive result, the lack of correspondence in aldosterone and the bias inherent to length of stay, make this result less appealing and with dubious external validity.

Activation of the RAAS causes reabsorption of Na^+ and the excretion of K^+ in various epithelia such as the distal nephron[29]. Higher renin and aldosterone levels at admission were also associated with lower urinary Na/K ratio levels, these findings were not reproduced when patients were submitted to MRA. These observations are consistent with the RAAS effects on distal nephron, i.e. higher levels of renin and aldosterone lead to higher Na^+ reabsorption and K^+ excretion, decreasing uNa/K ratio. On the other hand, in the group of patients submitted to spironolactone treatment, uNa/K ratio increased probably reflecting the MRA effect. These dynamic changes suggest that uNa/K ratio can serve as a potential biomarker for MRA[13].

Our study has several limitations that should be noticed. First, it was a single-centre of a small sample size study. Second, no randomization or concealed allocation was performed, therefore we cannot exclude a selection bias potentially affecting the external validity of our results, particularly on outcome results like length of stay, however we included internal control variables like renin, aldosterone and NTproBNP that are less likely to be affected by bias and appear to be consistent with previous reports. Third, this study was performed post-hoc, therefore it is subject to the potential biases inherent to analyses of observational data. Fourth, baseline blood samples were collected in the first 24h after admission and before spironolactone administration, but most patients had already received diuretics and/or vasodilators, as a consequence renin and aldosterone admission levels may not reflect the real decompensation

values. Fifth, high aldosterone cutoff was lowered to 100 ng/dL to increase test sensitivity, despite losing specificity we were able to detect a higher proportion of patients with aldosterone increments. Finally, our inclusion criteria by restricting the enrolment of patients with hyperkalemia, impaired renal function, and severe valvular disease may be responsible for the good treatment response of our population and therefore limit the external validity of our conclusions.

Conclusion

Higher levels of aldosterone are associated with higher mortality risk in ADHF patients. High-dose spironolactone added to standard ADHF therapy induces an additional increase in renin and aldosterone levels. Whether higher levels of renin and aldosterone due to the reactive response to full MRA still have prognostic value requires further investigation.

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Disclosures

The authors have no conflicts of interest to disclose.

Tables

Table 1. Population Characteristics, Laboratory Results and Spironolactone Dose at Admission Day (Day 1) and Day 3

Age (yrs)	76,0 ± 10,88		
Male Sex – %	39		
DM - %	45		
Ischemic HF - %	50		
EF < 40 - %	32		
AF - %	59		
RDW (%)	15,20 [14,40 – 16,0]		
Hemoglobin (g/dL)	12,43 ± 2,07		
Length of Stay (days)	8,86 ± 3,36		
	Day 1	Day 3	p Value
Spironolactone - %	50	50	1**
Spironolactone Dose (mg)	94,50 ± 23,31	62,74 ± 24,33	<0,001
ACEi/ARB - %	44	61	0,069**
ACEi/ARB Dose (mg)	4,79 ± 3,01	3,79 ± 2,63	0,034
Beta-Blocker - %	37	57	0,143**
Beta-Blocker Dose (mg)	4,06 ± 1,77	3,19 ± 1,54	0,026
Furosemide IV Dose (mg)	81,08 ± 22,08	67,57 ± 25,54	0,001
Oral Furosemide - %	0	63	<0,001**
Oral Furosemide Dose (mg)	0	74,60 ± 28,10	<0,001
SBP (mmHg)	139,79 ± 25,86	121,97 ± 16,20	<0,001
Sodium (mmol/L)	140,54 ± 4,38	140,68 ± 3,95	0,718
Potassium (mmol/L)	4,03 ± 0,51	4,04 ± 0,54	0,950
Plasma Creatinine (mg/dL)	1,04 [0,89 – 1,31]	1,06 [0,85 – 1,40]	0,082*
Plasma Urea (mg/dL)	55,21 ± 20,84	62,3 ± 25,47	0,001
Proteinuria (g/g)	0,289 [0,188 – 0,629]	0,299 [0,160 – 0,097]	0,045*
NTproBNP (pg/mL)	2750 [1672 – 6032]	1835 [902 – 3837]	<0,001*
UNa/K ratio	3,04 [1,52 – 5,76]	2,80 [1,50 – 4,78]	0,341*
Renin (pg/mL)	4,35 [2,30 – 10,78]	5,34 [3,14 – 16,30]	0,011*
Aldosterone (ng/dL)	35,0 [12,0 – 92,5]	67,0 [21,3 – 125,0]	0,002*
hsTnT (ng/mL)	0,033 [0,193 – 0,050]	0,030 [0,018 – 0,051]	0,039*

Continuous variables are presented as mean value ± standard deviation [SD], p value or median [inter-quartile range, IQR], p value. Categorical variables are presented as absolute number (%), p value.

*Non-parametric paired sample test; **Chi-square test.

Note: day 1 analysis were collected before spironolactone administration.

Legend: DM = diabetes mellitus; EF = left ventricular ejection fraction; HF = heart failure; AF = atrial fibrillation; SBP, systolic blood pressure; ACEi/ARB = angiotensin converting enzyme inhibitors/angiotensin receptor blockers; RDW = red cell distribution width; NTproBNP = N-terminal pro brain natriuretic peptide; UNa/K = urinary sodium to potassium; hsTnT = high sensitivity troponin T.

Table 2. Comparison Between Normal and High Renin and Aldosterone Values at Admission.

	Renin < 16,5 (n=80)	Renin ≥ 16,5 (n=20)	p Value	Aldosterone < 100 (n=77)	Aldosterone ≥ 100 (n=23)	p Value
Age	75,99 ± 10,33	76,05 ± 13,13	0,982	74,91 ± 10,70	76,65 ± 10,88	0,066
Male Sex – no. (%)	30 (37,5)	9 (45)	0,539	35 (45,5)	4 (17,4)	0,015
DM – no. (%)	38 (47,5)	7 (35)	0,315	34 (44,2)	11 (47,8)	0,756
EF <40% – no. (%)	26 (32,5)	5 (25)	0,517	25 (32,5)	6 (26,1)	0,562
Ischemic HF – no. (%)	37 (46,3)	13 (65)	0,134	37 (48,1)	13 (58,5)	0,476
AF – no. (%)	42 (52,5)	17 (85)	0,008	44 (57,1)	15 (65,2)	0,490
SBP	140,93 ± 26,08	135,25 ± 25,11	0,383	140,84 ± 26,72	136,26 ± 22,94	0,459
Beta Blocker – no. (%)	31 (38,8)	5 (25)	0,252	27 (35,1)	9 (39,1)	0,722
ACEi/ARB – no. (%)	34 (42,5)	8 (40)	0,839	32 (41,6)	10 (43,5)	0,870
Sodium	140,75 ± 4,41	139,70 ± 4,26	0,340	140,84 ± 4,54	139,52 ± 3,69	0,205
Potassium	4,05 ± 0,50	3,98 ± 0,55	0,586	3,97 ± 0,47	4,24 ± 0,57	0,023
Log Creatinine	0,02 ± 0,11	0,02 ± 0,14	0,888	0,02 ± 0,12	0,04 ± 0,11	0,495
Log Proteinuria	-0,44 ± 0,40	-0,45 ± 0,47	0.962	-0,51 ± 0,39	-0,23 ± 0,42	0,008
Log RDW	1,18 ± 0,03	1,21 ± 0,06	0,004	1,18 ± 0,04	1,20 ± 0,04	0,047
Log NTproBNP	3,49 ± 0,45	3,41 ± 0,35	0,444	3,42 ± 0,41	3,67 ± 0,47	0,014
Log UNa/K ratio	0,50 ± 0,38	0,22 ± 0,31	0,003	0,49 ± 0,37	0,29 ± 0,37	0,026
Log Aldosterone	1,53 ± 0,43	1,70 ± 0,49	0,135	1,39 ± 0,34	2,15 ± 0,12	<0,001
Log Renin	0,52 ± 0,33	1,46 ± 0,22	<0,001	0,69 ± 0,48	0,78 ± 0,50	0,402
Log hsTnT	-1,47 ± 0,39	-1,47 ± 0,33	0,995	-1,47 ± 0,39	-1,47 ± 0,34	0,988
Urea	53,78 ± 19,68	60,95 ± 24,68	0,170	52,44 ± 20,52	64,48 ± 19,60	0,014
Hemoglobin	12,50 ± 1,98	12,16 ± 2,44	0,512	12,39 ± 2,06	12,59 ± 2,17	0,689
Log Length of Stay	0,89 ± 0,17	1,01 ± 0,15	0,003	0,91 ± 0,16	0,91 ± 0,19	0,958

Legend: DM = diabetes mellitus; EF = left ventricular ejection fraction; HF = heart failure; AF = atrial fibrillation; SBP, systolic blood pressure; ACEi/ARB = angiotensin converting enzyme inhibitors/angiotensin receptor blockers; RDW = red cell distribution width; NTproBNP = N-terminal pro brain natriuretic peptide; UNa/K = urinary sodium to potassium; hsTnT = high sensitivity troponin T.

Table 3. Comparison Between Normal and High Renin and Aldosterone Values at Day 3.

	Renin < 16,5 (n=76)	Renin ≥ 16,5 (n=24)	p Value	Aldosterone < 100 (n=66)	Aldosterone ≥ 100 (n=34)	p Value
Age	76,58 ± 9,61	74,17 ± 14,25	0,346	78,02 ± 9,28	72,09 ± 12,70	0,009
Male Sex – no. (%)	28 (36,8)	11 (45,8)	0,431	25 (37,9)	14 (41,2)	0,749
DM – no. (%)	37 (48,7)	8 (33,3)	0,188	29 (43,9)	16 (47,1)	0,766
EF <40% – no. (%)	22 (28,9)	9 (37,5)	0,430	18 (27,3)	13 (38,2)	0,262
Ischemic HF – no. (%)	36 (47,4)	14 (58,3)	0,241	30 (45,5)	20 (68,8)	0,205
AF – no. (%)	43 (56,6)	16 (66,7)	0,264	42 (63,6)	17 (50)	0,189
SBP	123,55 ± 16,73	116,96 ± 13,52	0,082	122,55 ± 15,76	120,85 ± 17,20	0,623
Beta Blocker – no. (%)	46 (60,5)	11 (45,8)	0,205	38 (57,6)	19 (55,9)	0,871
ACEi/ARB – no. (%)	48 (63,2)	13 (54,2)	0,431	41 (62,1)	20 (58,8)	0,749
IV Furosemide – no. (%)	27 (35,5)	10 (41,7)	0,587	25 (37,9)	12 (35,4)	0,800
Spironolactone – no. (%)	34 (44,7)	16 (66,7)	0,050	28 (56)	22 (64,7)	0,035
Spironolactone Dose	63,89 ± 24,96	60,29 ± 23,48	0,620	60,00 ± 23,31	66,30 ± 25,68	0,355
Sodium	141,11 ± 3,85	139,33 ± 4,05	0,055	141,12 ± 4,33	139,82 ± 2,96	0,121
Potassium	4,01 ± 0,56	4,14 ± 0,49	0,299	4,00 ± 0,50	4,10 ± 0,61	0,402
Log Creatinine	0,03 ± 0,14	0,05 ± 0,16	0,675	0,02 ± 0,14	0,07 ± 0,14	0,058
Log Proteinuria	-0,46 ± 0,38	-0,59 ± 0,31	0.132	-0,49 ± 0,35	-0,49 ± 0,41	0,955
Log RDW	1,18 ± 0,03	1,21 ± 0,06	0,007	1,19 ± 0,04	1,18 ± 0,04	0,658
Log NTproBNP	3,27 ± 0,50	3,29 ± 0,51	0,881	3,28 ± 0,49	3,24 ± 0,52	0,679
Log UNa/K ratio	0,42 ± 0,40	0,34 ± 0,37	0,378	0,44 ± 0,38	0,31 ± 0,40	0,120
Log Aldosterone	1,64 ± 0,45	1,94 ± 0,51	0,007	1,47 ± 0,38	2,21 ± 0,19	<0,001
Log Renin	0,59 ± 0,34	1,52 ± 0,25	<0,001	0,69 ± 0,45	1,07 ± 0,54	<0,001
Log hsTnT	-1,51 ± 0,33	-1,52 ± 0,42	0,838	-1,51 ± 0,33	-1,51 ± 0,39	0,942
Urea	60,86 ± 25,20	66,92 ± 26,32	0,312	60,70 ± 26,83	65,44 ± 22,64	0,380
Hemoglobin	12,52 ± 2,11	12,16 ± 1,96	0,465	12,38 ± 2,11	12,53 ± 2,03	0,728
Log Length of Stay	0,89 ± 0,17	0,99 ± 0,13	0,008	0,89 ± 0,18	0,96 ± 0,13	0,043

Legend: DM = diabetes mellitus; EF = left ventricular ejection fraction; HF = heart failure; AF = atrial fibrillation; SBP, systolic blood pressure; ACEi/ARB = angiotensin converting enzyme inhibitors/angiotensin receptor blockers; RDW = red cell distribution width; NTproBNP = N-terminal pro brain natriuretic peptide; UNa/K = urinary sodium to potassium; hsTnT = high sensitivity troponin T.

