ORIGINAL RESEARCH ARTICLE

Effects of intravenous furosemide plus small-volume hypertonic saline solutions on markers of heart failure

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Abstract

Aims We sought to compare the effects of furosemide + hypertonic saline solution (HSS) treatment in patients with acute decompensated heart failure in comparison with furosemide alone and the response in a compensated state after an acute saline load with regard to serum levels of heart failure biomarkers.

Methods and results We enrolled 141 patients with acute decompensated heart failure with reduced ejection fraction admitted to our Internal Medicine ward from March 2017 to November 2019. A total of 73 patients were randomized to treatment with i.v. high-dose furosemide plus HSS, whereas 68 patients were randomized to i.v. high-dose furosemide alone. Patients treated with furosemide plus HSS compared with controls treated with furosemide alone showed a comparable degree of reduction in the serum levels of interleukin (IL)-6, soluble suppression of tumorigenicity 2 (sST2), and N-terminal pro-brain natriuretic peptide (NT-proBNP) in the 'between-group' analysis. Nevertheless, patients treated with high-dose furosemide + HSS showed significantly higher absolute delta values of IL-6 (2.3 ± 1.2 vs. 1.7 ± 0.9, $P < 0.0005$, and 2.0 ± 0.8 vs. 1.85 ± 1.1, $P = 0.034$), sST2 (41.2 ± 8.6 vs. 27.9 ± 7.6, $P < 0.0005$, and 37.1 ± 6.6 vs. 28.4 ± 6.7, $P < 0.0005$), high-sensitivity troponin T (0.03 ± 0.02 vs. 0.02 ± 0.01, $P = 0.001$, and 0.03 ± 0.02 vs. 0.02 ± 0.01, $P = 0.009$), NT-proBNP (7237 ± 7931 vs. 3244 ± 4159, $P < 0.005$, and 5381 ± 4829 vs. 4466 ± 4332, $P = 0.004$), and galectin-3 (15.7 ± 3.2 ng/mL vs. 11.68 ± 1.9 ng/mL, $P < 0.0005$, and 16.7 ± 3.9 ng/mL vs. 11.8 ± 2.4 ng/mL, $P < 0.0005$) than patients treated with furosemide alone. After acute saline load, patients treated with i.v. furosemide + HSS in comparison with subjects treated with furosemide alone showed a significantly lower increase in the serum concentrations of IL-6 (−0.26 ± 0.42 pg/mL vs. −1.43 ± 0.86 pg/mL, $P < 0.0005$), high-sensitivity troponin T (0 vs. −0.02 ± 0.02 ng/mL, $P < 0.0005$), sST2 (−8.5 ± 5.9 ng/mL vs. −14.6 ± 6.2 ng/mL, $P < 0.0005$), galectin-3 (−2.1 ± 1.5 ng/mL vs. −7.1 ± 3.6 ng/mL, $P < 0.0005$), and NT-proBNP (77 ± 1373 vs. −1706 ± 2259 pg/mL, $P < 0.0005$).

Conclusions Our findings concerning a comparable degree of reduction in the serum levels of three cardinal biomarkers indicate that a reduction in serum heart failure markers is not linked to the higher degree of congestion relief with a more rapid achievement of a clinical compensation state. This issue may have possible benefits on clinical practice concerning its therapeutic effects over and beyond the simple amelioration of clinical congestion signs and symptoms. Nevertheless, our findings of higher delta values after treatment with i.v. furosemide plus HSS indicate a possible higher efficacy by means of modulation of the stretching and fibrosis mechanisms.

Background

Heart failure (HF) is a clinical syndrome with symptoms and signs caused by a structural and/or functional cardiac abnormality that results in reduced cardiac output and/or elevated intracardiac pressures. The causes can be different and sometimes concomitant, but the most frequent is an abnormality of the myocardium, causing systolic and/or diastolic ventricular dysfunction.[1]

The reduction in cardiac contractility and the overload of cardiac work entail the activation of long-term counterproductive compensation mechanisms. The sympathetic nervous system is activated, with increased heart rate, cardiac inotropism, and arteriolar vasoconstriction.[2] The sympathetic nervous system achieves a reduction in renal blood flow and hydrosaline retention and the maintenance of this vasoconstriction through the renin-angiotensin-aldosterone system (RAAS).[2]

Cardiac remodelling is defined as the result of changes in the expression of the cellular genome of the myocardial tissue, which induces molecular changes in the cellular structure and interstitial matrix that produce changes in the weight, shape, and function of the heart.

Fibrosis, induced by the renin-angiotensin system (RAAS), increases stiffness and decreases the elasticity of the myocardium with diastolic dysfunction and can also affect the heart valves, alter the propagation of electrical impulses, and impair the supply of nutrients to the myocardial tissue by supporting the progression of remodelling.[3]

By definition, a biomarker is an objectively measurable variable that is an indicator of a normal biological process, of a pathological process, or of a response to drug therapy.[3] Biomarkers can have diagnostic, prognostic, or predictive significance[4] in the clinical setting of congestive HF (CHF).

Myocardiocytes produce suppression of tumorigenicity 2 (ST2) following mechanical stress, which is found in two forms: transmembrane or cellular (ST2L) and soluble (sST2).[5] It acts as a receptor for interleukin (IL)-33, a cytokine produced in the case of cellular damage, expressed by endothelial and epithelial cells.[6]

A higher concentration of sST2 would prevent IL-33 from binding ST2L, thus preventing its cardioprotective action observed in experimental models that occurs through the reduction of myocardial fibrosis, cardiomyocyte hypertrophy, and apoptosis.[7]

Galectin-3 expression, produced by different cell types and especially by macrophages, induces the proliferation of fibroblasts and the production of collagen at the heart level.[8–10] Some studies have shown the association between high levels of this molecule and newly diagnosed HF patients, but it is also useful as a prognosis and severity marker in patients with reduced and preserved ejection fraction (EF).[9–11]

Troponin is a sarcomere protein complex consisting of three subunits called I, C, and T. The increase in serum troponin levels is associated with the severity of the disease and mortality, while the reduction is associated with a better prognosis.[12]

Natriuretic peptides (NPs) in the heart are produced as a result of atrial and ventricular distension and neurohormonal activation.[13] Both B-type natriuretic peptide (BNP) and N-terminal pro-B-type natriuretic peptide (NT-proBNP) are widely used to aid diagnosis, assess the effect of therapy, and predict outcomes in HF and reduced EF.[14]

Inflammatory cytokines such as IL-6, IL-1, and tumour necrosis factor alpha (TNF-α) are overexpressed in patients with HF, and their concentrations are directly proportional to the New York Heart Association (NYHA) class as well as to the EF.[15–17]

Preliminary studies[18–21] confirmed the safety and tolerability of treatment with an intravenous combination of hypertonic saline solution (HSS) and high-dose furosemide in the treatment strategy of patients with CHF. These studies addressed the hypothesis that the maintenance of adequate vascular refilling and renal perfusion during treatment with high doses of furosemide may enhance their effectiveness. Both of these goals can be achieved by combining high-dose furosemide with the administration of hypertonic saline.

