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Original Article

C-reactive protein reduction with sacubitril-valsartan treatment in heart failure patients

Antonio Valentim Goncalves, Tiago Pereira-da-Silva, Ana Galrinho, Pedro Rio, Luísa Moura Branco, Rui Soares, Rita Ilhao Moreira, Rui Cruz Ferreira

Department of Cardiology, Hospital de Santa Marta, Centro Hospitalar Universitário de Lisboa Central, Lisbon, Portugal

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Abstract: Objective: C-Reactive Protein (CRP) has emerged as an accessible measured product of inflammation. Whether systemic inflammation, a common feature of Heart Failure (HF), can be reduced by HF treatments in not well established. Sacubitril/Valsartan had prognosis benefit demonstrated in the PARADIGM-HF trial and was able to reduce proinflammatory cytokines in preclinical animal studies. However, no human studies evaluated if the benefits of this therapy are mediated by anti-inflammatory effects too. The aim of this study was to prospectively compare CRP values before and six months after Sacubitril-Valsartan therapy. Methods: Prospective evaluation of chronic HF patients with left ventricular ejection fraction ≤ 40% despite optimized standard of care therapy, in which Sacubitril/Valsartan therapy was started and no additional HF treatment was expected to change. Clinical, laboratorial (including CRP values), electrocardiographic, transthoracic echocardiography and cardiopulmonary exercise test (CPET) data were gathered in the week before starting Sacubitril/Valsartan therapy and six months thereafter. Results: There were 42 patients with a mean age of 59 ± 11 years, of which 35 completed the six months of follow-up, since 2 patients died and 5 discontinued treatment for adverse events. Patients with baseline CRP values above the median (> 2.5 mg/L) had a significantly higher percentage of New York Heart Association class ≥ III (65% vs. 33%, P=0.028) and a reduced exercise time in CPET (361 ± 297 vs. 575 ± 265 seconds, P=0.034). After 6 months of Sacubitril-Valsartan therapy, 24 (69%) patients had an improvement in CRP values with a significantly reduction as compared to baseline (median 2.5 mg/L (Interquartile range (IQR) 1.3-5.0) vs. 2.2 mg/L (IQR 0.9-4.0), P=0.014 in the Wilcoxon test). In the group of 17 (49%) patients with at least 25% improvement in CRP values with Sacubitril/Valsartan therapy, the benefit of several clinical, CPET and echocardiographic parameters were not significantly different from the benefit of patients with no improvement or an improvement inferior to 25% in CRP values. Conclusion: Sacubitril/Valsartan therapy was able to reduce CRP values in a chronic HF population. Whether this reduction was only a consequence of clinical improvement with Sacubitril/Valsartan or an anti-inflammatory effect is also present should be further evaluated.

Keywords: Sacubitril-valsartan, anti-inflammatory effect, C-reactive protein, heart failure

Introduction

Systemic inflammation can be a cause or effect of Heart Failure (HF) [1], since it is an etiology of HF but is also a marker of poor outcome independent of other HF traditional prognosis predictors such as peak oxygen consumption (pVO_2), left ventricular ejection fraction and New York Heart Association (NYHA) functional class [2-4].

Previous trials validated the association between inflammation and HF [5]. However, clinical trials of therapies targeting inflammatory mechanisms in HF had not demonstrate proven benefit so far [6-10].

C-reactive protein (CRP) is elevated in HF patients [11-13], with higher levels associated with a worse outcome [14]. Whether treatments with prognosis benefit in HF patients, such as angiotensin-converting enzyme inhibitors (ACEI), angiotensin II receptor blocker (ARB), beta-blockers and mineralocorticoid receptor antagonist (MRA), can significantly reduce CRP levels have not been consistently demonstrated.

Sacubitril/Valsartan is a new HF treatment that is changing the prognosis of HF patients since the PARADIGM-HF trial publication. In this trial, the combination of neprilysin inhibition and Valsartan reduced the composite of HF hospi-

talization and cardiovascular mortality in 20% in comparison with Enalapril [15]. As a result, Sacubitril/Valsartan has a Class I recommendation as a replacement for an ACEI to ambulatory patients with HF reduced ejection fraction who remain symptomatic despite optimal treatment with an ACEI (or in alternative if not tolerated an ARB), a beta-blocker and a MRA [16]. In an animal study, Sacubitril/Valsartan was able to reduce proinflammatory cytokines [17]. However, no human studies evaluated if the benefits of this therapy are mediated by anti-inflammatory effects too.

The purpose of this study was to prospectively compare CRP values changes with Sacubitril/Valsartan therapy in a group of chronic HF patients with previous optimized HF therapy.

Methods

The investigation conforms to the principles outlined in the Declaration of Helsinki. The institutional ethics committee and the national committee for patient information protection approved the study protocol (CNPD authorization number 5962). All patients provided written informed consent.

Patient population

The study included a prospective single centre analysis, in which patients were included from October 2017 to June 2018.

During this period, all ambulatory patients with optimized standard of care therapy for chronic HF, left ventricular ejection fraction ≤ 40% and NYHA class ≥ II, were proposed to start Sacubitril/Valsartan therapy according to the current Guidelines [16].