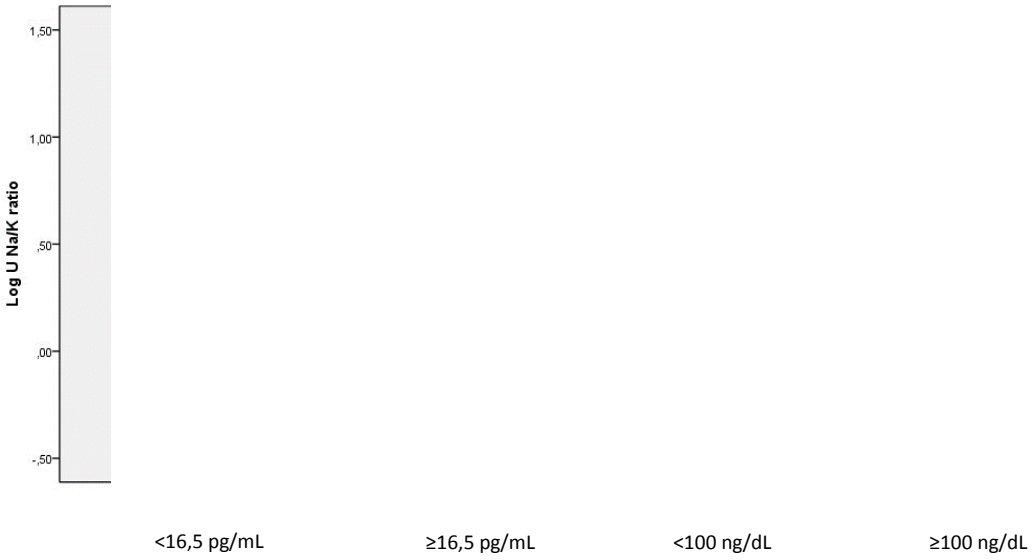
Table 4. Comparison of Renin and Aldosterone Levels at Admission and Day 3 within Spironolactone and Control Groups

	Day 1			Day 3		
	Spironolactone	Control	p Value	Spironolactone	Control	p Value
Renin > 16,5 – no. (%)	12 (24)	8 (16)	0,317	16 (32)	8 (16)	0,050
Aldosterone > 100 – no. (%)	8 (16)	15 (30)	0,096	22 (44)	12 (24)	0,035

Note: Day 1 analysis were performed before spironolactone administration

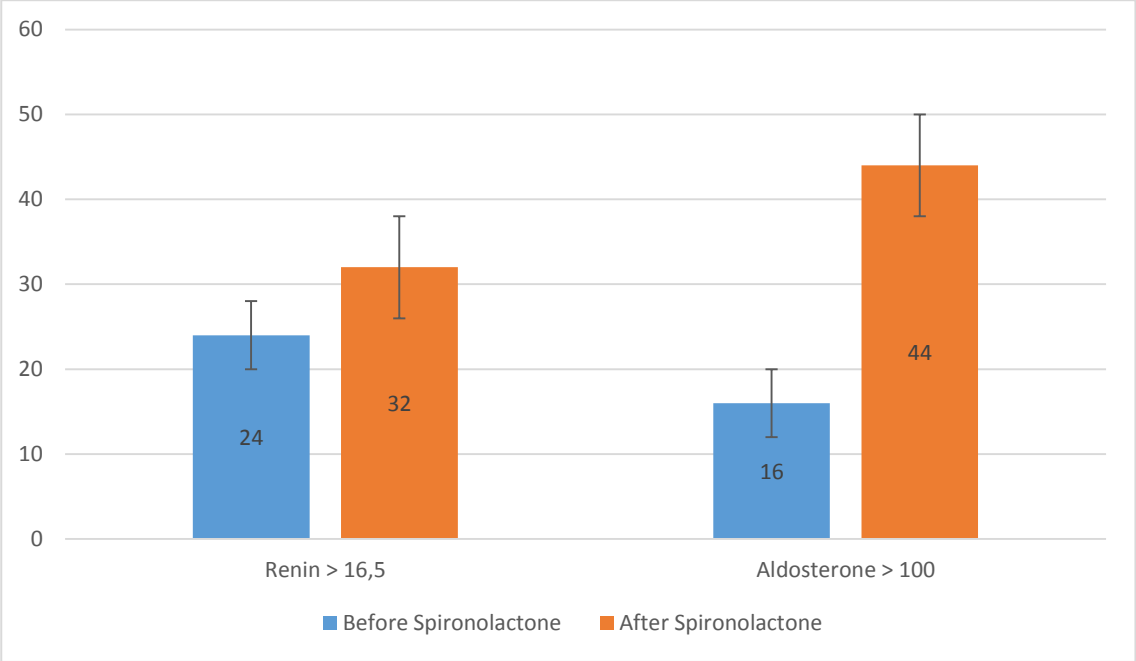
Figures

Figure 1. Comparison of Urinary Sodium to Potassium Ratio According to Renin and Aldosterone Levels at Admission.



Legend: Renin = Log Renin; Aldosterone = Log Aldosterone; U Na/K = urinary sodium to potassium.
Patients with higher renin and aldosterone levels at admission had lower urinary sodium to potassium ratios.

Figure 2. Comparison of Renin and Aldosterone Levels Before and After Spironolactone Treatment



Legend: Results are presented in percentage (%) of total. Renin is expressed in pg/mL and Aldosterone in ng/dL.
The proportion of patients with increased levels of renin and aldosterone is significantly higher in patients submitted to spironolactone treatment. The proportion of patients with renin levels above 16,5 pg/mL increases from 24% before spironolactone administration to 32% after spironolactone administration, p value = 0,003. The proportion of patients with aldosterone levels above 100 ng/dL increases from 16% before spironolactone administration to 44% after spironolactone administration, p value < 0,001.

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V. High Sensitivity Troponin T: A Biomarker for Diuretic Response in Decompensated Heart Failure Patients?

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Abstract

Background: Patients presenting with acutely decompensated heart failure (ADHF) and positive circulating cardiac troponins were found to be a high-risk cohort. The advent of high-sensitive troponins resulted in a detection of positive troponins in a great proportion of heart failure patients. However, the pathophysiological significance of this phenomenon is not completely clear.

Objectives The aim of this study is to determine the early evolution and clinical significance of high-sensitivity troponin T (hsTnT) in ADHF.

Methods: Retrospective, secondary analysis of a prospective study including 100 patients with ADHF.

Results: Globally, high-sensitivity troponin T decreased from day 1 to day 3 (p = 0,039). However, in the subgroup of patients who remained decompensated no significant differences in hsTnT from day 1 to day 3 were observed (p = 0,955), whereas in successfully compensated patients a significant reduction in hsTnT levels was observed (p = 0,025). High sensitivity troponin T decrease was correlated with NTproBNP reduction (p = 0,007). Patients with hsTnT increase had longer length of stay (p = 0,033).

Conclusions: Episodes of ADHF are associated with transient increases in the blood levels of hsTnT that are reduced with effective acute episode treatment. The decrease in hsTnT can translate less myocardial damage along with favourable ADHF treatment.

Key-words: Acute Heart Failure. High-Sensitivity Troponin T. Biomarkers. N-Terminal Pro-Brain Natriuretic Peptide

Introduction

Patients presenting with acutely decompensated heart failure (ADHF) and positive circulating cardiac troponins were found to be a high-risk cohort, requiring greater use of hospital resources, and having increased risk of in-hospital mortality[1]. Measurement of cardiac troponins in this setting adds important prognostic information and should be considered as part of an early assessment of risk[1,2].

Detectable troponins, even in the absence of acute coronary syndrome, are associated with impaired hemodynamics, progressive decline in left ventricular systolic function, and shortened survival[3-5].

Recent improvements in the sensitivity of troponin assays added additional challenges in the interpretation of these biomarkers in heart failure (HF). The increasing sensitivity of more contemporary assays has resulted in the detection of circulating troponin in a progressively greater proportion of HF patients. This phenomenon has led to increasing uncertainty about the clinical interpretation of troponin data from contemporary assays, particularly in patients with ADHF, since a substantial proportion of these patients have elevations of circulating troponins[1,6,7].

The aim of this study is to determine the early evolution, associations, and correlations of high-sensitivity troponin T (hsTnT) in ADHF.

Methods

Study Design

We analysed a database from a previous conducted prospective, interventional trial that we performed[8]. In that study we enrolled a 100 consecutive patients who presented in a Portuguese tertiary hospital with ADHF, between February 2012 and February 2013. They were assigned in a sequential 1:1 ratio to spironolactone plus standard ADHF therapy or standard ADHF therapy alone. Patients were eligible for enrollment if they presented with decompensation of chronic HF with symptoms leading to hospitalization. ADHF was diagnosed on the basis of the presence of history of chronic HF and at least one symptom (dyspnea, orthopnea, or edema) and one sign (rales, peripheral edema, ascites, or pulmonary vascular congestion on chest radiography). Exclusion criteria were: chronic use of mineralocorticoid receptor antagonists (MRAs), cardiac surgery within 60 days of enrollment, cardiac mechanical support, cardiac resynchronization-therapy within the last 60 days, comorbid conditions with an expected survival of less than 6 months, acute MI at time of hospitalization, hemodynamically significant uncorrected primary cardiac valvular disease, patients requiring intravenous vasodilators or inotropic agents, supine systolic arterial blood

pressure <90 mmHg, plasma creatinine (pCr) level >1,5 mg/dL, serum potassium level >5,0 mmol/L, hemoglobin (HgB) level <9 g/dL, and sepsis.

Institutional review board or ethics committee approval was obtained. All patients provided written informed consent to participate in the study.

Study assessments

Patient`s clinical assessment including physical examination, was prospectively recorded daily by the same assistant physician.

Medications and respective dosages were prospectively recorded by the investigators according to the assistant physician prescriptions.

Blood and spot urine samples were collected in the first 24 hours (h) after admission (day 1) of the patient to the hospital. The day 3 samples were collected between 72 and 96 h of hospitalization. An assessment of biomarkers, including pCr, plasma urea (pUr), electrolytes, N-terminal pro-brain natriuretic peptide (NTproBNP) and hsTnT was performed at a central core laboratory at day 1 and day 3. Clinical assessment and routine analyses were performed daily during hospital stay. Estimated glomerular filtration rate (eGFR) was determined using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation[8]. All patients performed a transthoracic echocardiography within 72 hours upon admission. Ejection fraction (EF) was calculated according to biplane Simpson method.

High sensitive troponin T was measured using COBAS Troponin T hs (high sensitive) STAT (short turn around time) (Roche Diagnostics®). According to the manufacturer a positive hsTnT test was considered when the value was above the upper reference limit (99th percentile) of 0,014 ng/mL.

Variable definitions

We studied hsTnT regarding the following covariates: comorbidities such as diabetes mellitus (DM), chronic obstructive pulmonary disease (COPD), and sleep apnea; body mass index (BMI); heart rate (HR); systolic blood pressure (SBP); atrial fibrillation (AF); HF etiology; echocardiographic parameters such as EF; furosemide dose, proportion of patients on angiotensin converting enzyme inhibitors (ACEi), beta-blockers (BB), and spironolactone; pCr, pUr, NTproBNP, sodium, potassium; HgB and serum albumin.

In order to determine the differences in hsTnT concentration between patients with faster diuretic response and patients with slower diuretic response after 3 days of inpatient treatment, patients were considered faster diuretic responders if they had decreased intravenous (i.v.) furosemide dose or switched to oral furosemide in the first three days of in-hospital treatment. On the other hand, patients were considered to be

slower diuretic responders if the assistant physician increased or maintained i.v. furosemide dosage after three days of in-hospital treatment.

Statistical Analysis

Normally distributed continuous variables are expressed as mean ± standard deviation (SD), and skewed distributions are presented as median [inter-quartile range, IQR].

Categorical variables are expressed in absolute numbers (no.) and proportions (%).

Comparison between groups was performed using parametric, non-parametric tests, or chi-square tests, as appropriate. Significant association was defined by a probability (p) value ≤ 0,05.

The positively skewed distributions were log transformed for analysis.

Correlations of log hsTnT were first examined by single variable linear or logistic regression and presented as non-adjusted coefficient (NAC) and 95% confidence interval [$_{95\%}$CI]. Factors with a p value ≤ 0,05 by single variable regression analyses were included in a multivariable linear regression model, presented as adjusted coefficient (AC) [$_{95\%}$CI].

Statistical analysis was performed using SPSS software (version 19, Chicago, IL, USA).

Results

Baseline Characteristics and Early Changes

Mean ± SD age of the 100 patients admitted due to ADHF was 76 ± 10,9 years. Thirty-nine (39%) patients were male; 50 patients had documented ischemic heart disease (IHD); 59 had AF; and mean ± SD EF (%) was 43,46 ± 11,73 - Table 1. All patients were admitted in New York Heart Association (NYHA) class IV. Patient characteristics, medications and comparison of lab results between admission day (day 1) and the third day of inpatient treatment is shown in Table 1.

Globally, high-sensitivity troponin T was likely to decrease from day 1 to day 3 (median [IQR], 0,033 [0,019 – 0,050] vs. 0,030 [0,018 – 0,051], p = 0,039) – Table 1. However, in the subgroup of patients considered to have slower diuretic response no significant differences in hsTnT from day 1 to day 3 were observed (median [IQR], from 0,046 [0,033 - 0,087] to 0,055 [0,032 - 0,072], p = 0,955), whereas in the group of patients considered to have a faster diuretic response a significant reduction in hsTnT levels was observed (median [IQR], from 0,032 [0,017 - 0,048] to 0,028 [0,017 - 0,045], p = 0,025) – Table 2 and Figure 1. The hsTnT variation did not differ between groups (median [IQR], -0,0005 [-0,043 to 0,004] vs. -0,0010 [-0,020 to 0,002], p = 0,51) –

Table 2. The majority of patients with negative hsTnT at day 1 remained negative at day 3 (76,9%). On the other hand only a small proportion (3,1%) of patients with positive hsTnT at day 1 turned negative at day 3 – Table 3.

High-Sensitivity Troponin T Correlations

Bivariate analysis of hsTnT at day 1 found positive correlations with day 1 Log NTproBNP (NAC [$_{95\%}$CI], 0,481 [0,267 to 0,574], p < 0,001), pUr (NAC [$_{95\%}$CI], 0,309 [0,002 to 0,009], p = 0,002), and Log pCr (NAC, [$_{95\%}$CI], 0,345 [0,500 to 1,704], p < 0,001). A negative correlation was found with Log eGFR (NAC [$_{95\%}$CI], -0,275 (-1,231 to -0,216), p = 0,006). Day 3 hsTnT was also positively correlated with day 3 Log NTproBNP (NAC [$_{95\%}$CI], 0,486 [0,218 to 0,464], p < 0,001), and Log pCr (NAC, [$_{95\%}$CI], 0,439 [0,630 to 1,503], p < 0,001), and negatively correlated with Log eGFR (NAC [$_{95\%}$CI], -0,399 (-1,232 to -0,455), p = 0,006) – Table 4. High sensitivity troponin T decrease was correlated with NTproBNP reduction (NAC [$_{95\%}$CI], 0,267 [0,044 to 0,276], p = 0,007) – Table 4. and Figure 2. By multivariate analysis, hsTnT correlated with NTproBNP at day 1 and day 3 (AC [$_{95\%}$CI], 0,400 [0,185 to 0,513], p < 0,001, and 0,381 [0,146 to 0,389], p< 0,001, respectively) – Table 4.

Determinants of hsTnT Change

High-sensitivity troponin T was transformed according to the pattern of change (decrease or increase) during the first 3 days of treatment – Table 5.

Patients with hsTnT increase had lower NTproBNP decrease (median [IQR], -1167 [-2337 to -367] vs. -379 [-1273 to 319,5], p = 0,003), had longer length of stay (median [IQR], 8 [6 to 11] vs. [9 [7 to 12], p = 0,033), and had higher proportion of AF (49,2% vs. 75,7%, p = 0,009). Diuretic dosages, other HF medications, renal function and length of stay did no differ between groups – Table 5.