Furthermore, our group[22] reported that in subjects with decompensated CHF, treatment with furosemide + HSS compared with treatment with furosemide alone resulted in a significant lowering of plasma levels of atrial natriuretic peptide (ANP), BNP, TNF-α, IL-1β, and IL-6. We also reported that an acute saline load (15 mL/kg of 0.9% NaCl) administered after an 8 day course of the furosemide + HSS regimen resulted in these same groups of patients having a lower percentage increase in serum levels of ANP, BNP, TNF, and IL-1β compared with the control groups.

Study hypothesis

Our study hypothesis involves the evaluation of the effect of moderate/high doses of treatment with intravenous furosemide plus small volumes of HSS on serum markers of HF, such as NT-proBNP, high-sensitivity troponin T (hsTnT), galectin-3, IL-6, ST2, and C-reactive protein (CRP), in patients with acute decompensated heart failure (ADHF) due to HF with reduced ejection fraction (HFrEF).

We hypothesized that there would be a higher degree of reduction in the serum levels of HF biomarkers after treatment with i.v. furosemide + HSS in comparison with i.v. furosemide alone and a lower degree of increase in these biomarkers after an acute saline load in a compensated state intended to represent partial or complete remission of congestive symptoms and signs, due to possible better modulation of stretching overload and of inflammation due to the

addition of HSS to the intravenous furosemide treatment protocol.

Aims of the study

We sought to evaluate the efficacy of treatment with i.v. furosemide + HSS by comparing the reduction degree of serum levels of some chosen markers of HF and the response degree of these markers in a compensated state after an acute saline load in comparison with treatment with i.v. furosemide alone.

Materials and methods

All consecutive patients aged >18 years with a diagnosis of acute decompensated CHF due to HFrEF admitted to our Internal Medicine ward were enrolled from March 2017 to November 2019. Enrolled patients were randomly assigned to undergo treatment with moderate/high doses of i.v. furosemide plus HSS or i.v. furosemide alone using a computer-generated random sequence (1:1).

Study protocol

The study protocol is shown in *Figure 1*.

Patients were randomly assigned to undergo treatment with i.v. furosemide plus HSS or i.v. furosemide alone using a computer-generated random sequence (1:1).

Patients underwent three different evaluations at T0 (at admission before treatment with moderate/high-dose furosemide plus HSS or furosemide alone), T1 (after 6 days of treatment with i.v. furosemide + HSS or furosemide alone), and T2 (after a saline load) by venipuncture to obtain venous blood samples for the determination of serum concentrations of NT-proBNP, hsTnT, sST2, galectin-3, IL-6, and CRP.

Inclusion criteria

We enrolled all consecutive patients who were admitted to our Internal Medicine ward from December 2017 to December 2019 with a diagnosis of congestive HFrEF.

Exclusion criteria

The exclusion criteria were acute myocarditis, active pulmonary and liver disease, autoimmune disorders, infections, malignant diseases, muscle disorders, renal insufficiency (serum creatinine ≥ 2.5 mg/dL), chronic inflammatory diseases, rheumatological diseases, haematological diseases, and regular treatment with anti-inflammatory drugs.

Figure 1 Study protocol. Cases: patients randomized to i.v. high-dosage furosemide plus small volume of hypertonic saline solutions (HSS). Controls: patients randomized to i.v. high-dosage furosemide alone.
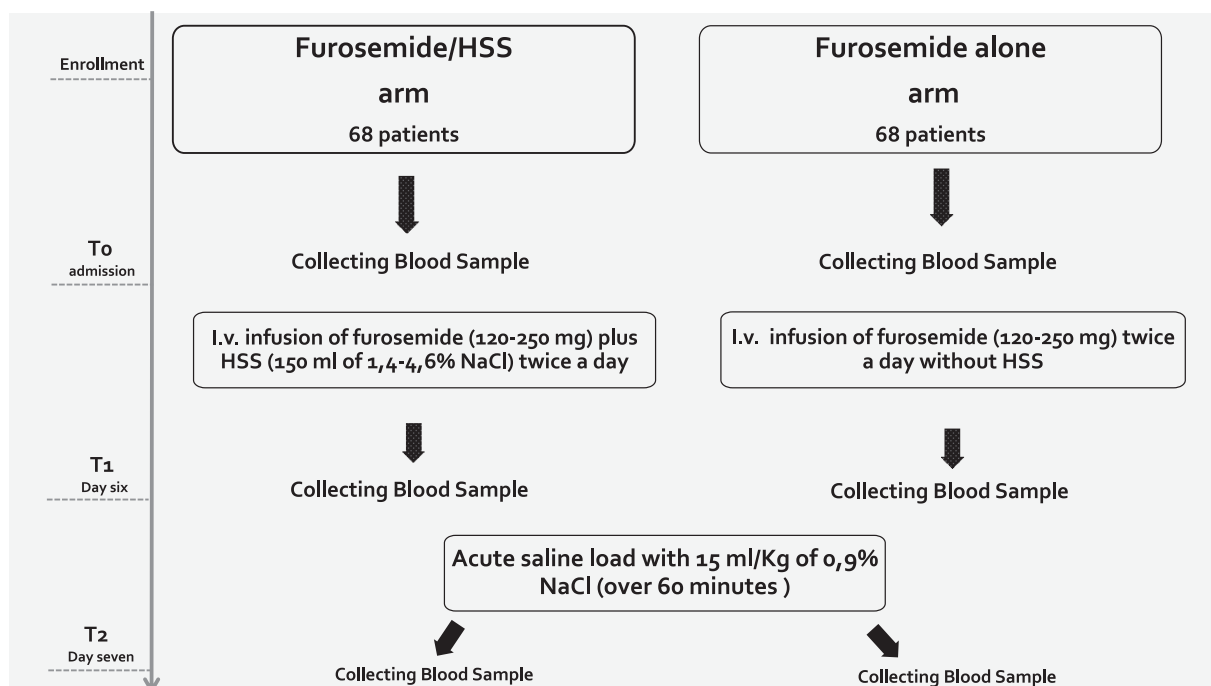
Definition of diseases

We defined HF according to the European Society of Cardiology (ESC) criteria, namely, as appropriate symptoms or signs of CHF (NYHA functional class II or worse) plus objective evidence of cardiac dysfunction.[23]

We defined ADHF as a clinical condition with signs and symptoms of congestion and fluid retention (weight gain, exertional dyspnoea, orthopnoea, and dependent oedema) associated with CHF.[23]

Heart failure with reduced ejection fraction was defined according to the ESC guidelines[23,24] as subjects with reduced left ventricular (LV) EF (<40%).