Discussion

The major finding of this study is that episodes of ADHF are associated with transient increases in the blood levels of hsTnT that are reduced with acute episode effective treatment. This statement is corroborated by the higher levels of hsTnT in patients who maintained or increased i.v. furosemide dose after 3 days of hospitalization, by a decrease in hsTnT levels in patients with faster response to diuretic therapy, by the correlation between troponin T decrease and NTproBNP reduction, and by the longer length of stay and lower decrease in NTproBNP levels in the group of patients who had increase in hsTnT from day 1 to day 3.

Improvements in analytical sensitivity have transformed circulating troponin from a biomarker that was only detectable in a minority of patients to one that is detectable in the vast majority of patients with HF[1]. The high sensitivity of the test can

detect very small changes in the circulating troponin levels[1,7], providing a potential explanation for the high proportion of patients who remained above the 99th percentile after 3 days of treatment.

In our study over 80% of the patients had hsTnT levels above the 99th percentile, this prevalence of detectable hsTnT was higher than in previously published reports[1-3,6,9,10]. The most likely explanation for this finding is the type of tests, assay platforms and the cutoff limits used in those studies. For example, the Acute Decompensated Heart Failure National Registry (ADHERE) study used a higher cutoff limit of 0,1 ng/mL and they did not control the assay platform[1], and in the Enhanced Feedback for Effective Cardiac Treatment (EFFECT) study the cuttof limit used was 0,5 ng/mL[3]. However, in the another study by Metra M., et al[6] the used cutoff was 0,01 ng/mL where levels above this value were considered abnormal. In that study, 51 (48%) of the 107 patients discharged alive from the hospital had detectable troponin in at least one measurement. Despite the differences in the type of test and assay platform, the cutoff limit was similar to the cutoff used in our study. One possible explanation for this discrepancy is the mean ± SD age of the patients included in the present study. Our patients are older than patients included in the study by Metra *et al* (76 ± 10,9 vs. 66 ± 13 years, respectively). Troponin levels are likely to have a Gaussian or near Gaussian distribution, with higher levels found in older age groups[11].

Elevations in baseline troponin levels were demonstrated to be independent predictors of events during the acute hospitalization (worsening or persistent HF, death, and increased length of stay) and also independent predictors of post-discharge outcomes[3,6,9,12-14]. In our study, an increase in hsTnT levels was also associated with longer length of stay consistent with the previous cited reports.

Changes in troponin status during initial treatment for ADHF have been proposed as potentially important targets for drug development[15]. In the biomarker analysis from the Relaxin in Acute Heart Failure (RELAX-AHF) development program[16], changes in markers of cardiac (hsTnT), renal (pCr and cystatin-C), and hepatic (aspartate transaminase and alanine transaminase) damage and of decongestion (NTproBNP) at day 2, improved with Serelaxin administration. These findings were consistent with the prevention of organ damage and faster decongestion. Our study also showed a reduction in hsTnT levels in the first days of HF treatment in patients who were able to reduce i.v. furosemide dose or switch it to oral route and in patients with higher reduction in natriuretic peptides, possibly traducing less myocardial damage in patients with more favourable therapeutic response, *i.e*. faster decongestion. This finding provides additional data supporting the use of troponin as a biomarker for ADHF severity and therefore a potential therapeutic target. In addition,

NTproBNP and hsTnT are independent markers of increased mortality risk in HF[6,14,17,18] and natriuretic peptides have shown to correlate with changes in ventricular wall stress, being inversely related to the severity of left ventricular dysfunction[17,19-21]. A decline in NTproBNP plasma levels during the initial hospitalisation was observed in our study, a finding consistent with previous reports[22-25]. Furthermore, this study demonstrates that patients with hsTnT decrease have a more pronounced NTproBNP reduction, and a weak but positive correlation between hsTnT and NTproBNP was found. Despite the weak correlation between hsTnT and NTproBNP, these results may suggest that congestion and ventricular wall stress relief can be translated into natriuretic peptide and hsTnT reduction. However the different pathophysiological mechanisms targeted by these biomarkers may explain the weak correlation between them described in this study. Nevertheless, this finding was not observed in other studies involving patients with heterogeneous HF presentations[6,26].

The ADHF episodes are associated with increased mechanical strain on the heart, activation of neurohormonal systems, and increased and oxidative stress[25]. These stimuli are known to mediate myocardial injury, accelerating myocyte loss[25]. Troponin T is highly specific for cardiac myocytes, but circulating levels may also be elevated due to renal insufficiency. However, this mechanism does not seem to underlie our observations, since hsTnT is positively correlated with pCr and negatively correlated with eGFR at day 1 and day 3, but the changes in hsTnT during treatment are not correlated with changes in renal function. Thus, patients with impaired renal function are likely to have higher hsTnT levels, but hsTnT reduction is independent of renal function changes. Thus, we believe that the elevation in hsTnT reflects increased release from the myocardium, and thus, may indicate myocyte injury and/or death.

In the group of patients with hsTnT increase a higher proportion of patients with AF was observed. These findings are consistent with previous larger trials, in which a positive hsTnT was detected in almost all patients with AF, with hsTnT levels carrying strong and independent prognostic information with a gradual increase in the risk of stroke, cardiac and total death[27]. Our study was underpowered for major cardiovascular events and death, but a longer length of stay was observed in patients with hsTnT increase as discussed above.

Limitations

Our study has several limitations that need to be considered. It was a single-centre investigation of a small sample, which limits our inferential analysis. The decision to withdraw diuretic therapy was based on subjective assessment of congestive signs and symptoms so we cannot rule out the inter-observer variability. However, in real-life clinical practice, the decision to step down diuretic therapy is also

based on subjective clinical evaluation. Our study protocol defined that the first blood sample would be collected in the first 24 h, so at the time of venous blood sampling patients could had been treated already with diuretics. Although we are not comparing diuretic-naïve patients at day 1 measurements, the overall effect of this bias would be an underestimated difference between day 1 and day 3, which does not significantly affect the internal validity of our study conclusions. Finally, the external validity of our conclusions is limited to normo-hypertensive and fluid overloaded HF patients with normal or mildly impaired renal function, since all these factors were considered inclusion criteria. On the other hand, our conclusions can be reproducible in this set of patients widely common in clinical practice.

Conclusions

Episodes of ADHF are associated with transient increases in the blood levels of hsTnT that are reduced with effective acute episode treatment. The decrease in hsTnT and NTproBNP can translate ventricular wall stress relief and less myocardial damage along with favourable ADHF treatment. Further studies are needed to examine the value of combining necrosis markers and natriuretic peptides in the clinical management of ADHF patients.

Disclosures

The authors have no conflicts of interest to disclose.

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Tables

Table 1. Population Characteristics, and Comparison of Clinical Variables, Laboratory Results and Medications Between Admission Day (Day 1) and Day 3

Age (yrs)	76,0 ± 10,88		
Male Sex – %	39		
Diabetes Mellitus - % Glycated HgB (%)	45 7,02 ± 0,96		
COPD - %	17		
Dementia - %	12		
Sleep Apnea - %	18		
Non-Invasive Ventilation - %	17		
Ischemic Heart Disease -%	50		
Atrial Fibrillation - %	59		
LV Ejection Fraction (%)	43,46 ± 11,73		
LV Ejection Fraction ≥ 40% - %	68		
	Day 1	Day 3	p Value
Body Mass Index (Kg/m^2)	29,44 ± 6,17	28,35 ± 6,23	< 0,001
Heart Rate (bpm)	93,65 ± 24,35	76,41 ± 11,96	< 0,001
SBP (mmHg)	139,79 ± 25,86	121,97 ± 16,2	< 0,001
Plasma Creatinine (mg/dL)	1,04 [0,89 – 1,31]	1,06 [0,85 – 1,40]	0,082*
eGFR (mL/min/1,73 m^2)	58,0 [44,0 – 72,0]	58,0 [39,25 – 72,75]	0,171*
Plasma Urea (mg/dL)	55,21 ± 20,84	62,3 ± 25,47	0,001
Serum Potassium (mmol/L)	4,03 ± 0,51	4,04 ± 0,54	0,95
Serum Sodium (mmol/L)	140,54 ± 4,38	140,68 ± 3,95	0,72
Hemoglobin (g/dL)	12,43 ± 2,07	-	-
Albumin (mg/dL)	3,68 ± 0,40	-	-
NTproBNP (pg/mL)	2750 [1672 – 6032]	1835 [902 – 3837]	< 0,001*
hsTnT (ng/mL)	0,033 [0,019 – 0,050]	0,030 [0,018 – 0,051]	0,039*
IV Furosemide - %	100	37	< 0,001**
IV Furosemide Dose (mg/d)	75,80 ± 21,52	67,57 ± 25,54	0,001
Oral Furosemide - %	0	63	-
Oral Furosemide Dose (mg/d)	0	74,6 ± 28,1	-
Furosemide Dose Reduction or Oral Route - %	-	84	-
ACEi - %	44	61	< 0,001**
Ramipril Eq. Dose (mg/d)	3,15 ± 2,04	3,36 ± 2,14	0,474
Beta-Blocker - %	37	57	< 0,001**
Bisoprolol Eq. Dose (mg/d)	3,01 ± 1,08	2,96 ± 1,89	0,474
Spironolactone - %	50	50	1**
Spironolactone Dose (mg/d)	94,50 ± 23,31	62,74 ± 24,33	< 0,001

Continuous variables are presented as mean value ± standard deviation [SD], p value or median [inter-quartile range, IQR], p value. Categorical variables are presented as % of total (100 patients), p value.

*Non-parametric paired sample test; ** Chi-square test.

Legend: COPD = chronic obstructive pulmonary disease; LV = left ventricular; eGFR = estimated glomerular filtration rate; NTproBNP = N-terminal pro brain natriuretic peptide; hsTnT = high sensitivity troponin T; IV = intra-venous; ACEi = angiotensin converting enzyme inhibitors.

Table 2. Comparison of TnT Levels Between Patients Who Responded to Diuretic Therapy versus Patients Who Needed to Increase Diuretic Dose

	Furosemide Maintenance or Increase (n=16)	Furosemide Decrease or Oral Administration (n=84)	p Value Between Groups
hsTnT (ng/mL)			
Day 1	0,046 [0,033 to 0,087]	0,032 [0,017 to 0,048]	0,026*
Day 3	0,055 [0,032 to 0,072]	0,028 [0,017 to 0,045]	0,004*
Δ hsTnT	-0,0005 [-0,043 to 0,004]	-0,0010 [-0,020 to 0,002]	0,51*
	p Value Within Group	p Value Within Group	
	0,955*	0,025*	

Continuous variables are presented as median [inter-quartile range, IQR], p value.
*Non-parametric test.
Legend: hs TnT = high-sensitivity troponin T

Table 3. Comparison of hsTnT Values Below (Negative) and Above (Positive) the 99th Percentile (≥ 0,014 ng/mL)

		Day 1			
		Negative hsTnT – no. (%)	Positive hsTnT – no. (%)	Total	p Value
Day 3	Negative hsTnT – no. (%)	10 (76,9)	3 (3,4)	13 (13)	< 0,001**
	Positive hsTnT – no. (%)	3 (23,1)	84 (96,6)	87 (87)	< 0,001**
	Total	13 (13)	87 (87)	100	

** Chi-square test. Legend: hsTnT = high sensitivity troponin T.

Table 4. Associations With Log hsTnT at Day 1, Day 3, and Changes Between Day 1 and Day 3 (Δ)

	Nonadjusted Coefficient for Log hsTnT	95%CI	p Value	Adjusted Coefficient for Log hsTnT	95%CI	p Value
Age	0,119	-0,001 to 0,005	0,240			
Male Sex	-0,066	-0,086 to 0,043	0,515			
DM	0,095	-0,033 to 0,093	0,349			
HgBA1c	0,058	-0,046 to 0,067	0,707			
LVEF	0,089	-0,001 to 0,004	0,376			
Ischemic HF	-0,078	-0,087 to 0,038	0,442			
Beta Blocker	-0,004	-0,065 to 0,062	0,969			
ACEi	0,023	-0,057 to 0,072	0,820			
Spironolactone	-0,116	-0,099 to 0,026	0,251			
BMI						
Day 1	-0,205	-0,025 to 0,000	0,042			
Day 3	-0,087	-0,016 to 0,006	0,391			
Δ BMI	-0,008	-0,019 to 0,018	0,937			
HR						
Day 1	0,063	-0,002 to 0,004	0,533			
Day 3	-0,078	-0,008 to 0,004	0,438			
Δ HR	0,013	-0,001 to 0,001	0,899			
SBP						
Day 1	0,102	-0,001 to 0,004	0,314			
Day 3	0,098	-0,002 to 0,006	0,333			
Δ SBP	0,207	0,000 to 0,003	0,039			
Log NTproBNP						
Day 1	0,481	0,267 to 0,574	<0,001	0,400	0,185 to 0,513	<0,001
Day 3	0,486	0,218 to 0,464	<0,001	0,381	0,146 to 0,389	<0,001
Δ Log NTproBNP	0,267	0,044 to 0,276	0,007	-	-	-
Log Albuminuria						

Day 1	0,131	-0,035 to 0,172	0,193			
Day 3	0,220	0,012 to 0,203	0,028	0,088	-0,041 to 0,128	0,311
Δ Log Albuminuria	0,099	-0,038 to 0,113	0,325			
Log eGFR						
Day 1	-0,275	-1,231 to -0,216	0,006	0,165	-0,503 to 1,372	0,360
Day 3	-0,399	-1,232 to -0,455	<0,001	0,034	-0,812 to 0,957	0,870
Δ Log eGFR	0,068	0,203 to 0,413	0,502			
Log pCr						
Day 1	0,345	0,500 to 1,704	<0,001	0,270	-0,224 to 1,951	0,118
Day 3	0,439	0,630 to 1,503	<0,001	0,256	-0,393 to 1,641	0,226
Δ Log pCr	-0,040	-0,443 to 0,297	0,696			
pUrea						
Day 1	0,309	0,002 to 0,009	0,002	0,116	-0,002 to 0,007	0,342
Day 3	0,382	0,003 to 0,008	<0,001	0,121	-0,002 to 0,005	0,335
Δ pUrea	-0,172	-0,003 to 0,000	0,087			
Albumin at Day 1	-0,049	-0,099 to 0,060	0,626			
Hemoglobin at Day 1	0,076	-0,009 to 0,021	0,451			

Day 1 values are compared with day 1 hsTnT; day 3 values are compared with day 3 hsTnT; Δ, age, sex, DM, HgBA1c, LVEF, ischemic HF, and medications are compared with changes (Δ) in hsTnT between day 1 and day 3 (day 3 – day 1).

Legend: DM = diabetes mellitus; HgBA1c = glycated hemoglobin; LVEF = left ventricular ejection fraction; HF = heart failure; ACEi = angiotensin converting enzyme inhibitors; BMI = body mass index; HR = heart rate; SBP = systolic blood pressure; NTproBNP = N-terminal pro brain natriuretic peptide; hsTnT = high sensitivity troponin T; eGFR = estimated glomerular filtration rate; pCr = plasma creatinine; pUrea = plasma urea; Δ = changes between day 3 and day 1 (day 3 – day 1).

Table 5. Determinants of hsTnT Dichotomic Changes

	hsTnT		
	Decrease (n = 63)	Increase (= 37)	p Value
Age (years)	75,94 ± 11,92	76,11 ± 8,97	0,940
Male Sex – no. (%)	22 (34,9)	17 (45,9)	0,275**
DM – no. (%)	24 (38,1)	21 (56,8)	0,070**
HGA1c (%)	6,93 ± 0,94	7,13 ± 1,00	0,475
Sleep Apnea – no. (%)	7 (36,8)	11 (44)	0,632**
NIV – no. (%)	10 (15,9)	7 (18,9)	0,695**
IHD – no. (%)	32 (50,8)	18 (48,6)	0,836**
AF – no. (%)	31 (49,2)	28 (75,7)	0,009**
LVEF (%)	43,37 ± 12,68	43,62 ± 10,08	0,917
LVEF ≥ 40% - no. (%)	41 (65,1)	26 (70,3)	0,594**
HgB (g/dL)	12,32 ± 1,95	12,62 ± 2,28	0,478
Albumin (mg/dL)	3,68 ± 0,41	3,67 ± 0,39	0,924
Δ BMI (Kg/m^2)	-1,08 ± 1,70	-1,10 ± 1,76	0,964
Δ HR (bpm)	-17,05 ± 20,71	-17,57 ± 29,15	0,917
Δ SBP (mmHg)	-18,56 ± 23,32	-16,57 ± 27,63	0,702
Δ pCr (mg/dL)	0,03 [-0,1 to 0,18]	0,02 [-0,06 to 0,11]	0,803*
Δ eGFR ($ml/min/1,73m^2$)	-2,0 [-9,0 to 7,0]	-1,0 [-11,0 to 6,0]	0,937*
Δ pUrea (mg/dL)	7,40 ± 20,59	6,59 ± 20,65	0,851
Δ NTproBNP (pg/mL)	-1167 [-2337 to -367]	-379 [-1273 to 319,5]	0,003*
Δ hsTnT (ng/mL)	-0,004 [-0,014 to -0,001]	0,004 [0,002 to 0,009]	<0,001*
Δ Albuminuria (mg/g)	-6,10 [-38,50 to 2,40]	-23,70 [-90,75 to 11,05]	0,337*
IV Furosemide at Day 1 (mg)	78,83 ± 21,61	74,05 ± 21,53	0,537
IV Furosemide Dose Maintenance or Increase at Day 3 – no. (%)	9 (14,3)	7 (18,9)	0,542**
ACEi – no. (%)	30 (47,6)	14 (37,8)	0,341**
Beta Blocker – no (%)	22 (34,9)	15 (40,5)	0,574**
Spironolactone – no. (%)	30 (47,6)	20 (54,1)	0,534**
Length of Stay (days)	8,0 [6,0 to 11,0]	9,0 [7,0 to 12,0]	0,033*

Continuous variables are presented as mean value ± standard deviation [SD], p value or median [inter-quartile range, IQR], p value. Categorical variables are presented as absolute number (%), p value.

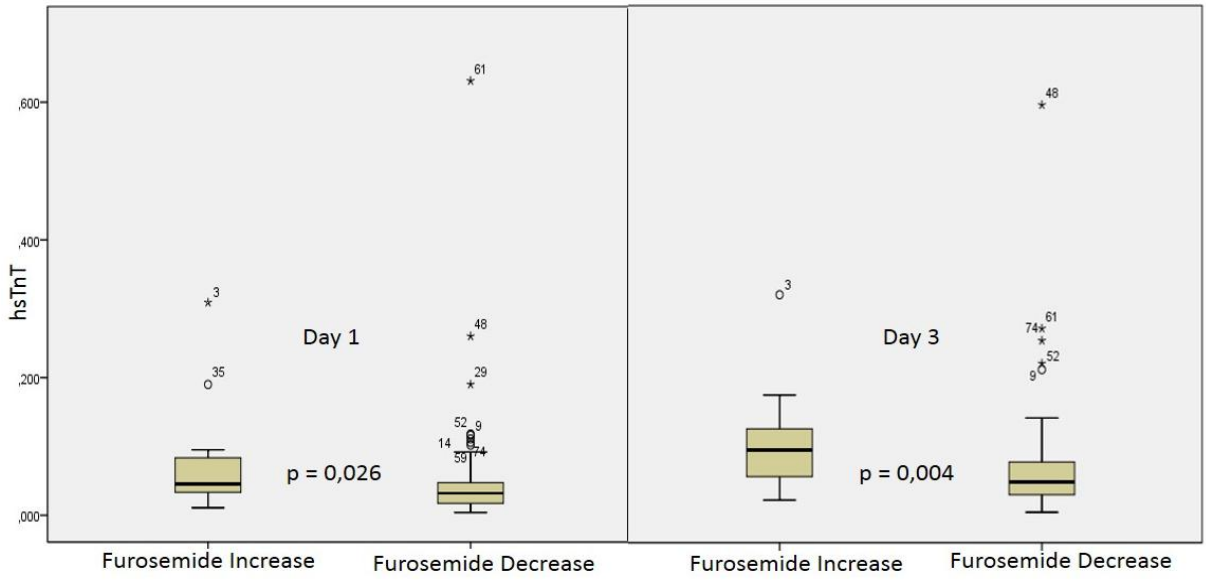
*Non-parametric paired sample test; ** Chi-square test.

DM = diabetes mellitus; HgBA1c = glycated hemoglobin; NIV = non-invasive ventilation; IHD = ischemic heart disease; AF = atrial fibrillation; HgB = hemoglobin; BMI = body mass index; HR = heart rate; SBP = systolic blood pressure; eGFR = estimated glomerular filtration rate; pCr = plasma creatinine; pUrea = plasma urea;

NTproBNP = N-terminal pro brain natriuretic peptide; hsTnT = high sensitivity troponin T; IV = intra-venous; ACEi = angiotensin converting enzyme inhibitors; Δ = changes between day 3 and day 1 (day 3 – day 1).

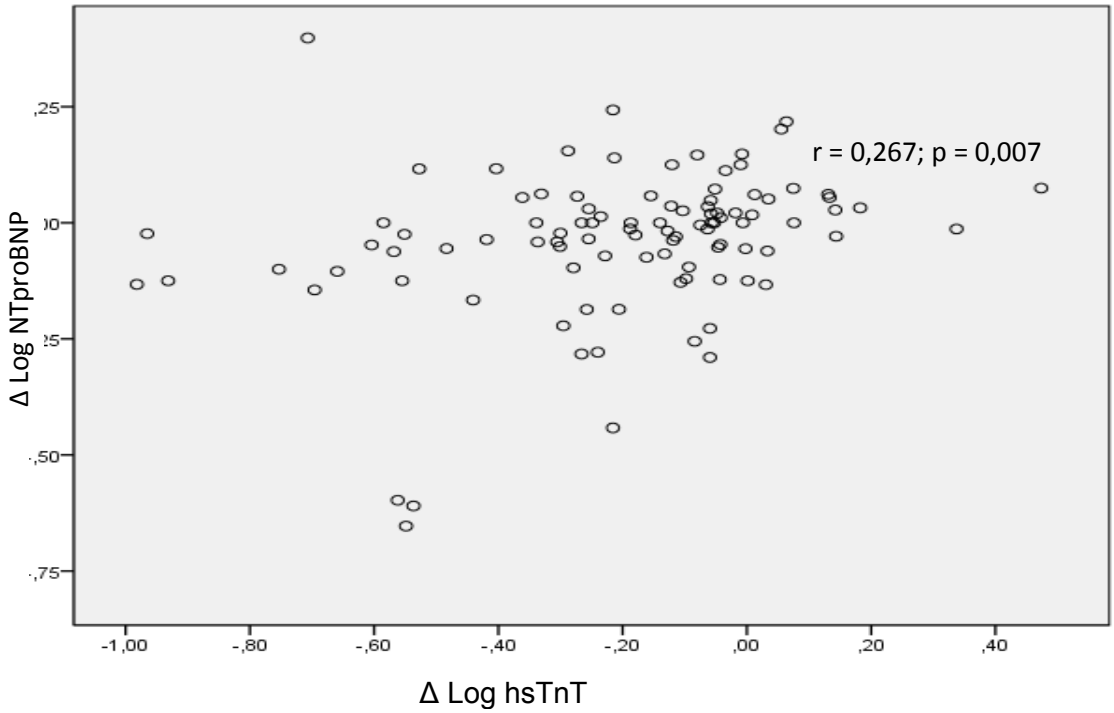
Figures

Figure 1. Differences in hsTnT Between Faster Diuretic Responders and Slower Diuretic Responders at Day 1 and Day 3



Legend: hsTnT = high sensitivity troponin T (ng/mL)

Figure 2. Correlation Between Δ Log hsTnT and Δ Log NT-pro BNP



Legend: hsTnT = high sensitivity troponin T (ng/mL); NT-pro BNP = N-terminal pro-brain natriuretic peptide (pg/mL); Δ = changes between day 3 and day 1 (day 3 – day 1).

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VI. Urinary Sodium to Potassium Ratio: Biomarker of Mineralocorticoid Receptor Antagonism in Decompensated Heart Failure

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Abstract

Acutely decompensated heart failure (ADHF) is characterized by abnormal neurohormonal activation. Reliable biomarkers are warranted to establish the activity of mineralocorticoid receptor antagonism (MRA). Urinary sodium to potassium (uNa/K) ratio is a candidate biomarker of MRA. The aim of the present study is to examine the uNa/K ratio as a potential biomarker of MRA in ADHF patients.

This was a retrospective secondary analysis of a study including 100 patients with ADHF. In that study 50 patients were submitted to spironolactone treatment. Baseline log renin was negatively correlated with log uNa/K ratio (p=0,001), in control group, day 3 log aldosterone and log renin were also negatively correlated with log uNa/K ratio (both, p<0,05). Higher levels of aldosterone, renin, and uNa/K ratio were observed in patients who were submitted to spironolactone treatment (all, p<0,05), and log aldosterone and log renin were correlated in this group (p=0,011). The variations (day 3 – baseline) in the levels of aldosterone and renin were both correlated to the variation in uNa/K ratio levels (p<0,05). Furthermore, uNa/K ratio showed a fair accuracy for spironolactone use (AUC=0,657).

Our pilot study results support the use of uNa/K ratio as a surrogate marker of MRA.

Key-words: mineralocorticoid receptor antagonism; biomarkers; urinary sodium to potassium ratio.

Introduction

The pathophysiology of acutely decompensated heart failure (ADHF) is characterized by abnormal neurohormonal activation, including the renin-angiotensin-aldosterone system (RAAS). A consequence of the perpetual RAAS overactivation is the increase of aldosterone. This adrenal hormone acts on the epithelium of distal nephron promoting the luminal secretion of potassium and the reabsorption of sodium. Beyond this physiological role, by acting on the mineralocorticoid receptor (MR) that is widespread expressed in several non-renal tissues, aldosterone promotes fibrosis, increases oxidative stress and apoptosis, amongst others deleterious mechanisms. Neurohormonal modulation, including angiotensin-enzyme-converting inhibitors (ACEi), angiotensin II receptor blockers (ARB) and mineralocorticoid receptor antagonism (MRA), had demonstrated significant morbidity and mortality reductions in chronic heart failure (HF) and post-myocardial infarction patients (MI)[1-3]. In these patients, higher aldosterone and renin levels were associated with poor outcomes[4,5]. Regarding the acute setting, adding high dose spironolactone to standard ADHF therapy as shown to be safe and likely to induce greater congestion relief translated into a more pronounced natriuretic peptide reduction[6]. In agreement, quantifying plasma levels of different neurohormones (NH) have been proposed for risk stratification of patients with HF[7,8]. Although the pharmacological blockade of RAAS is one of the mainstays of HF treatment, a suboptimal neurohormonal inhibition is still a critical issue in standard practice. Measuring the activation and the effective blockade of RAAS would help us to enhance its inhibition and to tailor these therapies to the individual HF patients[9]. Thus reliable and practical biomarkers are warranted to establish the RAAS blockade in clinical practice[10]. Taking advantage of the renal physiological action of aldosterone, the urinary sodium to potassium (uNa/K) ratio is an interesting candidate biomarker of RAAS modulation and MRA[10].

The aim of the present study is to examine the uNa/K ratio as a potential biomarker of MRA in ADHF patients.

Methods

Study Design

This study is based on analysed data from a previous prospective, interventional, clinical trial that we performed[6]. In that study we enrolled 100 consecutive patients who presented in a Portuguese tertiary hospital with ADCHF, between February 2012 and February 2013. They were non-randomly assigned in a sequential 1:1 ratio to spironolactone plus standard ADCHF therapy or standard ADCHF therapy alone, 50 patients within each arm (*i.e*. patients were alternatively

assigned to spironolactone arm or standard ADCHF therapy arm in a sequential manner - the first patient to one arm and the next to the other arm. This sequence was repeated until we reach 100 patients, 50 patients within spironolactone group and 50 patients within control group). Patients were blinded to the allocation, and the clinicians were not blinded to the allocation. The recommended spironolactone dose was 100 mg/day, however the assistant physician could decrease the spironolactone dose to 50 mg/day after 48h upon admission. After 72h the study was open label. Furosemide dose and form of administration was liberal.

Patients were eligible for enrollment if they presented with decompensation of chronic HF with symptoms leading to hospitalization. ADHF was diagnosed on the basis of the presence of history of chronic HF and at least one symptom (dyspnea, orthopnea, or edema) and one sign (rales, peripheral edema, ascites, or pulmonary vascular congestion on chest radiography). Exclusion criteria were: chronic use of MRAs, cardiac surgery within 60 days of enrollment, cardiac mechanical support, cardiac resynchronization-therapy within the last 60 days, comorbid conditions with an expected survival of less than 6 months, acute MI at time of hospitalization, hemodynamically significant uncorrected primary cardiac valvular disease, patients requiring intravenous vasodilators or inotropic agents, supine systolic arterial blood pressure <90 mmHg, plasma creatinine (pCr) level >1,5 mg/dL, serum potassium level >5,0 mmol/L, hemoglobin (HgB) level <9 g/dL, and sepsis.