The pathogenesis of HF was determined on the basis of clinical history and clinical records showing a history of hypertension, myocardial infarction, angina, valvular disease, diabetes, and non-ischaemic dilated cardiomyopathy.

The study was approved by the Ethics Committee of the Policlinico 'P. Giaccone' of Palermo, Italy. Written informed consent was obtained from all patients.

The trial has been registered on ClinicalTrials.gov (ClinicalTrials.gov Identifier: NCT04628325).

Daily clinical and laboratory evaluation

An accurate assessment of body weight and measurement of 24 h of urinary volume were performed every day for cases and controls. Fasting blood samples were drawn to determine serum Na, K, Cl, albumin, uric acid, creatinine, urea, and glycaemia daily during hospitalization and continued until the clinically compensated status was reached at the end of the treatment period.

The total daily urine output was collected for daily dieresis and urinary Na, K, and Cl measurements.

Treatment protocol

The study protocol is shown in *Figure 1*.

Subjects in the furosemide + HSS group received a 30 min of i.v. infusion of furosemide (120–250 mg) plus HSS (150 mL of 1.4–4.6% NaCl) twice a day for 6 days from admission (*Figure 1*). The daily dosage of furosemide was defined considering urinary volume, blood pressure values, and severity of signs and symptoms of congestion. The dose of HSS was determined in each patient according to the following schedules:

- for serum Na values < 135 mEq/L, the HSS concentration was 3.5%;
- for serum Na values > 135 mEq/L, the HSS concentration varied between 1.4% and 2.4% (empirically based on serum Na values); and

- KCL (20–40 mEq i.v.) was administered to prevent hypokalaemia.

Subjects in the furosemide group received a 30 min of i.v. infusion of furosemide (120–250 mg) twice a day without HSS for 6 days from admission. The daily dosage of furosemide was defined considering urinary volume, blood pressure values, and the severity of signs and symptoms of congestion.

Once the treatment period was reached, i.v. administration of furosemide was stopped, and it was replaced with oral furosemide (25–250 mg/day). The diuretic dosage was established on the basis of the severity of LV dysfunction, NYHA class, patient symptoms due to signs of congestion, and blood pressure values.

During the study period, subjects in the 'furosemide/HSS' group and the 'furosemide alone' group received angiotensin-converting enzyme inhibitors, digitalis, and nitrates, and they were maintained at moderate physical activity when symptoms were mild or moderate (NYHA class II) and at bed rest when symptoms were severe (NYHA class III–IV).

All enrolled subjects were followed for 10 days with a low intake sodium diet [1.61 g/Na/day (70 mmol/day); 4.0 g NaCl/day].

Acute saline load

The day after the end of the treatment period (6 days), all enrolled patients in the furosemide + HSS and furosemide alone groups underwent an acute saline load with 15 mL/kg of 0.9% NaCl (over 60 min) as previously described in a study by our group.[22]

Blood sample collection

Blood samples from each enrolled subject were drawn after at least 30 min of bed rest in a supine position, within 24 h of admission (T0), after 6 days of treatment (T1), and after an acute saline load administered 24 h after the treatment period (6 days) with i.v. furosemide + HSS or i.v. furosemide alone (T2).

Patients were subjected to blood sample collection at T0, T1, and T2 for the measurement of serum concentrations of ST2, NT-proBNP, hsTnT, galectin-3, IL-6, and CRP.

Laboratory analysis

Non-fasting blood samples were obtained by venipuncture. The collected material was centrifuged at 1700 *g*/relative centrifugal force, after which citrate, EDTA, heparin, and trasylol plasma were separated, as well as blood serum. Buffy coats were collected from EDTA tubes to enable an analysis of genetic factors. Dimethylsulfoxide was added to an

additional EDTA tube for cryopreservation of blood cells. All blood aliquots were subsequently stored at a temperature of −80°C within 2 h after venipuncture.

Galectin-3 measurements

Galectin-3 concentrations were determined in serum using the BGM Galectin-3 Test as instructed by the manufacturer (BG Medicine, Inc., Waltham, MA, USA).

Determination of N-terminal pro-B-type natriuretic peptide

The NT-proBNP concentrations were determined in heparin plasma using the Elecsys NT-proBNP assay on a Cobas 8000 analyser (Roche Diagnostics Limited, Rotkreuz, Switzerland).

Determination of interleukin-6 serum concentration

The serum IL-6 concentration was measured in samples from 138 patients using an enzyme-linked immunosorbent assay (Intertest 6; Genzyme, Boston, MA, USA) according to the kit procedure. The limit of detection of the test was 76 pg/mL, and lower levels were considered undetectable.

Determination of serum C-reactive protein concentration

The CRP concentration was measured using a fluorescence polarization immunoenzymatic assay (Abbott Laboratories, Chicago, IL, USA). The limit of detection of the CRP assay is 5 mg/L.

Determination of soluble suppression of tumorigenicity 2 serum levels

Soluble ST2 was measured with a sandwich double monoclonal antibody ELISA method (Medical & Biological Laboratories).

Statistical analysis

Continuous data are expressed as the mean ± standard deviation, unless otherwise specified. Baseline differences between cases and controls were assessed by the χ^2 test or Fisher's exact test, as needed for categorical variables, and by univariate analysis of variance for parametric variables. Friedman's test for paired data was used to compare, both for cases and for controls, the trends of variables in basal conditions, after 6 days of treatment, and after saline load, and post hoc analysis with the Tukey test was used to determine if there were any intragroup (i.v. furosemide + HSS group and furosemide alone group) differences in pairs. Data were analysed by IBM SPSS Software Version 22 (IBM Corp., Armonk, NY, USA). All *P*-values were two-sided, and $P \leq 0.05$ was considered statistically significant.

Results

We enrolled 141 patients with acute decompensated HFrEF admitted to our Internal Medicine ward from March 2017 to November 2019.

Five patients were excluded based on the presence of exclusion criteria. Thus, 68 patients were randomized to treatment with i.v. high-dose furosemide plus HSS, whereas 68 patients were randomized to i.v. high-dose furosemide alone.

General, demographic, and laboratory variables in subjects treated with furosemide plus HSS versus controls are listed in *Table 1*.