Institutional review board or ethics committee approval was obtained. All patients provided written informed consent to participate in the study.

Study assessments

Patient`s clinical status including physical examination and was prospectively recorded by the same assistant physician at day 1 and day 3.

Medications and respective dosages were prospectively recorded by the investigators according to the assistant physician prescriptions.

Blood and spot urine samples were collected in the first 24 hours (h) after admission (day 1) of the patient to the hospital. The first dose of spironolactone was only administered after samples collection. Fifty patients had daily oral spironolactone according to the study protocol described above. The day 3 samples were collected between 72 and 96 h of hospitalization. All samples were collected in the morning with the patient in supine position, and first-morning spot urine was used. All patients had low-salt, low-calorie hospital diet. Extra fruit and vegetables administration was not allowed. An assessment of biomarkers (including pCr, plasma urea [pUr], electrolytes, N-terminal pro-brain natriuretic peptide [NTproBNP], high-sensitivity troponin T [hsTnT]

and proteinuria) was performed at a central core laboratory at day 1 and day 3. Clinical assessment and routine analyses were performed daily during hospital stay. All patients performed a transthoracic echocardiography within 72 hours upon admission. Left ventricle ejection fraction (EF) was calculated according to biplane Simpson method.

Aldosterone was measured using radioimmunoassay (RIA) Coat-a-Count® (Siemens) and renin with RIA (DiaSource).

Variable definitions

We studied aldosterone (ng/dL) and renin (pg/mL) and their variation (delta) regarding the following covariates: age; sex; diabetes mellitus (DM); ischemic HF; EF (%); systolic blood pressure (SBP); intravenous (IV) furosemide dose; proportion of patients with furosemide dose reduction or switch to oral route from baseline to day 3; proportion of patients on angiotensin converting enzyme inhibitors (ACEi), angiotensin receptor blockers (ARB), beta-blockers (BB), and spironolactone; pCr (mg/dL), pUr (mg/dL), NTproBNP (pg/mL), hsTnT (ng/mL), sodium (mmol/L), potassium (mmol/L), uNa/K ratio, proteinuria (g/g) and HgB (g/dL).

Statistical Analysis

Normally distributed continuous variables are expressed as mean ± standard deviation (SD), and skewed distributions are presented as median [inter-quartile range, IQR].

Because of the positively skewed distributions of aldosterone, renin, pCr, proteinuria, RDW, NTproBNP, hsTnT and uNa/K ratio, these variables were log transformed for analysis.

Categorical variables are expressed in proportions (%).

Comparison between groups was performed using parametric, non-parametric tests, or chi-square tests, as appropriate. Significant association was defined by a probability (p) value ≤0,05.

Correlations of log aldosterone and log renin with the mentioned variables were first examined by single variable linear or logistic regression and presented as non-adjusted coefficient (NAC) and 95% confidence interval [$_{95\%}$CI]. Factors with a p value ≤0,05 by single variable regression analyses were included in a multivariable linear regression model, presented as adjusted coefficient (AC) [$_{95\%}$CI].

Statistical analysis was performed using SPSS software (version 19, Chicago, IL, USA).

Results

Baseline Characteristics, Medications and Lab Results

Mean ± SD age of the 100 patients admitted due to ADHF was 76,0 ± 10,9 years. Thirty-nine (39%) patients were male; 50 patients had documented ischemic heart disease (IHD); 32 had EF < 40%; spironolactone was administered to 50 patients starting on the admission day.

Patient characteristics, and comparison of systolic blood pressure (SBP), lab results and medication dosage between admission day (day 1) and the third day of inpatient treatment is shown in Table 1.

Plasma urea, proteinuria, NTproBNP and hsTnT reduced between day 1 and day 3 (all, $p < 0,05$). Furosemide dose was reduced from day 1 to day 3 (75,80 ± 21,52 vs. 67,57 ± 25,54, $p < 0,001$). Spironolactone dose was also reduced according to study protocol (94,50 ± 23,31 vs. 62,74 ± 24,33). The proportion of patients on ACEi/ARB, BB and oral furosemide increased from day 1 to day 3 (all, $p < 0,05$). Renin and aldosterone increased during the study period (these results were not compared because day 1 samples were all collected before spironolactone administration and at day 3 fifty patients were on spironolactone). No changes were found between day 1 and day 3 regarding pCr and uNa/K ratio – Table 1.

Day 1 Aldosterone and Renin Correlations

Day 1 log aldosterone was positively correlated with ischemic HF (3,029 [1,197 to 7,668], $p = 0,019$), pUr (0,231 [0,001 to 0,009], $p = 0,021$), and log proteinuria (0,215 [0,021 to 0,443], $p = 0,031$). These associations remained significant after adjustment – Table 2.

Day 1 log renin was negatively correlated with log uNa/K ratio (-0,332 [-0,671 to -0,184], $p=0,001$) and SBP (-0,223 [-0,008 to -0,001], $p = 0,026$). Correlation was positive with ischemic HF (2,743 [1,150 to 6,542], $p=0,023$). After adjustment log renin remained significantly correlated with log uNa/K ratio and SBP – Table 2.

Aldosterone, Renin, and uNa/K ratio Changes

Previous to spironolactone administration (day 1) log aldosterone levels did not differ between groups, although a trend to higher log aldosterone levels was observed in control group (1,484 ± 0,436 vs. 1,651 ± 0,446, $p=0,062$), this tendency was inverted after 3 days of spironolactone use (1,789 ± 0,461 vs. 1,654 ± 0,500, $p=0,166$) with higher levels of aldosterone in spironolactone group and a significant variation in the log aldosterone levels (0,305 ± 0,484 vs. 0,004 ± 0,432, $p=0,001$) – Table 3.

At day 1 log renin levels were not significantly different between groups, but in this case higher log renin levels were observed in spironolactone group (0,801 ± 0,491 vs. 0,625 ± 0,475, $p=0,071$), these differences were accentuated after spironolactone

administration (0,934 ± 0,547 vs. 0,704 ± 0,454, p=0,025) – Table 3. A similar scenario was found in uNa/K ratio analysis with significant differences being achieved after spironolactone administration (0,487 ± 0,394 vs. 0,312 ± 0,370, p=0,024) – Table 3.

Day 3 Aldosterone and Renin Correlations Within Spironolactone and Control Groups

Within control group, both day 3 log aldosterone and log renin were correlated with log uNa/K ratio (-0,450 [-0,955 to -0,257], p = 0,001 and -0,347 [-0,756 to 0,091], p = 0,014, respectively) – Table 4. and Figure 1; log aldosterone was also associated with log pUr (0,302 [0,069 to 1,588], p = 0,033) – Table 4., and log renin was correlated to log pCr (0,297 [0,062 to 1,750], p = 0,036), and negatively correlated to beta-blocker use (-0,290 [-0,512 to -0,011], p = 0,041) – Table 4.

Within spironolactone group log aldosterone and log renin were correlated (0,359 [0,074 to 0,530], p = 0,011) – Table 4.; log aldosterone was also positively correlated with furosemide dose reduction (0,334 [0,142 to 1,415], p = 0,018), and log renin was negatively correlated with serum sodium (-0,337 [-0,094 to -0,010], p = 0,017) – Table 4.

Associations Between Delta (Δ) Log Renin, Δ Log Aldosterone and Δ Log UNa/K ratio

Changes (day 3 – day 1) in log aldosterone and log renin were correlated with the changes in log uNa/K ratio within spironolactone group (-0,288 [-0,518 to -0,009], p=0,042 and -0,512 [-0,740 to -0,256], p <0,001, respectively) – Table 5.

Na/K ratio: Receiver Operating Curve Analysis

Na/K ratio showed a sensitivity of 86% and a specificity of 44% (area under curve [AUC], 0,657 [0,550 to 0,765], p = 0,007) in predicting spironolactone use – Figure 2.

Comparison Between Spironolactone and Control Groups Regarding Potential Confounders

No differences were observed regarding SBP, serum sodium and potassium, ACEi/ARB and beta-blocker use, and i.v. furosemide dose. A greater proportion of patients were on oral furosemide at day 3 within spironolactone group (22 vs. 41, p < 0,001).

Discussion

The present study tested if uNa/K ratio was independently associated with plasma levels of aldosterone and renin, and if the levels of these biomarkers changed with the introduction of spironolactone in a cohort of patients with ADHF, half of them treated with spironolactone. Renin, aldosterone and uNa/K ratio levels increased in the

group of patients submitted to spironolactone treatment. In this group of patients, the variation in aldosterone and renin levels from baseline to day 3 were correlated with the variation in the uNa/K ratio levels. In addition, uNa/K ratio showed a fair accuracy for spironolactone use. Taken together, our results support the potential usefulness of this simple urinary measure as biomarker of MRA.

Aldosterone regulates sodium balance by increasing the expression of the epithelial Na^+ channel and the Na^+/K^+-ATPase pump found on the distal nephron epithelial cells[11], promoting reabsorption of Na^+ and the excretion of K^+[12]. Previous reports had indicated a measurable effect of MRA on uNa/K ratio consistent with effects of aldosterone on electrolyte balance[13,14]. Mineralocorticoid receptor blockade elicited an increase in uNa/K ratio in rats in a dose-dependent manner[10], supporting the use of uNa/K ratio as a translatable biomarker of MRA with the potential to enable dose selection for clinical trials[10]. In our study, renin was negatively associated with uNa/K ratio at day 1, and at day 3 both renin and aldosterone were independently associated with uNa/K ratio in the group of patients not submitted to spironolactone treatment. These findings are consistent with the RAAS effects on distal nephron, i.e. higher levels of renin and aldosterone lead to higher Na^+ reabsorption and K^+ excretion, decreasing uNa/K ratio[15]. In addition, we also observed an increase of uNa/K ratio in the group of spironolactone-treated patients, despite the higher increase in aldosterone and renin levels in this group of patients, suggesting a compensatory response to MRA[10,16,17]. These observations were supported by the significant correlation between the variation (day 3 – baseline) in aldosterone and renin levels with the variation in the uNa/K ratio levels. Together, these dynamic changes demonstrate an effective MRA by spironolactone and suggest that uNa/K ratio can serve as a surrogate biomarker for MRA. No significant differences between groups (spironolactone vs. control) were observed regarding ACEi/ARB use, IV furosemide dose, SBP, plasma potassium, serum sodium or diet, therefore it is likely that the observed dynamic changes were due to the spironolactone effect. Noteworthy, a higher proportion of patients on oral furosemide at day 3 was observed in spironolactone group, potentially translating a faster diuretic response within this group[18], however the less aggressive diuretic strategy would theoretically decrease urinary sodium excretion leading to a lower uNa/K, yet this was not observed in our study, by the contrary, spironolactone treated patients had higher uNa/K ratio corroborating that the observed dynamic changes in uNa/K ratio were driven by MRA effect.

Our preliminary results, are the first to show the potential usefulness of uNa/K as a surrogate marker for MRA. The implications of these observations have the

potential to be translated into clinical trials and also to daily practice, helping in the MRA dose titration and clinical effect monitorization.

In our study uNa/K ratio has demonstrated to have a fair accuracy in determining spironolactone use, with patients on spironolactone having higher uNa/K ratio (high sensitivity), but the test has not demonstrated to be accurate in excluding patients without spironolactone treatment (low specificity). Whether the accuracy of the test can be increased with higher MRA dose or tighter potential confounder control (in a randomized and controlled trial) needs to be determined in future studies. However we find our results very encouraging towards the use of this inexpensive and readily available biomarker.

We validated uNa/K ratio against aldosterone and renin plasma concentrations. Although there are some caveats in the interpretation of these tests, they are recommended and used in standard clinical practice to screen and diagnose diseases associated to increased SRAA activity. The median aldosterone and renin levels found in our study were higher than the median values found in previous reports of patients with chronic HF[4,5,7,19] and acute HF[9]. This finding may be explained by the lower proportion (only 44%) of the patients on baseline ACEi/ARB, potentially leading to higher levels of renin and aldosterone[19,20], similar to the levels found before the widespread use of the RAAS inhibitors[7,8]. MRA induced an increase in serum aldosterone and renin levels. These findings are concordant with previous reports in which the physiological elevation in plasma renin activity (PRA) and aldosterone were demonstrated in response to eplerenone and spironolactone treatment[10,16,17,21,22].

Our study has several limitations that need to be considered. First, it was a single-centre of a small sample size study. Second, diuretic therapy is could independently increase both renin and aldosterone plasma concentrations and uNa/K ratio. However, this would attenuate the inverse correlation between these variables that we found. Third, the plasma renin and aldosterone measure the circulating RAAS ativity, while uNa/K ratio is influenced by systemic and intra-renal SRAA activity. Fourth, whether the accuracy of uNa/K ratio as a biomarker for MRA use can be increased with higher MRA doses or tighter variable control is yet to be determined. Finally, our inclusion criteria by restricting the enrolment of patients with hyperkalemia, severe impaired renal function limit the external validity of our conclusions.

Conclusions

In an ADHF patients cohort, we demonstrated that uNa/K ratio is independently associated with renin and aldosterone at different time points and is also influenced by MRA therapy. Therefore, our findings disclose the potential role of uNa/K

ratio as a non-invasive and inexpensive biomarker of MRA therapy in acute HF patients. Nevertheless, how clinical management will be tailored according to the activation level of the RAAS is still unclear.

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Disclosures

The authors have no conflicts of interest to disclose.