General, laboratory, and clinical variables in subjects treated with i.v. furosemide + HSS versus subjects treated with i.v. furosemide alone

Subjects in the furosemide + HSS group versus control group subjects showed a significantly higher frequency of hypertension, higher mean age (77.9 ± 9.3 years vs. 74.5 ± 6.0 years, *P* = 0.012), systolic blood pressure (134.2 ± 20.5 mmHg vs. 120.0 ± 14.4 mmHg, *P* < 0.0005), higher mean body weight (82.9 ± 14.5 kg vs. 73.3 ± 13.8 kg, *P* < 0.0005), higher body mass index (28.7 ± 5.6 kg/m^2 vs. 25.7 ± 4.3 kg/m^2, *P* = 0.001), higher mean white blood cell count (9344.3 ± 3448.4 vs. 7384.7 ± 2144.5, *P* < 0.0005), higher mean serum total cholesterol (134.6 ± 38.2 mg/dL vs. 97.5 ± 82.8 mg/dL, *P* = 0.001), higher mean serum triglycerides (92.9 ± 36.2 vs. 64.8 ± 27.5 mg/dL, *P* < 0.0005), and lower mean serum HDL (41.9 ± 14.2 mg/dL vs. 94.6 ± 77.7 mg/dL, *P* < 0.0005).

After the treatment period, we observed a higher degree of increase of diuresis in the furosemide + HSS group versus the furosemide alone group (1031.62 ± 212.29 vs. 2260.74 ± 466.37 mL, *P* = 0.0001, and 1001.47 ± 167.72 vs. 1907.35 ± 269.36 mL, *P* = 0.360). We also observed a higher degree of body weight reduction in the furosemide + HSS group versus the furosemide alone group (73.76 ± 5.16 vs. 67.50 ± 5.32 and 72.99 ± 4.07, *P* = 0.0008, vs. 69.69 ± 4.08, *P* = 0.330) after the treatment period with i.v. furosemide + HSS than in subjects treated with i.v. furosemide alone.

Table 1 General, demographic, and laboratory variables in subjects treated with i.v. furosemide plus HSS versus control group treated with i.v. furosemide alone

	Pts treated with i.v. furosemide plus HSS ($n = 68$)	Controls ($n = 68$)	P
Age (years)	77.9 ± 9.3	74.5 ± 6.0	0.012
Sex (M/F)	39/29	28/40	0.086
SBP (mmHg)	134.2 ± 20.5	120.0 ± 14.4	<0.0005
DBP (mmHg)	70.7 ± 9.3	70.2 ± 11.6	0.782
Weight (kg)	82.9 ± 14.5	73.3 ± 13.8	<0.0005
Height (cm)	170.1 ± 5.3	168.7 ± 8.4	0.233
BMI	28.7 ± 5.6	25.7 ± 4.3	0.001
WBC	9344.3 ± 3448.4	7384.7 ± 2144.5	**<0.0005**[*]
Platelets	244 044.1 ± 105 758.0	191 573.5 ± 44 369.9	<**0.0005**[*]
Total cholesterol (mg/dL)	134.6 ± 38.2	97.5 ± 82.8	**0.001**[*]
Triglycerides	92.9 ± 36.2	64.8 ± 27.5	<**0.0005**[*]
HDL cholesterol	41.9 ± 14.2	94.6 ± 77.7	<**0.0005**[*]
FPG (mg/dL)	132.4 ± 57.8	122.9 ± 47.3	0.295
Estimated GFR	45.1 ± 22.0	39.8 ± 20.6	0.150
LVEF%	57.4 ± 11.1	55.3 ± 10.1	0.242
LAVI (mL/m^2)	33.0 ± 4.1	32.6 ± 3.7	0.529
LVMI (g/m^2)	110.3 ± 17.9	106.8 ± 13.9	0.213
CAD, n (%)	31 (45.6)	29 (42.6)	0.863
Previous cerebrovascular diseases, n (%)	15 (22)	10 (14.7)	0.376
PAD, n (%)	10 (14.7)	7 (10.3)	0.605
Chronic renal disease, n (%)	22 (32.3)	21 (30.88)	0. 561
Diabetes, n (%)	23 (33.82)	28 (35.8)	0.485
Hypertension, n (%)	66 (97.0)	53 (77.9)	**0.001**[*]
Valvular heart disease, n (%)	20 (29.4)	20 (29.4)	1.0
Ischaemic dilated cardiomyopathy	33 (48.52)	31 (45.58)	0.45
Non-ischaemic dilated cardiomyopathy	11 (16.17)	10 (14.70)	0.37
Atrial fibrillation, n (%)	30 (44.1)	22 (32.3)	0.217
Anaemia, n (%)	13 (19.1)	11 (16.1)	0.822
Smoking, n (%)	21 (30.8)	18 (26.4)	0.705
Resting dyspnoea, n (%)	12 (17.64)	11 (16.17)	0.97
Work/effort dyspnoea	61 (89.7)	61 (89.7)	1.0
Peripheral oedema, n (%)	64 (94.1)	63 (92.6)	1.0
Jugular vein distension, n (%)	14 (20.5)	11 (16.17)	0.42
Causes of decompensation (%)			
Inappropriate drug reduction	38 (55.8)	36 (52.9)	0.33
Uncontrolled hypertension	16 (23.5)	15 (22.05)	0.37
Arrhythmias	14 (20.5)	17 (25)	0.36
NYHA class			
II	12 (17.64)	11 (16.17)	0.32
III	44 (64.7)	46 (67.64)	0.37
IV	12 (17.64)	11 (16.17)	0.42
Mean daily dosage of intravenous furosemide (mg)	156 ± 22	160 ± 26	0.152
ACE inhibitors, n (%)	51 (75)	49 (72.05)	0.375
ARBs, n (%)	8 (11.7)	10 (14.7)	0.235
Beta-blockers, n (%)	42 (61.7)	41 (60.2)	0.333
MRA (%)	12 (17.6)	13 (10.11)	0.221

Pts treated with i.v. furosemide plus HSS: patients randomized to i.v. high-dosage furosemide plus small volume of hypertonic saline solutions (HSS); controls: patients randomized to i.v. high-dosage furosemide alone. ARBs, angiotensin II receptor blockers; BMI, body mass index; CAD, coronary artery disease; DBP, diastolic blood pressure; FPG, fasting plasma glucose; GFR, glomerular filtration rate; HDL, high density lipoprotein; LAVI, left atrial volume index; LDL, low density lipoprotein; LVEF, left ventricular ejection fraction; LVMI, left ventricular mass index; MRA, mineralocorticoid receptor antagonist; NYHA, New York Heart Association; PAD, peripheral artery disease; SBP, systolic blood pressure; WBC, white blood cells.
Bold values indicate significant value of the $P < 0.05$.
[*]$P < 0.05$.