Tables

Table 1. Population Characteristics, Laboratory Results and Spironolactone Dose at Admission Day (Day 1) and Day 3

Age (yrs)	76,0 ± 10,88		
Male Sex – %	39		
DM - %	45		
Ischemic HF - %	50		
EF < 40 - %	32		
Hemoglobin (g/dL)	12,43 ± 2,07		
	Day 1	Day 3	p Value
SBP (mmHg)	139,79 ± 25,86	121,97 ± 16,2	< 0,001
IV Furosemide - %	100	37	< 0,001**
IV Furosemide Dose (mg/d)	75,80 ± 21,52	67,57 ± 25,54	0,001
Oral Furosemide - %	0	63	-
Oral Furosemide Dose (mg/d)	0	74,6 ± 28,1	-
ACEi - %	44	61	< 0,001**
Ramipril Eq. Dose (mg/d)	3,15 ± 2,04	3,36 ± 2,14	0,474
Beta-Blocker - %	37	57	< 0,001**
Bisoprolol Eq. Dose (mg/d)	3,01 ± 1,08	2,96 ± 1,89	0,474
Spironolactone - %	50	50	1
Spironolactone Dose (mg/d)	94,50 ± 23,31	62,74 ± 24,33	< 0,001
Plasma Creatinine (mg/dL)	1,04 [0,89 – 1,31]	1,06 [0,85 – 1,40]	0,082*
Plasma Urea (mg/dL)	55,21 ± 20,84	62,3 ± 25,47	0,001
Plasma Potassium (mmol/L)	4,03 ± 0,51	4,04 ± 0,54	0,950
Serum Sodium (mmol/L)	140,54 ± 4,38	140,68 ± 3,95	0,72
Proteinuria (g/g)	0,289 [0,188 – 0,629]	0,299 [0,160 – 0,970]	0,045*
NTproBNP (pg/mL)	2750 [1672 – 6032]	1835 [902 – 3837]	< 0,001*
UNa/K ratio	3,04 [1,52 – 5,76]	2,80 [1,50 – 4,78]	0,341*
Renin (pg/mL)	4,35 [2,30 – 10,78]	5,34 [3,14 – 16,30]	- **
Aldosterone (ng/dL)	35,0 [12,0 – 92,5]	67,0 [21,3 – 125,0]	- **
hsTnT (ng/mL)	0,033 [0,019 – 0,050]	0,030 [0,018 – 0,051]	0,039*

Continuous variables are presented as mean value ± standard deviation [SD], p value or median [inter-quartile range, IQR], p value. Categorical variables are presented as absolute number (%), p value.

*Non-parametric paired sample test.

**These results were not compared because day 1 samples were all collected before spironolactone administration and at day 3 fifty patients were on spironolactone.

Note: day 1 analysis were collected before spironolactone administration.

Legend: DM = diabetes mellitus; HF = heart failure; EF = ejection fraction; ACEi/ARB = angiotensin converting enzyme inhibitors/angiotensin receptor blockers; NTproBNP = N-terminal pro brain natriuretic peptide; UNa/K = urinary sodium to potassium; hsTnT = high sensitivity troponin T.

Table 2. Day 1 Log Aldosterone and Log Renin Associations

	NAC for Log Aldosterone	95%CI	p Value	AC for Log Aldosterone	95%CI	p Value
Age	0,102	-0,004 to 0,120	0,311			
Male Sex	0,458	-0,180 to 1,162	0,100			
DM	0,965	-0,398 to 2,342	0,938			
EF <40%	0,515	-0,194 to 1,370	0,184			
Ischemic HF	3,029	1,197 to 7,668	0,019	0,259	0,068 to 0,393	0,006
SBP	-0,154	-0,006 to 0,001	0,126			
IV Furosemide Dose	0,096	-0,002 to 0,006	0,340			
Beta Blocker	1,690	-0,669 to 4,271	0,267			
ACEi/ARB	1,088	-0,455 to 2,659	0,853			
Spironolactone	-0,187	-0,342 to 0,008	0,062			
Log Creatinine	0,150	-0,170 to 1,310	0,130			
Urea	0,231	0,001 to 0,009	0,021	0,200	0,000 to 0,008	0,034
Potassium	0,145	-0,047 to 0,303	0,149			
Sodium	-0,011	-0,032 to 0,009	0,267			
Log Proteinuria	0,215	0,021 to 0,443	0,031	0,202	0,020 to 0,416	0,031
Log NTproBNP	0,189	-0,008 to 0,397	0,059			
Log UNa/K ratio	-0,180	-0,440 to 0,020	0,073			
Log Renin	0,156	-0,039 to 0,323	0,122			
Log hsTnT	-0,032	-0,273 to 0,198	0,754			
Hemoglobin	0,087	-0,024 to 0,062	0,388			
	NAC for Log Renin	95%CI	p Value	AC for Log Renin	95%CI	p Value
Age	0,001	-0,008 to 0,010	0,804			
Male Sex	1,217	-0,533 to 2,777	0,642			
DM	0,634	-0,277 to 1,449	0,280			
EF <40%	0,691	-0,283 to 1,685	0,416			
Ischemic HF	2,743	1,150 to 6,542	0,023	0,173	-0,010 to 0,347	0,064
SBP	-0,223	-0,008 to -0,001	0,026	-0,232	-0,008 to -0,001	0,013
IV Furosemide Dose	-0,09	-0,002 to 0,007	0,372			
Beta Blocker	0,639	-0,270 to 1,514	0,309			
ACEi/ARB	1,100	-0,487 to 2,488	0,819			
Spironolactone	0,181	-0,016 to 0,368	0,071			
Log Creatinine	0,108	-0,377 to 1,263	0,286			
Urea	0,169	-0,001 to 0,009	0,092			
Potassium	-0,100	-0,289 to 0,096	0,322			
Sodium	-0,034	-0,026 to 0,019	0,740			
Log Proteinuria	-0,064	-0,312 to 0,160	0,525			
Log NTproBNP	-0,038	-0,268 to 0,183	0,709			
Log UNa/K ratio	-0,332	-0,671 to -0,184	0,001	-0,322	-0,651 to -0,178	0,001
Log	0,156	-0,039 to 0,323	0,122			

Aldosterone						
Log hsTnT	-0,031	-0,055 to 0,040	0,759			
Hemoglobin	-0,031	-0,055 to 0,040	0,759			

Note: day 1 analysis were collected before spironolactone administration.
Legend: NAC = non-adjusted coefficient; AC = adjusted coefficient; DM = diabetes mellitus; LVEF = left ventricular ejection fraction; HF = heart failure; ACEi/ARB = angiotensin converting enzyme inhibitors/angiotensin receptor blockers; NTproBNP = N-terminal pro brain natriuretic peptide; UNa/K = urinary sodium to potassium; hsTnT = high sensitivity troponin T.

Table 3. Comparison Between Groups (Spironolactone vs. No Spironolactone) at Day 1 and Day 3

	Spironolactone	No Spironolactone	p Value*
Log Aldosterone			
Day 1	1,484 ± 0,436	1,651 ± 0,446	0,062
Day 3	1,789 ± 0,461	1,654 ± 0,500	0,166
ΔLog Aldosterone	0,305 ± 0,484	0,004 ± 0,432	0,001
Log Renin			
Day 1	0,801 ± 0,491	0,625 ± 0,475	0,071
Day 3	0,934 ± 0,547	0,704 ± 0,454	0,025
ΔLog Renin	0,133 ± 0,513	0,079 ± 0,309	0,531
Log UNa/K ratio			
Day 1	0,477 ± 0,407	0,409 ± 0,350	0,374
Day 3	0,487 ± 0,394	0,312 ± 0,372	0,024
ΔLog UNa/K ratio	0,010 ± 0,528	-0,097 ± 0,401	0,255

*Independent sample T test.
Note: day 1 analysis were collected before spironolactone administration.
Legend: UNa/K, urinary sodium to potassium; Δ, variation.

Table 4. Day 3 Log Aldosterone and Log Renin Associations Within Spironolactone and Control Groups

	Spironolactone Group			Control Group		
	NAC for Log Aldosterone	95%CI	p Value	NAC for Log Aldosterone	95%CI	p Value
SBP	-0,157	-0,012 to 0,004	0,277	-0,063	-0,011 to 0,007	0,685
Furosemide Reduction	0,334	0,142 to 1,415	0,018	-0,082	-0,409 to 0,229	0,572
Beta Blocker	0,195	-0,083 to 0,447	0,174	-0,015	-0,303 to 0,274	0,920
ACEi/ARB	0,123	-0,155 to 0,386	0,394	-0,166	-0,457 to 0,121	0,248
Log Creatinine	0,021	-0,543 to 0,992	0,559	0,255	-0,086 to 1,798	0,074
Urea	0,085	-0,940 to 1,090	0,883	0,302	0,069 to 1,588	0,033
Potassium	0,088	-0,174 to 0,327	0,542	0,067	-0,205 to 0,328	0,646
Sodium	0,011	-0,036 to 0,039	0,939	0,080	-0,024 to 0,043	0,581
Log Proteinuria	-0,031	-0,425 to 0,344	0,832	0,103	0,237 to 0,499	0,477
Log NTproBNP	-0,081	-0,351 to 0,197	0,575	-0,107	-0,398 to 0,183	0,460
Log UNa/K ratio	-0,006	0,347 to 0,332	0,964	-0,450	-0,955 to -0,257	0,001
Log Renin	0,359	0,074 to 0,530	0,011	0,189	-0,105 to 0,522	0,188
Log hsTnT	0,039	-0,348 to 0,457	0,787	0,053	-0,327 to 0,473	0,715
	NAC for Log Renin	95%CI	p Value	NAC for Log Renin	95%CI	p Value
SBP	-0,010	-0,010 to 0,005	0,944	-0,079	-0,011 to 0,006	0,587
Furosemide Reduction	-0,091	-1,051 to 0,546	0,528	-0,213	-0,497 to 0,070	0,137
Beta Blocker	-0,148	-0,480 to 0,154	0,307	-0,290	-0,512 to -0,011	0,041
ACEi/ARB	-0,106	-0,440 to 0,204	0,465	-0,073	-0,333 to 0,198	0,613
Log Creatinine	0,102	-0,777 to 1,621	0,483	0,297	0,062 to 1,750	0,036
Urea	0,162	-0,391 to 1,414	0,260	0,253	-0,070 to 1,330	0,077
Potassium	0,070	-0,225 to 0,370	0,628	0,166	-0,100 to 0,378	0,249
Sodium	-0,337	-0,094 to -0,010	0,017	-0,006	-0,031 to 0,030	0,969
Log Proteinuria	-0,246	-0,830 to 0,055	0,084	-0,133	-0,487 to 0,180	0,359
Log NTproBNP	-0,028	-0,358 to 0,295	0,848	0,065	-0,205 to 0,324	0,655
Log UNa/K ratio	-0,172	-0,636 to 0,158	0,232	-0,347	-0,756 to 0,091	0,014
Log Aldosterone	0,359	0,074 to 0,530	0,011	0,189	-0,105 to 0,522	0,188
Log hsTnT	-0,065	-0,584 to 0,371	0,656	-0,002	-0,366 to 0,362	0,991

Legend: SBP, systolic blood pressure; IV, intra venous; ACEi/ARB, angiotensin converting enzyme inhibitors/angiotensin receptor blockers; NTproBNP, N-terminal pro brain natriuretic peptide; UNa/K, urinary sodium to potassium; hsTnT = high sensitivity troponin T.

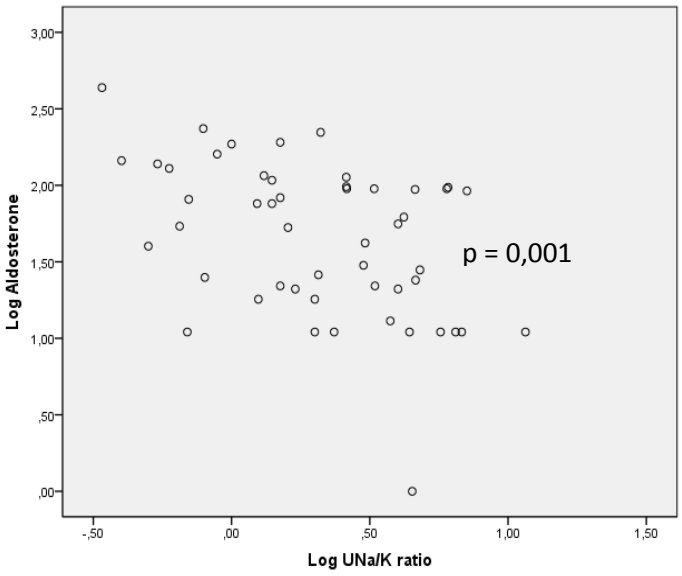
Table 5. Associations Between Delta (Δ) Log Renin, Δ Log Aldosterone and Δ Log UNa/K ratio Within Spironolactone and Control Groups

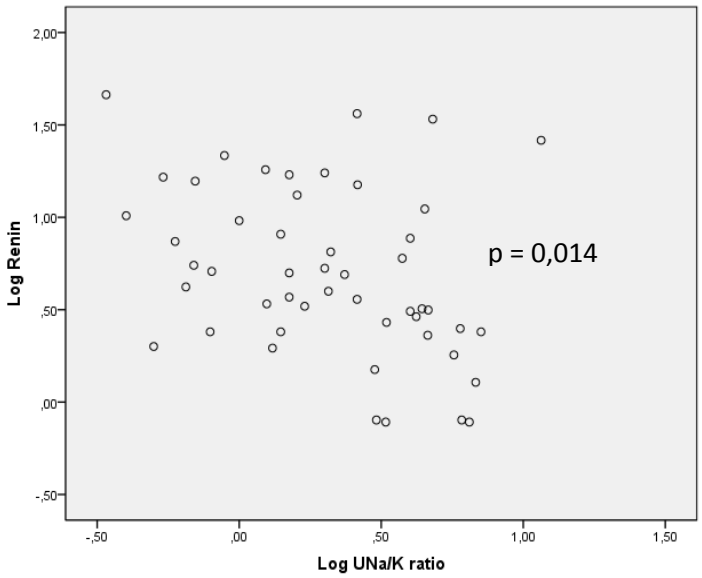
	Spironolactone Group			Control Group		
	NAC for Δ Log Aldosterone	95%CI	p Value	NAC for Δ Log Aldosterone	95%CI	p Value
Δ Log Renin	0,384	0,123 to 0,692	0,006	-0,119	-0,568 to 0,237	0,411
Δ Log UNa/K ratio	-0,288	-0,518 to -0,009	0,042	0,063	-0,244 to 0,380	0,663
	NAC for Δ Log Renin			NAC for Δ Log Renin		
Δ Log Aldosterone	0,384	0,123 to 0,692	0,006	-0,119	-0,568 to 0,237	0,411
Δ Log UNa/K ratio	-0,512	-0,740 to -0,256	<0,001	-0,165	-0,348 to 0,093	0,252

Legend: NTproBNP, N-terminal pro brain natriuretic peptide; UNa/K, urinary sodium to potassium; Δ, changes (day 3 – day 1).

Figure

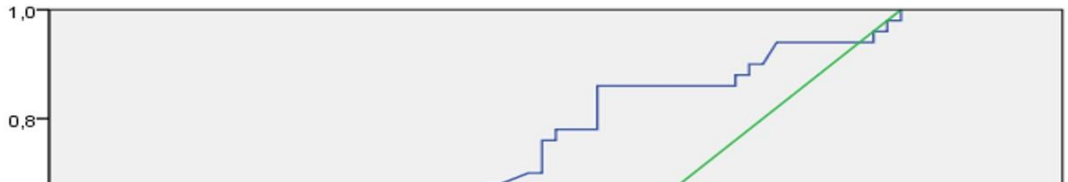
Figure 1. Log Aldosterone and Log Renin Correlations with Log UNa/K ratio at Day 3 in Control Group (No Spironolactone Use)





Legend: UNa/K, urinary sodium to potassium

Figure 2. Receiver Operating Curve: uNa/K ratio Biomarker Potential of Spironolactone Use



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VII. The Influence of Spironolactone on Matrix Metalloproteinases in Acute Decompensated Heart Failure

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Abstract

Background: Matrix metalloproteinases (MMPs) are a family of enzymes important for the resorption of extracellular matrices (ECM), control of vascular remodelling and repair. Increased activity of MMP2 has been demonstrated in heart failure (HF) and in acutely decompensated heart failure (ADHF) a decrease of circulating MMPs has been demonstrated along with successful treatment.

Objectives: Our aims are to test the influence of spironolactone in MMP2 levels.

Methods: Secondary analysis of a prospective, interventional study including 100 patients with ADHF. Fifty patients were non-randomly assigned to spironolactone 100 mg/day plus standard ADHF therapy (intervention group) or standard ADHF therapy alone (control group).

Results: Patients within spironolactone group were younger, had lower creatinine and urea levels (all $p < 0,05$). Baseline MMP2, NT-pro BNP and weight did not differ between spironolactone and control groups. A trend to a more pronounced decrease of MMP2 from baseline to day 3 was observed in spironolactone group (-21 [-50 to 19] vs 1,5 [-26 to 38] ng/mL, $p = 0,06$), NT-pro BNP and weight also had a greater decrease in spironolactone group. The proportion of patients with a decrease in MMP2 levels from baseline to day 3, was also likely to be greater in spironolactone group (50% vs 66,7%), but without statistical significance. Correlations between MMP2, NT-pro BNP and weight variation were not statistically significant.

Conclusions: MMP2 levels are increased in ADHF. Patients submitted to spironolactone treatment may have greater reduction in MMP2 levels.

Key-words: matrix metalloproteinase-2; decompensated heart failure; spironolactone.

Introduction

Matrix metalloproteinases (MMPs) are a family of zinc-dependent interstitial enzymes important for the resorption of extracellular matrices (ECM) in both health and disease[1]. ECM are a dynamic structure central to the control of vascular remodelling and repair[1], mostly due to the ability of MMPs to reabsorb and digest excessive amounts of ECM responsible for structural disruption[2,3].

Elevated MMPs promote loss of cardiac contractility via cell proteolysis and alterations in the ECM, contributing to cardiac and extra-cardiac remodelling processes[4]. In fact, clinical and experimental heart failure (HF) models of dilated and ischemic cardiomyopathy demonstrated an increased activity of matrix metalloproteinase-2 (MMP2)[2,5-7]. In patients with HF, increased levels of MMP2 were associated with all-cause mortality[8]. Concordantly, in the acutely decompensated heart failure (ADHF) setting a decrease of circulating MMPs has been demonstrated along with successful ADHF treatment[3,9]. Previous studies have suggested a therapeutic benefit of spironolactone in ADHF setting[10]. But no studies had looked to the effect of spironolactone in the ECM remodelling.

In the present study, we aimed to examine the influence of spironolactone on the ECM remodeling in ADHF patients. We hypothesized that MMP-2 plasma levels of ADHF patients will have steeper decrease if spironolactone is added to standard treatment.

Methods

Study Design

We analysed data from a previous pilot, prospective, interventional, clinical trial that we performed. That study was performed between February 2012 and February 2013 and during that period we enrolled 100 consecutive patients who presented into a Portuguese tertiary hospital with ADHF. Patients were eligible for enrollment if they presented with decompensation of chronic HF with symptoms leading to hospitalization. ADHF was diagnosed on the basis of the presence of history of chronic HF and at least one acute symptom (dyspnea, orthopnea, or edema) and one sign (rales, peripheral edema, ascites, or pulmonary vascular congestion on chest radiography). Patients were non-randomly assigned in a sequential 1:1 ratio to spironolactone plus standard ADHF therapy or standard ADHF therapy alone, 50 patients within each arm. Patients were alternatively assigned to spironolactone arm or standard ADHF therapy arm in a sequential manner - the first patient to one arm and the next to the other arm. This sequence was repeated until we reach 100 patients, 50 patients within spironolactone group and 50 patients within control group. Patients were

blinded to the allocation, but not the clinicians. The recommended spironolactone dose was 100 mg/day, however the assistant physician could decrease the spironolactone dose to 50 mg/day after 48h upon admission. Furosemide dose and route of administration was adjusted clinically according to the hydration status of the patients.

Exclusion criteria were: chronic use of mineralocorticoid receptor antagonists, cardiac surgery within 60 days of enrollment, cardiac mechanical support, cardiac resynchronization-therapy within the last 60 days, comorbid conditions with an expected survival of less than 6 months, acute myocardial infarction at time of hospitalization, hemodynamically significant uncorrected primary cardiac valvular disease, patients requiring intravenous vasodilators or inotropic agents, supine systolic arterial blood pressure <90 mmHg, plasma creatinine level >1,5 mg/dL, serum potassium level >5,0 mmol/L, hemoglobin level <9 g/dL, and sepsis.

Institutional review board or ethics committee approval was obtained. All patients provided written informed consent to participate in the study.

Clinical assessment of participants

Patient`s clinical status including physical examination was prospectively recorded by the same assistant physician at day 1 and day 3. Medications and respective dosages were prospectively recorded by the investigators according to the assistant physician prescriptions.

Blood samples were collected in the first 24 hours (h) after admission (baseline) of the patient to the hospital, and the day 3 samples were collected between 72 and 96 h of hospitalization. Samples were analysed at a central core laboratory, and included plasma creatinine and urea, electrolytes, NT-pro BNP and MMP2. Clinical assessment and routine analyses were performed daily during hospital stay. Estimated glomerular filtration rate (eGFR) was determined using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation[11]. All patients performed a transthoracic echocardiography within 72 hours upon admission. Left ventricular ejection fraction (LVEF) was calculated according to biplane Simpson method.

MMP2 was measured by enzyme-linked immunoabsorbent assays (ELISA) - Quantikine Elisa Human MMP-2 Immunoassay, by the manufacturer R&D Systems, Inc®. The normal range of MMP2 values published by the manufacturer are median [inter-quartile range$_{25\text{-}75}$], 199 [161 – 301] ng/mL. The assay sensitivity is 0,047 ng/mL.

Of the 100 studied patients we analysed baseline (day 1) and day 3 blood samples from 87. Thirteen (13) samples were not analysed due to transport and/or sampling processing errors. Samples were collected in the morning with patients supine. Serum was separated and stored at -80 ºC until sample analysis.

Variable definitions

We classified patients according to spironolactone use and their response to diuretic therapy.

We studied the relationships between baseline characteristics, day 3 and changes (Δ, difference between day 3 and baseline values) in MMP2, NT-proBNP and weight regarding the spironolactone use and diuretic response.

Statistical Analysis

Normally distributed continuous variables are expressed as mean ± standard deviation (SD), and skewed distributions are presented as median [inter-quartile range$_{25\text{-}75}$, IQR].

Categorical variables are expressed in proportions (%).

Comparison between groups was performed using parametric (independent samples t-test), non-parametric (Mann-Whitney test), or Chi-square tests, as appropriate.

Correlations of MMP2 were examined by single variable linear regression and presented as correlation coefficient and 95% confidence interval [$_{95\%}$CI].

A p value < 0,05 was considered statistically significant.

Statistical analysis was performed using SPSS software (version 19, Chicago, IL, USA).

Results

Baseline Patients Characteristics in Control and Spironolactone Groups

Patients in control group were older (78,8 ± 9,3 versus [vs.] 73,2 ± 11,7 years, p = 0,01), and had higher creatinine and urea levels (1,15 ± 0,27 vs. 1,03 ± 0,30 mg/dL, p = 0,026 and 59,32 ± 22,27 vs. 51,10 ± 18,63 mg/dL, p = 0,048). No differences between groups were found regarding sex, diabetes mellitus, chronic obstructive pulmonary disease, dementia, sleep apnea, non-invasive ventilation, ischemic heart disease, atrial fibrillation, LVEF, weight, systolic blood pressure, potassium, sodium, hemoglobin, albumin, NT-pro BNP, MMP2, furosemide dose, hospital length of stay, and in the proportion of patients on angiotensin converting enzyme inhibitors and beta-blockers – Table 1.

Spironolactone Influence on MMP2, NT-pro BNP and Weight Dynamic Changes

No differences between control and spironolactone groups were observed regarding baseline and day 3 MMP2 levels – Table 2. However, MMP2 decreased from baseline to day 3 in spironolactone group, while in control group MMP2 levels

increased, leading to a tendency for a reduction in MMP2 levels in spironolactone group (1,5 [-26 to 38] vs. -21 [-50 to 19] ng/mL, p = 0,06) – Table 2. and Figure 1. The proportion of patients in which a decrease in MMP2 levels, from baseline to day 3, was observed was also greater in spironolactone group, however this difference did not reach statistical significance – number (%), 21 (50) in controls versus 30 (66,7) in spironolactone group, p = 0,115.

No differences were observed in NT-pro BNP levels at baseline, however at day 3 the group of patients who underwent spironolactone treatment presented lower levels of NT-pro BNP (248 [923 – 5502] vs. 1555 [722 – 2554] pg/mL, p = 0,05). No differences between groups were observed in the variation of NT-pro BNP levels probably due to the lower levels (although not significantly lower) of NT-pro BNP at baseline in the spironolactone group, leading to a smaller amplitude of variation in this group – Table 2.

A greater weight decrease was also observed in the spironolactone-treated patients (-2,9 ± 2,4 vs. -4,8 ± 2,8 Kg, p < 0,001) – Table 2.

Δ MMP2, Δ NT-pro BNP and Δ Weight Correlations

No significant correlations were observed between Δ in MMP2, NT-pro BNP and weight - Table 3.

Discussion

In the present study we observed an increased baseline levels of MMP2 in patients with ADHF. Those patients treated with spironolactone showed a tendency to a greater reduction of MMP2 levels. These results are consistent with previous findings demonstrating the impact of mineralocorticoid receptor antagonists on the ECMs remodelling and highlight the potential interest of spironolactone in the treatment of ADHF where those mechanisms are strongly exacerbated.

Increased serum levels of MMP2 have been demonstrated in the ADHF setting[3,9]. In our study the median [IQR] MMP2 levels at admission day were 260 [225 - 312] ng/mL. These values are above the normal range defined by the manufacturer, 199 [161 – 301] ng/mL, and are concordant with previous reports on patients with HF decompensation. A previous study by Shirakabe A., *et al*[3], also showed increased serum MMP2 levels in ADHF, with a rapid decrease along with HF compensation. Furthermore, an interventional placebo-controlled trial performed by Tziakas DN., et al[9], showed a significant reduction in MMP2 levels in the group of patients treated with levosimendan. In animal models, exacerbated neurohormonal activation leads to an increase in the levels of several myocardial MMPs subtypes[12-14]. MMPs are important for proteolysis that can affect the composition of ECM and consequently myocardial

remodelling. Additionally, increased ECM turnover may be associated with pathological myocardial remodelling, that may be accelerated in decompensated HF[3,15]. Consequently, a reduction in markers of ECM turnover may serve as a surrogate marker for deceleration of myocardial turnover and remodelling. Our study showed a greater decrease of MMP2 in the group of patients submitted to spironolactone treatment. Mineralocorticoid receptor antagonists improve survival and reduce morbidity in patients with heart failure with reduced ejection fraction, and mild-to-severe symptoms, and in patients with left ventricular systolic dysfunction and heart failure after acute myocardial infarction[16-18], additionally, used in natriuretic doses, the mineralocorticoid receptor antagonists are likely to improve congestion in ADHF with good tolerability and few side effects[19]. Several proposed mechanisms explain how MRAs improve HF outcomes, and these pathways include a reduction of myocardial remodelling[20]. Our study provides important information towards a better understanding of ECM turnover processes. The steeper MMP2 reduction observed in patients submitted to spironolactone treatment provides a real demonstration of potential mitigation of harmful remodelling through spironolactone use. Interestingly, patients without MMP2 reduction or increase, after an acute HF episode, had poorer prognosis[3]. Therefore, changes in MMP2 levels are a potentially useful prognostic marker in patients admitted due to ADHF.

NT-pro BNP is a well-validated, widely used, and very accurate biomarker for the diagnosis and risk stratification of HF[21]. Patients submitted to spironolactone treatment had lower levels of NT-pro BNP at day 3, and a more pronounced weight reduction when compared to controls, and patients with slower diuretic response had higher NT-pro BNP levels at day 3 of hospitalization, a tendency to lower NT-pro BNP reduction, and less weight loss. However, the changes in MMP2 values did not correlate with the variation in NT-pro BNP or weight. The small sample size, the NT-proBNP and MMP2 elevated variance, and the different mechanisms influencing those biomarkers may all explain the absence of this correlation.

Several limitations in our study should be noticed. First, this was a single-centre, non-randomized trial with a small number of patients with mixed HF etiologies and treatments. Second, this post-hoc analysis has limitations inherent to observational studies. Third, the decision to withdraw diuretic therapy was based on subjective assessment of congestive signs and symptoms so we cannot rule out the inter-observer variability. However, in real-life patients, the decision to step down diuretic therapy is also based on subjective clinical evaluation. Fourth, our study excluded HF patients significant renal impairment, since plasma creatinine level of less than 1,5 mg/dL was an inclusion criteria, leading to a potential selection of a subset of low risk

patients, which can affect the external validity of our results. Fifth, the group of patients submitted to spironolactone treatment were younger and had lower plasma creatinine and urea levels which can positively affect the response to this drug. Finally, only MMP2 was evaluated and other forms of MMPs may have different effects and responses in ADHF patients.

Conclusion

The present study showed that MMP2 levels can be increased in ADHF, and that patients treated with spironolactone may have greater reduction in MMP2 levels. Whether these findings have prognostic significance requires further investigation.

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Disclosures

The authors have no conflicts of interest to disclose.