Congestive heart symptoms frequency in subjects treated with i.v. furosemide + HSS versus subjects treated with i.v. furosemide alone

With regard to congestive heart symptoms, 12 (17.64%) subjects in the furosemide + HSS group and 11 (16.17%) subjects in the furosemide alone group complained of resting dyspnoea, 61 (89.7%) in the furosemide + HSS group and 61 (89.7%) in the furosemide alone group complained of work/effort dyspnoea, and 64 (94.1%) in the furosemide + HSS group and 63 (92.6%) in the furosemide alone group complained of peripheral oedema.

At T1 after the 6 day course of therapy with high-dose furosemide + HSS or furosemide alone, two (2.94%;

intragroup P = 0.013) subjects in the furosemide + HSS group and four (5.88%; intragroup P = 0.81) subjects in the furosemide alone group complained of resting dyspnoea (between-group P = 0.24), 11 (16.17%, intragroup P = 0.0001) in the furosemide + HSS group and 21 (30.88%; intragroup P = 0.0001) in the furosemide alone group complained of work/effort dyspnoea (between-group P = 0.044), and 14 (20.58%; intragroup P = 0.0001) in the furosemide + HSS group and 25 (36.76%; intragroup P = 0.0001) in the furosemide alone group complained of peripheral oedema (between-group P = 0.038).

Inflammatory, stretching, and myocardial stress marker serum levels after 6 days of treatment with i.v. furosemide + HSS versus treatment with i.v. furosemide alone

Concerning inflammatory, stretching, and myocardial stress markers, subjects treated with furosemide plus HSS versus subjects treated with i.v. furosemide alone showed no significant difference in the mean serum levels of IL-6, hsTnT, galectin-3, and NT-proBNP at baseline, whereas in subjects treated with furosemide + HSS in comparison with subjects treated with furosemide alone, we observed a significantly higher mean serum value of ST2 (41.2 ± 8.6 pg/mL vs. 37.1 ± 6.6 pg mL, P = 002).

'In-group' and 'between-group' analyses of changes of inflammatory, stretching, and myocardial stress marker serum levels after 6 days of treatment with i.v. furosemide + HSS versus treatment with i.v. furosemide alone

In 'in-group' analyses, between T0 and T1, patients treated with intravenous moderate/high-dose furosemide plus HSS and controls treated with i.v. furosemide alone showed a significant degree of reduction in the serum levels of IL-6 (2.3 ± 1.2 vs. 1.7 ± 0.9, $P < 0.0005$, and 2.0 ± 0.8 vs. 1.85 ± 1.1, P = 0.034, respectively), sST2 (41.2 ± 8.6 vs. 27.9 ± 7.6, $P < 0.0005$, and 37.1 ± 6.6 vs. 28.4 ± 6.7, $P < 0.0005$), hsTnT (0.03 ± 0.02 vs. 0.02 ± 0.01, P = 0.001, and 0.03 ± 0.02 vs. 0.02 ± 0.01, P = 0.009), NT-proBNP (7237 ± 7931 vs. 3244 ± 4159, $P < 0.005$, and 5381 ± 4829 vs. 4466 ± 4332, P = 0.004), and galectin-3 (15.7 ± 3.2 ng/mL vs. 11.68 ± 1.9 ng/mL, $P < 0.0005$, and 16.7 ± 3.9 ng/mL vs. 11.8 ± 2.4 ng/mL, $P < 0.0005$) (*Table 2*).

Nevertheless, in the 'between-group' analyses, we observed no significant difference in the degree of reduction in the serum levels of IL-6, hsTnT, NT-proBNP, ST2, galectin-3, and sST2 between patients treated with i.v. furosemide plus HSS in comparison with patients treated with i.v. furosemide alone (*Table 2*).

Table 2 Myocardial stress variable values, after furosemide + HSS or furosemide therapy at T0 (baseline), at T1 (after 6 days of treatment with i.v. furosemide + HSS or i.v. furosemide alone), and at T2 after saline load (after a saline load administered after the end of treatment with i.v. furosemide) in subjects with acute decompensated HFrEF

Variable	Groups	Admission T0	Between-group P	T1 after 6 days of treatment	Between-group P	In-group P	T2 after acute saline load	Between-group P	In-group P
IL-6 (pg/mL)	Furosemide alone group	2.0 (0.8)	0.171	1.85 (1.1)	0.359	0.034*	3.3 (1.2)	<0.0005*	<0.0005*
	Furosemide + HSS group	2.3 (1.2)		1.70 (0.9)		<0.0005*	1.9 (0.9)		<0.0005*
hsTnT (ng/mL)	Furosemide alone group	0.03 (0.02)	0.596	0.02 (0.01)	0.962	0.009*	0.03 (0.03)	<0.0005*	0.006*
	Furosemide + HSS group	0.03 (0.02)		0.02 (0.01)		0.001*	0.02 (0.01)		1.0
sST2 (ng/mL)	Furosemide alone group	37.1 (6.6)	0.002*	28.4 (6.7)	0.678	<0.0005*	43.0 (6.8)	<0.0005*	<0.0005*
	Furosemide + HSS group	41.2 (8.6)		27.9 (7.6)		<0.0005*	36.3 (7.6)		<0.0005*
Gal-3 (ng/mL)	Furosemide alone group	16.7 (3.9)	0.122	11.8 (2.4)	0.668	<0.0005*	18.9 (3.2)	<0.0005*	<0.0005*
	Furosemide + HSS group	15.7 (3.2)		11.6 (1.9)		<0.0005*	13.8 (2.1)		<0.0005*
CRP (mg/dL)	Furosemide alone group	2.2 (0.68)	0.769	2.1 (0.69)	0.979	0.304	2.3 (0.72)	0.226	0.304
	Furosemide + HSS group	2.2 (0.82)		2.1 (0.66)		0.056	2.1 (0.65)		0.056
NT-proBNP (pg/mL)	Furosemide alone group	5381 (4829)	0.102	4466 (4332)	0.096	0.004*	6173 (5214)	<0.0005*	<0.0005*
	Furosemide + HSS group	7237 (7931)		3244 (4159)		<0.0005*	3167 (4041)		<0.0005*

CRP, C-reactive protein; Gal-3, galectin-3; HFrEF, heart failure with reduced ejection fraction; HSS, hypertonic saline solution; hsTnT, high-sensitivity troponin T; IL-6, interleukin-6; NT-proBNP, N-terminal pro-brain natriuretic peptide; sST2, soluble suppression of tumorigenicity 2.
Bold values indicate significant value of the $P < 0.05$.
*$P < 0.05$.

Analysis of absolute and percentage 'in-group' delta (Δ) values of inflammatory, stretching, and myocardial stress marker serum levels after 6 days of treatment with i.v. furosemide + HSS versus treatment with i.v. furosemide alone

Furthermore, the evaluation of the comparison of absolute 'in-group' delta (Δ) values at T0–T1, representing the difference in the values of serum levels of the chosen markers between baseline (T0) and after the treatment period with furosemide + HSS or furosemide alone (T1), showed that patients treated with high-dose furosemide + HSS compared with patients treated with furosemide alone had a significantly higher Δ value of IL-6 (0.57 ± 0.73 vs. 0.18 ± 0.75 pg/mL, $P = 0.002$), sST2 (13.3 ± 8.3 vs. 8.7 ± 4.6, $P < 0.0005$), and NT-proBNP (3992 ± 5438 vs. 915 ± 2136, $P < 0.0005$) (Table 3), whereas we found no significant difference with regard to the 'in-group' Δ values of hsTnT, galectin-3, and CRP (Table 3).

The evaluation of the 'in-group' percentage delta (% Δ) value at T0–T1, representing the percentage value of the increase or decrease in serum levels of the chosen markers between baseline (T0) and after the treatment period with furosemide + HSS or furosemide alone (T1), showed that patients treated with high-dose furosemide + HSS compared with patients treated with furosemide alone have a significantly higher 'in-group' % Δ value of IL-6 with a percentage reduction of 25.11% versus 8.87% ($P < 0.005$), an sST2 serum level percentage reduction of 32.28% versus 23.50% ($P < 0.0005$), and an NT-proBNP percentage reduction of 55.16% versus 17% ($P < 0.0005$) (Table 4).

Analysis of absolute and percentage 'in-group' delta (Δ) values of inflammatory, stretching, and myocardial stress marker serum levels after acute saline load in subjects treated with i.v. furosemide + HSS versus subjects treated with i.v. furosemide alone

After acute saline load (at T2), analysis of the absolute 'in-group' delta (Δ) value at T1–T2, representing the difference value between serum levels of the chosen markers after the treatment period with furosemide + HSS or furosemide alone (T1) and these serum levels after an acute saline load (T2), showed that patients treated with high-dose furosemide + HSS compared with patients treated with furosemide alone had significantly lower 'in-group' absolute Δ values of IL-6 (−0.26 ± 0.42 pg/mL vs. −1.43 ± 0.86 pg/mL, $P < 0.0005$), hsTnT (0 vs. −0.02 ± 0.02 ng/mL, $P < 0.0005$), sST2 (−8.5 ± 5.9 ng/mL vs. −14.6 ± 6.2 ng/mL, $P < 0.0005$), galectin-3 (−2.1 ± 1.5 ng/mL vs. −7.1 ± 3.6 ng/mL,

Table 3 Absolute delta values (Δ) in cases and controls at T1 (T0–T1) and T2 time (T1–T2) in subjects treated with i.v. furosemide + HSS or i.v. furosemide alone

Variable	Groups	Delta 0–1	Between-group P	Delta 0–2	Between-group P	Delta 1–2	Between-group P
Δ IL-6	Furosemide alone group	0.18 (0.75)	**0.002***	−1.25 (0.96)	**<0.0005***	−1.43 (0.86)	**<0.0005***
	Furosemide + HSS group	0.57 (0.73)		0.31 (0.66)		−0.26 (0.42)	
Δ hsTnT	Furosemide alone group	0.01 (0.02)	0.470	−0.005 (0.02)	**<0.0005***	−0.02 (0.02)	**<0.0005***
	Furosemide + HSS group	0.008 (0.01)		0.008 (0.01)		0.0 (0.0)	
Δ sST2	Furosemide alone group	8.7 (4.6)	**<0.0005***	−5.89 (6.3)	**<0.0005***	−14.6 (6.2)	**<0.0005***
	Furosemide + HSS group	13.3 (8.3)		4.85 (7.6)		−8.5 (5.9)	
Δ Gal-3	Furosemide alone group	4.89 (4.2)	0.210	−2.2 (4.2)	**<0.0005***	−7.1 (3.6)	**<0.0005***
	Furosemide + HSS group	4.09 (3.1)		1.97 (3.4)		−2.1 (1.5)	
Δ CRP (mg/dL)	Furosemide alone group	0.05 (0.82)	0.771	−0.09 (0.96)	0.236	−0.14 (0.91)	0.281
	Furosemide + HSS group	0.09 (0.57)		0.09 (0.82)		0.004 (0.66)	
Δ NT-proBNP (pg/mL)	Furosemide alone group	915 (2136)	**<0.0005***	−791 (1245)	**<0.0005***	−1706 (2259)	**<0.0005***
	Furosemide + HSS group	3992 (5438)		4069 (5566)		77 (1373)	

CRP, C-reactive protein; Gal-3, galectin-3; HSS, hypertonic saline solution; hsTnT, high-sensitivity troponin T; IL-6, interleukin-6; NT-proBNP, N-terminal pro-brain natriuretic peptide; sST2, soluble suppression of tumorigenicity 2.
Bold values indicate significant value of the $P < 0.05$.
*$P < 0.05$.

Table 4 Percentage delta (% Δ) values in cases and controls at T1 (T0–T1) and T2 time (T1–T2)

Variable	Groups	T0	T1	T2	Delta 0–1	D 0–1%	Delta 0–2	D 0–2%	Delta 1–2	D 1–2%
% Δ IL-6	Furosemide alone group	2.03	1.85	3.28	0.18	**8.87***	−1.25	−61.58	−1.43	**77.30***
	Furosemide + HSS group	2.27	1.7	1.96	0.57	**25.11****	0.31	13.66	−0.26	**15.29*****
% Δ hsTnT	Furosemide alone group	0.03	0.02	0.03	0.01	33.33	−0.005	−16.67	−0.02	**100.00***
	Furosemide + HSS group	0.03	0.02	0.02	0.008	26.67	0.008	26.67	0	**0.00*****
% Δ sST2	Furosemide alone group	37.1	28.4	43	8.72	**23.50***	−5.89	−15.88	−14.6	**51.41***
	Furosemide + HSS group	41.2	27.9	36.3	13.3	**32.28*****	4.85	11.77	−8.5	**30.47*****
% Δ galectin-3	Furosemide alone group	16.7	11.8	18.9	4.89	29.28	−2.2	−13.17	−7.1	**60.17***
	Furosemide + HSS group	15.7	11.6	13.8	4.09	26.05	1.97	12.55	−2.1	**18.10*****
% Δ CRP	Furosemide alone group	2.2	2.1	2.3	0.05	2.27	−0.09	−4.09	−0.14	6.67
	Furosemide + HSS group	2.2	2.1	2.1	0.09	4.09	0.09	4.09	0.004	0.19
% Δ NT-proBNP	Furosemide alone group	5381	4466	6173	915	**17.00***	−791	−14.70	−1706	**38.20***
	Furosemide + HSS group	7237	3244	3167	3992	**55.16*****	4069	56.22	77	**2.37*****

CRP, C-reactive protein; HSS, hypertonic saline solution; hsTnT, high-sensitivity troponin T; IL-6, interleukin-6; NT-proBNP, N-terminal pro-brain natriuretic peptide; sST2, soluble suppression of tumorigenicity 2.
Bold values indicate significant value of the $P < 0.05$.
*$P < 0.05$.
**$P < 0.005$.
***$P < 0.0005$.