Tables

Table 1. Baseline Population Characteristics, Laboratory Results, Medications, and Hospital Length of Stay in Treatment and Control Groups

	Control Group (n = 50)	Spironolactone Group (n = 50)	p Value
Age (yrs)	78,8 ± 9,3	73,2 ± 11,7	0,010
Male Sex – %	34	44	0,31**
Diabetes Mellitus - %	50	40	0,31**
COPD - %	10	26	0,32**
Dementia - %	16	8	0,22**
Sleep Apnea - %	10	26	0,32**
Non-Invasive Ventilation - %	14	20	0,42**
Ischemic Heart Disease -%	48	52	0,69**
Atrial Fibrillation - %	68	50	0,07**
LV Ejection Fraction < 40% - %	56	68	0,22**
Weight (Kg)	75,6 ± 16,3	76,1 ± 16,4	0,89
SBP (mmHg)	140,5 ± 23,9	139 ± 27,9	0,80
Plasma Creatinine (mg/dL)	1,15 ± 0,27	1,03 ± 0,30	0,03
eGFR (mL/min/1,73 m^2)	54,5 ± 16,5	68,3 ± 23,6	0,001
Plasma Urea (mg/dL)	59,3 ± 22,3	51,1 ± 18,6	0,05
Serum Potassium (mmol/L)	4,1 ± 0,4	4,0 ± 0,6	0,33
Serum Sodium (mmol/L)	140,5 ± 5,0	140,6 ± 3,7	0,96
Hemoglobin (g/dL)	12,2 ± 1,8	12,7 ± 2,3	0,22
Albumin (mg/dL)	3,7 ± 0,4	3,6 ± 0,4	0,63
NTproBNP (pg/mL)	3102 [1797 – 8204]	2701 [1463 – 5004]	0,17*
MMP2 (ng/mL)	260 [226 – 299]	268 [207 – 336]	0,52*
IV Furosemide Dose (mg/d)	75,6 ± 20,7	76,0 ± 25,5	0,93
ACEi/ARB – %	38	50	0,20**
Beta-Blocker - %	42	32	0,30**
Spironolactone - %	-	100	-
Spironolactone Dose (mg/d)	-	94,5 ± 23,3	-
Hospital Length of Stay (days)	9,0 ± 3,7	8,7 ± 3,0	0,59

Continuous variables are presented as mean value ± standard deviation [SD], p value or median [inter-quartile range, IQR], p value. Categorical variables are presented as absolute number (%), p value.

*Non-parametric paired sample test; ** Chi-square test.

Legend: COPD - chronic obstructive pulmonary disease; LV - left ventricular; eGFR - estimated glomerular filtration rate; NT-pro BNP - N-terminal pro brain natriuretic peptide; hsTnT - high sensitivity troponin T; MMP2 - matrix metalloproteinase -2; IV - intra-venous; ACEi - angiotensin converting enzyme inhibitors

Table 2. Comparison of MMP2, NT-pro BNP and Weight at Baseline, Day 3, and Change (Delta, Δ) from Baseline to Day 3 Between the Study Groups

	Control Group	Spironolactone Group	p Value
MMP2			
Baseline	260 [226 – 299]	268 [207 – 336]	0,52*
Day 3	266 [227 – 298]	261 [212 – 307]	0,49*
Δ (day 3 – baseline)	1,5 [-26 to 38]	-21 [-50 to 19]	0,06*
NT-pro BNP			
Baseline	3102 [1792 – 8204]	2701 [1463 – 5004]	0,17*
Day 3	2488 [923 – 5502]	1555 [722 – 2554]	0,05*
Δ (day 3 – baseline)	-945 [-2249 to -62]	-816 [-1833 to -106]	0,75*
Weight			
Baseline	75,6 ± 16,3	76,1 ± 16,4	0,89
Day 3	72,8 ± 16,3	71,3 ± 16,2	0,66
Δ (day 3 – baseline)	-2,9 ± 2,4	-4,8 ± 2,8	<0,001

Continuous variables are presented as mean value ± standard deviation [SD], p value or median [inter-quartile range, IQR], p value and independent samples t-test or independent samples non-parametric test were used, respectively.
*non-parametric test.
Legend: NT-pro BNP - N-terminal pro brain natriuretic peptide; MMP2 - matrix metalloproteinase -2; Δ - delta or difference between day 3 and baseline values.

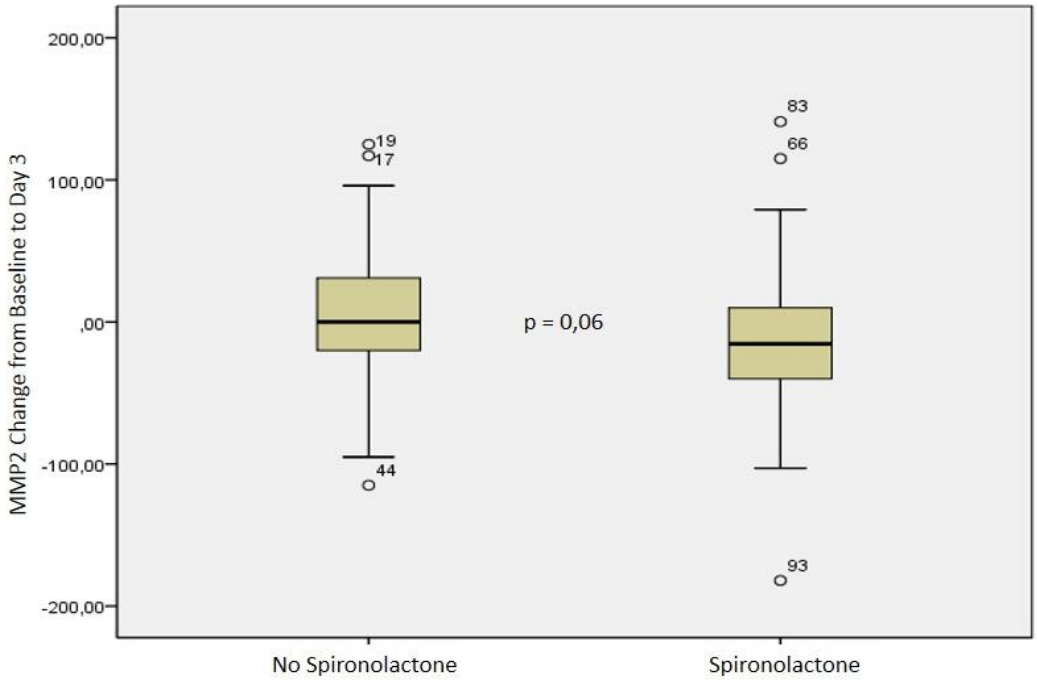
Table 3. Correlation Coefficients and 95% Confidence Intervals (CI) Between Delta (Δ) MMP2, Δ NT-pro BNP, and Δ Weight

	Δ MMP2 Correlation Coefficient	95%CI	p Value
Δ NT-pro BNP	0,11	-0,003 to 0,01	0,33
Δ Weight	0,12	-1,97 to 7,05	0,27

Legend: MMP2 - matrix metaloproeinase-2; NT-pro BNP - N-terminal pro-brain natriuretic peptide; Δ - delta or difference between day 3 and baseline values

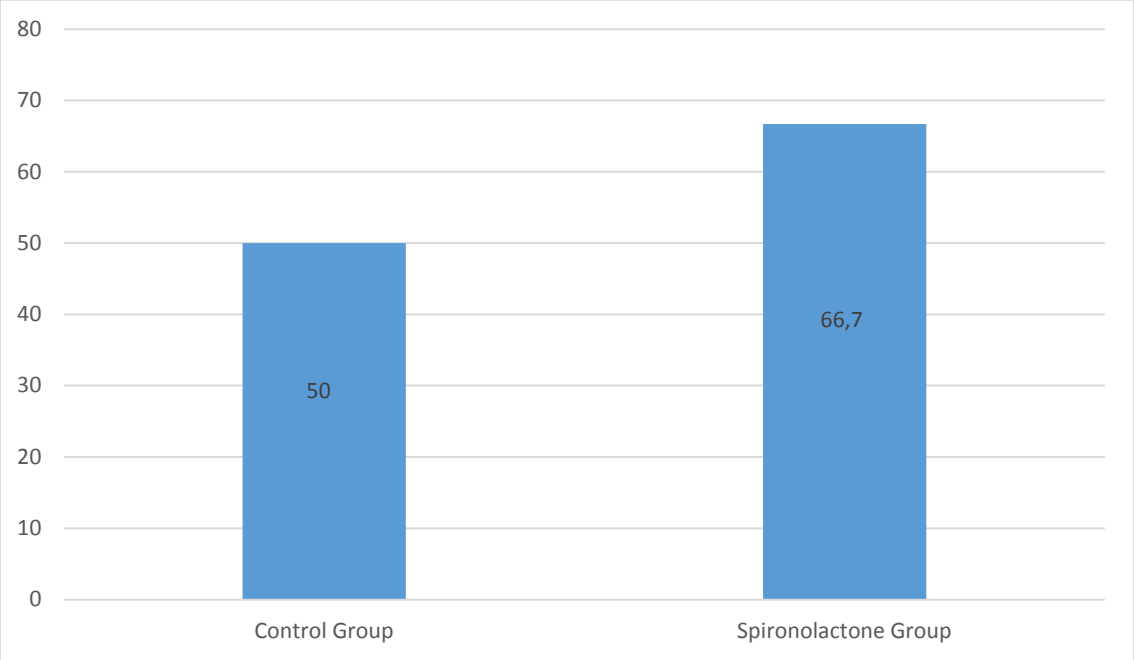
Figures

Figure 1. Comparison of Δ MMP2 Levels from Baseline to Day 3 Between Control and Spironolactone Groups.



A trend towards a more pronounced reduction in MMP2 levels was observed in spironolactone group.
Analysis was performed comparing the variation (Δ, day 3 - baseline) of MMP2 levels between control and spironolactone groups using non-parametric independent sample tests (1,5 [-26 to 38] vs. -21 [-50 to 19], p = 0,06).
Legend: MMP2 = matrix metalloproteinase-2; vs. = versus.

Figure 2. Proportion (%) of Patients with MMP2 Decrease from Day 1 to Day 3



Legend: The proportion of patients in which a decrease in MMP2 levels, from baseline to day 3, was observed was greater in spironolactone group, however this difference did not reach statistical significance, p = 0,115

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General Discussion and Limitations

This pilot study and its sub-analysis provide original evidence towards the use of natriuretic spironolactone doses in ADHF, particularly regarding its safety profile, natriuretic effect and mitigation of end-organ damage.

This discussion will focus three cornerstone findings: safety, congestion relief and end-organ protection.

Our results suggest that the use of spironolactone in ADHF is safe and not associated with renal dysfunction or hyperkalemia. The concomitant use of intravenous diuretics with kaliuretic properties could have contributed to the absence of hyperkalemia, however we observed a trend to hypokalemia in the group of patients not receiving spironolactone, and reducing the risk of hypokalemia is a significant issue due to the fact that low potassium levels are deleterious in heart failure. Moreover, worsening renal function occurred more frequently in control group, possibly due to the lack of an adequate neurohormonal blockade in patients not taking spironolactone, with subsequent arteriolar vasoconstriction and greater renal damage.

We also found that patients submitted to high-dose spironolactone had a faster resolution of congestive signs and an earlier switch to oral furosemide. The diuretic effect of doses greater than 50 mg per day of spironolactone can provide a plausible explanation to this finding, which was corroborated by a greater reduction in natriuretic peptides within the spironolactone group.

Additionally, our study was the first to demonstrate a greater reduction in albuminuria in ADHF patients submitted to spironolactone treatment. The rationale for this effect may rely on effective neurohormonal blockade, suggesting a potential renal protective effect of this treatment. Patients in spironolactone group also had a more pronounced matrix metalloproteinase-2 reduction, which may represent a potential reduction of harmful remodelling. Additionally, this study also showed a tendency to a greater cardiac troponin reduction in patients submitted to spironolactone treatment that can represent a potential reduction of myocardial injury.

We also would like to highlight the potential use of urinary sodium to potassium ratio as a simple, inexpensive and widely available surrogate marker for mineralocorticoid receptor antagonism.

This study has several limitations. The most important general limitations are: 1) the lack of true randomization or concealed allocation, which can determine a selection bias with impact on the external validity of our results; 2) the assistant physicians performed the congestive signs assessment, therefore, we cannot exclude an ascertainment bias that can affect the internal validity of the comparison of subjective outcomes such as congestive signs or symptoms. Though, we had included an

important internal control such as plasma NTproBNP that is unaffected by this bias and appear to be consistent with the earlier resolution of the congestive signs in the treatment group; 3) our study was underpowered to detect the differences of the expected low rate of adverse events between groups; 4) the post-hoc analysis have the limitations inherent to observational studies.

Conclusions

The use of high-dose spironolactone in ADHF is safe, and possibly associated with greater congestion relief, faster diuretic response and less end-organ injury. The urinary sodium to potassium may serve as a potential surrogate marker for mineralocorticoid receptor antagonism.

Abstract

We aimed to add original evidence on the use of high-dose spironolactone in acutely decompensated heart failure (ADHF).

We demonstrated that doses equal or superior to 50 mg a day of spironolactone can provide a more intense mineralocorticoid receptor antagonism (MRA), mitigating neurohormonal activation inherent to ADHF and also providing a natriuretic effect that can help loop diuretics in congestion relief.

In our pilot study we provided real-world evidence that higher spironolactone dosages are safe, can reduce natriuretic peptides - translating greater congestion relief, can decrease albuminuria - traducing less renal damage, and probably decrease matrix-metalloproteinases and cardiac troponins translating less myocardial injury. We also showed that spironolactone increases renin, aldosterone and urinary sodium to potassium ratio providing potential surrogate markers of MRA.

Resumo

A nossa intenção com este estudo era fornecer evidência original para o uso de doses elevadas de espironolactona na insuficiência cardíaca descompensada (ICD).

Demonstrámos que a espironolactona em doses iguais ou superiores a 50 mg por dia pode proporcionar um bloqueio eficaz dos recetores mineralocorticóides, diminuindo a ativação neurohormonal presente na ICD e, concomitantemente, aumentado o efeito natriurético dos diuréticos da ansa, funcionando como uma terapêutica adjuvante para o alívio da congestão hídrica.

Neste estudo piloto, em doentes do *mundo-real*, conseguimos demonstrar o perfil de segurança de doses elevadas de espironolactona na ICD, o potencial da espironolactona reduzir peptídeos natriuréticos traduzindo maior alívio congestivo, diminuir a albuminúria traduzindo menor lesão renal e o potencial de proporcionar uma maior redução nos níveis séricos de metaloproteinases da matriz e nas troponinas cardíacas traduzindo menor dano cardíaco potencial. Demonstrámos também que a espironolactona aumenta os níveis de renina, aldosterona e razão sódio-potássio urinário favorecendo a utilização destes marcadores como tradutores do uso deste fármaco.

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