$P < 0.0005$), and NT-proBNP (77 ± 1373 vs. −1706 ± 2259 pg/mL, $P < 0.0005$) (Table 3).

The evaluation of the 'in-group' percentage delta (% Δ) value of T1–T2, representing the percentage change in the levels of the analysed markers after the treatment period with furosemide + HSS or furosemide alone (T1) and these serum levels after an acute saline load (T2), showed a significantly lower 'in-group' % Δ in subjects treated with furosemide + HSS versus subjects treated with furosemide alone with a percentage increase in serum IL-6 values of 15.29% versus 77.30% ($P < 0.0005$), in serum ST2 of 30.47% versus 51.41% ($P = 0.02$), in hsTnT delta of 0% versus 100% ($P < 0.0005$), in galectin-3 of 18.10% versus 60.17% ($P < 0.0005$), and in NT-proBNP of 2.37 versus 38.20 ($P < 0.0005$) (Table 4).

Discussion

Our findings show that in subjects with acute decompensated HFrEF, treatment with moderate/high doses of i.v. furosemide plus small volumes of hypertonic saline compared with treatment with i.v. furosemide alone is associated in both groups with a significant but comparable reduction in the serum levels of IL-6, sST2, hsTnT, galectin-3, and NT-proBNP.

A previous study[25] conducted in 94 patients with refractory CHF who received intravenous furosemide (500 to 1000 mg) plus HSS twice a day showed a significant increase in daily diuresis and natriuresis and a significantly faster reduction in BNP levels, shorter hospitalization stay, and lower incidence in readmissions in the 30 days of study period.

Another recent study[25] aimed to examine whether there was a difference in the reduction in plasma BNP levels and

in LV functional recovery between CHF patients treated with long-acting diuretics (the azosemide group) and short-acting diuretics (the furosemide group). This study reported that the decrease in plasma BNP levels was larger in the azosemide group than in the furosemide group ($P < 0.01$).

Thus, our study is consistent with previous studies[19–21,25] reporting a more significant clinical improvement after treatment with i.v. furosemide + HSS, whereas no previous studies analysed the effects of treatment with intravenous furosemide on other HF markers, such as inflammatory and fibrosis markers. Nevertheless, our study reports comparable degrees of reduction in serum levels of the inflammatory marker IL-6, fibrosis markers sST2 and galectin-3, the myocardial damage marker hsTnT, and the stretching marker NT-proBNP in patients randomized to intravenous furosemide plus HSS in comparison with subjects randomized to furosemide alone.

Our findings concerning stretching and inflammatory markers (NT-proBNP and IL-6) seem to be inconsistent with our previous study[22] reporting that after treatment with high-dose furosemide + HSS compared with treatment with furosemide alone, we observed a significant decrease in ANP, BNP, TNF-α, IL-1β, and IL-6. Nevertheless, with the exception of IL-6 in the current study, we evaluated different novel HF markers, such as fibrosis and myocardial damage markers, and we used different furosemide dosages, thus partially explaining the different findings of our current study. Furthermore, in our current study, we evaluated NT-proBNP serum levels.

Although the 'between-group' analysis showed no significant differences in the degree of reduction of the chosen markers, the 'in-group' analysis showed a significant difference in the 'in-group' absolute and percentage delta values at T1 of HF markers. These findings may partially corroborate the conclusion of our previous study[25] reporting higher

efficacy of high-dose furosemide plus HSS on the modulation of the neurohormonal cascade related to HF pathogenesis. Nevertheless, these conflicting results may be because the evaluation of the 'in-group' absolute delta performed significantly better than the simple 'between-group' comparison to detect the higher efficacy of i.v. furosemide plus HSS in modulating the serum levels of the chosen biomarkers.

It has been identified that biologically active molecules such as cytokines are expressed in the setting of HF.[26-31] TNF-α and IL-6 levels are elevated and correlate with disease severity in HF.[9] Previous studies have noted that optimization of background standard therapy of HF with diuretics, angiotensin-converting enzyme inhibitors, beta-blockers, and digoxin can result in significant reductions in circulating levels of TNF and IL-6.[30]

Among a great number of new candidates, sST2 is the most promising biomarker according to recent studies, and ST2 is a member of the IL-1 receptor family, also known as IL-1 receptor-like 1.[32–34] Some authors[32,35] reported that it could be expressed by cardiac cells in response to myocardial stress.

In another study,[36] the explanatory baseline biomarker model of a poor diuretic response included low potassium, chloride, haemoglobin, and myeloperoxidase and high blood urea nitrogen, albumin, triglycerides, ST2, and neutrophil gelatinase-associated lipocalin. This result could be consistent with a possible direct relationship between sST2 level reduction and the therapeutic response to high-dosage furosemide. Previous studies by our own group demonstrated higher efficacy in terms of relief of signs of congestion of therapy with high-dose furosemide plus HSS than furosemide alone. Thus, the findings of our current study concerning a comparable degree of reduction in the serum levels of three cardinal biomarkers do not seem to be directly linked to the degree of congestion relief with a more rapid achievement of a clinical compensation state. Previous studies[19–21] have reported a higher efficacy of furosemide plus HSS in comparison with furosemide alone regarding congestion signs. These findings were also confirmed in our current study, with a better profile of mean diuresis increase, body weight reduction,[34] and clinical improvement in patients treated with high-dose furosemide + HSS in comparison with the i.v. furosemide alone group. The explanation of our findings could be that HSS do not exert immediate direct possible effects on the remodelling process with possible effects on inflammatory and fibrotic pathways, as hypothesized in our previous study.[22] Nevertheless, the addiction of HSS on intravenous furosemide therapy may have positive effects on clinical practice in terms of possible pleiotropic effects on remodelling and inflammatory markers linked to CHF syndrome.

Several pathophysiological stimuli, such as pressure and volume overload, trigger the remodelling cascade, a process that initially confers protection to the heart as a compensatory mechanism.[37]

Mehta and Griendling[38] reported the effects of HSS on the expression of human polymorphonuclear leukocyte adhesion molecules, suggesting a sodium-specific inhibitory effect on the up-regulation of b2-integrins of N-formyl-L-methionyl-L-leucyl-phenylalanine (fMLP)-stimulated human polymorphonuclear leukocytes (PMNLs).

We also observed that in subjects treated with high-dose furosemide plus HSS, after reaching the clinical compensation state and being subjected to a volume load challenge with an acute saline load obtained by means of rapid intravenous administration of saline solution, they experienced an increase in the serum levels of IL-6, sST2, and galectin-3 but not of NT-proBNP. We reported a significantly lower degree of increase in IL-6, sST2, and galectin-3 in subjects treated with intravenous furosemide + HSS than in subjects treated with furosemide alone.

Although the exact mechanism of action of hypertonic saline is unclear, a few hypotheses have been implicated. Furosemide reaches the intraluminal site of nephrons, where it exerts its function via active secretion from proximal tubules. Most patients with decompensated HF develop hypovolemia and decreased renal blood flow, which impairs the active secretion process.[37] Administration of hypertonic saline has been shown to increase intraluminal furosemide concentrations as well as to increase 24 h of diuresis, urinary sodium levels, and urinary osmolarity.[39] Another aspect of reduced renal blood flow is the overactivation of the tubuloglomerular feedback mechanism, which may be defined as a vasomotor response to tubular osmolarity and sodium concentrations detected by macula densa cells.[40] Correction of such a compensatory feedback mechanism by means of hypertonic saline treatment and many other drugs, such as mannitol and dextran, that may attract extravascular volume towards intravascular compartments has been proposed.[41,42] Moreover, hypertonic saline was shown to cause a decrease in plasma renin activity and ANP levels.[43–47]

Few studies have examined the effect of intravenous diuretic treatment on markers of neurohormonal and immunoinflammatory pathways. The effect of reducing IL-6 and other markers can be reasonably attributed to the HSS, which would therefore enhance the action of furosemide. Haemodynamic overload resulted in an albeit transient increase in markers, suggesting significant stretching of cardiomyocytes. Treatment with high doses of furosemide and hypertonic solution could, by reducing the volume overload and causing a rapid elevation of extracellular NaCl concentrations, be responsible for a reduction in the stretching of cardiomyocytes and affect NPs and immune-inflammatory serum markers.[22] Thus, our findings regarding a more significant in-group increase in IL-6, sST2, and NT-proBNP serum levels after an acute saline load in subjects treated with furosemide alone in comparison with subjects treated with high-dose furosemide plus HSS are possibly related to the

modulation of the myocardial stretching response to volume overload.

In our present study, at a compensated state, reached by both groups of enrolled subjects, those treated with combination therapy showed a lower degree of release of markers of inflammation, stretching, and fibrosis, such as IL-6, sST2, and galectin-3, after a saline load. This finding may be due to a possible better de-remodelling due to treatment with high-dose furosemide plus HSS or to a better clinical compensated state now fully expressed only by the NYHA class in these subjects but that could also be expressed by the lower response in terms of fibrosis, stretching, and inflammatory markers to an acute saline load.

Myocardial stretch, as a result of acute haemodynamic overload, is one of the most frequent challenges to the heart, and the ability of the heart to intrinsically adapt to it is essential to prevent circulatory congestion. Cardiac stretch also stimulates cardiomyocytes to release cardiac natriuretic hormones, namely, ANP and BNP. Both exert their cardiac effect by activating cell surface-associated particulate guanylate cyclase A, which in turn increases the concentration of cGMP in the subsarcolemmal compartment.[39,40] The myocardial response to acute stretch represents a fundamental adaptive capacity of the heart. Nevertheless, our findings concerning a lower degree of increase in inflammatory and myocardial stretching markers after an acute saline load could suggest a possible modulatory role of HSS plus a moderate/high dose of furosemide in subjects with HFrEF. This issue may have possible benefits on clinical practice concerning its therapeutic effects over and beyond the simple amelioration of clinical congestion signs and symptoms. The association between i.v. furosemide plus HSS may permit to obtain a possible de-remodelling effect in terms of atrial stretch and fibrosis modulation.

Possible limitations

Our study has limitations related to the number of patients enrolled, which is certainly worthy of further increase, to the different dosages of furosemide administered, and to the different aetiologies (ischaemic, dilative, valvular, etc.). A continuation (already underway) of the recruitment and stratification of patients by type of decompensation and pathogenesis is therefore desirable.

Another possible limitation is due to the higher serum values of sST2 at time T0 in subjects randomized to i.v. furosemide + HSS compared with subjects treated with i.v. furosemide alone.

We also observed that after acute saline load (at T2), patients treated with i.v. furosemide + HSS for 6 days showed an increase in the serum concentrations of IL-6, sST2, and galectin-3 (albeit less significantly), which were greater than at T1 (end of i.v. therapy) but still lower than the basal T0

sample. Instead, the concentrations of NT-proBNP continued to decrease despite the saline load. This could be explained by the slower kinetics of this molecule, in accordance with what was also proposed by Maningas *et al*.,[41] so we could have observed an increase by further sampling at 24–48 h. However, no significant changes in PCR and hsTnT were observed.

Furthermore, considering the higher mean systolic blood pressure values observed in subjects of the furosemide/HSS group, this may have had a confounding effect on our results.

Another possible limitation is due to the discrete variability of i.v. furosemide dosages in both enrolled groups; thus, it is not possible to clearly evaluate the real effect of the furosemide dosage on CHF biomarkers after both treatment periods.

Conclusions

Our findings confirm higher clinical efficacy of i.v. high-dose furosemide plus HSS in comparison with treatment with i.v. furosemide alone. Nevertheless, we showed a comparable degree of reduction in the serum levels of markers of atrial stretching, fibrosis, and inflammation after treatment with high-dose furosemide plus HSS. Thus, the addition of HSS does not have additional effects on fibrosis and stretching markers of CHF. These findings do not seem to be directly linked to the degree of congestion relief with a more rapid achievement of a clinical compensation state in the furosemide + HSS group. Nevertheless, we showed that after a saline load, subjects treated with high-dose furosemide plus HSS showed a lower increase in some serum markers of HF, as expressed by lower delta values in terms of differences between the serum levels of these markers prior to and after the saline load.

Conflict of interest

No conflicts or competing interests are declared.

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Author contributions

A.T. drafted the manuscript and performed the study conception. C.M. drafted the manuscript and collected the data. D. D.R. and R.F. collected the data. V.V. collected the data and

critically revised the manuscript. M.G.P., T.D., A.M., A.D., M.D., and A.O. collected the data. A.P. critically revised the manuscript and supervised the study.

Ethics approval

The study was approved by the Ethics Committee of the Policlinico 'P. Giaccone' of Palermo, Italy.

Consent to participate

Written informed consent to participate in the study was obtained from all patients.

Consent for publication

Written informed consent to participate in the study was obtained for all patients.

Availability of data and material

The database is available on figshare.

Code availability

Not applicable.

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