Definition of chronic HF with optimized standard of care therapy

Optimized standard of care therapy for chronic HF was defined as treatment for more than six months of maximum tolerated dose of an ACEI or ARB as appropriated, a beta-blocker and a MRA. ICD and/or cardiac resynchronization therapy (CRT) if indicated by the current Guidelines and if the subject has been adequately treated per applicable standards for coronary artery disease and mitral regurgitation [16] and no new treatment was expected for the next six months.

Patients were excluded if one of the following

Age < 18 years; Percutaneous coronary revascularization, cardiac surgery, ICD/CRT implantation, atrial fibrillation ablation or percutaneous mitral regurgitation treatment in the last 6 months; Planned percutaneous coronary revascularization, cardiac surgery, ICD/CRT implantation, atrial fibrillation ablation or mitral regurgitation treatment for the following 6 months; Glomerular filtration rate < 30 ml/min; Baseline potassium values > 5.5 mEq/L; Child-Pugh class B or C; Anti-inflammatory treatment, except for the vascular dose of Aspirin.

Study protocol

All patients provided written informed consent. Thereafter clinical, laboratorial, electrocardiographic, transthoracic echocardiography and cardiopulmonary exercise test (CPET) were obtained in the week before starting Sacubitril/Valsartan therapy.

A washout period of 36 hours was used to allow switching from an ACEI to Sacubitril/Valsartan. Sacubitril/Valsartan therapy was preferentially started at 49/51 mg twice daily or 24/26 mg twice daily in patients with a dose < 10 mg/day of Enalapril or equivalent. The dose was attempted to be doubled every 2 to 4 weeks to reach the target maintenance dose of 97/103 mg twice daily except in patients with systolic blood pressure < 100 mmHg, symptomatic hypotension, hyperkalaemia > 5.5 mEq/L or a decrease in glomerular filtration rate to less than 60 ml/min as assessed by the Cockcroft-Gault equation.

All patients were followed-up for six months from the date of completion of the exams and clinical, laboratorial, electrocardiographic, transthoracic echocardiography and CPET were repeated after the six months of Sacubitril/Valsartan therapy.

CRP and data collection

Plasma CRP (mg/L) and the rest of the laboratory analysis were measured by the ARCHITECT c16000 (Abbott) clinical chemistry analyser before and six months after Sacubitril/Valsartan therapy. Glomerular filtration rate was calculated by the Cockcroft-Gault equation.

Full evaluation was completed before and 6 months after Sacubitril/Valsartan with: A stan-

dard 12-lead electrocardiogram, consisting of 3 limb leads (I, II and III), 3 augmented limb leads (aVR, aVL and aVF) and six precordial leads (V_1-V_6) [18]. A complete transthoracic echocardiogram study, performed by two experienced echocardiographists of our center, using the GE Vivid E95 ultrasound system. LV ejection fraction was calculated by the biplane Simpson's method of discs. A maximal symptom-limited treadmill CPET was performed using the modified Bruce protocol (GE Marquette Series 2000 treadmill). Gas analysis was preceded by calibration of the equipment. Minute ventilation, oxygen uptake and carbon dioxide production were acquired breath-by-breath, using a SensorMedics Vmax 229 gas analyser. The pVO_2 was defined as the highest 30-second average achieved during exercise and was normalized for body mass index [19]. The VE/VCO_2 slope was calculated by least squares linear regression, using data acquired throughout the whole exercise. Patients were encouraged to perform exercise until the respiratory exchange ratio (RER) was ≥ 1.10.

Statistical analysis

Baseline characteristics, were summarized as frequencies (percentages) for categorical variables, as means and standard deviations for continuous variables when normality was verified and as median and interquartile range (IQR) when normality was not verified by the Kolmogorov-Smirnov test (CRP and BNP were the only two variables with no normal distribution). A t test or χ^2 test were used to compare clinical, echocardiographic and CPET information of two groups defined by the median CRP value.

The paired samples t-Test for independent samples or the Wilcoxon test when normality was not verified were used for the analysis of the variables before and after Sacubitril/Valsartan therapy. Statistical differences with a *p* value < 0.05 were considered significant. Data was analysed using the software Statistical Package for the Social Science for Windows, version 24.0 (SPSS Inc, Chicago IL).

Results

Overview of the study population

A total of 42 patients were enrolled in the study. Of the 42 patients, 35 (83.3%) completed the six months follow-up with Sacubitril/Valsartan, since 2 (4.8%) patients died (1 patient with intracranial haemorrhage after trauma and 1 patient with sudden cardiac death) and 5 (11.9%) patients discontinued treatment for adverse events (2 patients with reversible acute kidney injury and 3 patients with symptomatic hypotension with the lowest Sacubitril/Valsartan dose). No patient was lost during the six months follow-up.

Mean age was 58.6 ± 11.1 years, with 29 (82.9%) male patients and an ischemic etiology in 15 (42.9%) patients. These patients were highly symptomatic, as revealed by a NYHA class ≥ III in 51% and by 43% of hospitalizations for worsening HF in the year previously to Sacubitril/Valsartan therapy. All patients were previously doing an ACEI or ARB and a beta-blocker and 94% were doing an MRA. An ICD were previously implanted in 30 (86%) patients, out of which 7 (20%) had a CRT-D system. There were no significant changes regarding the dose expressed as per cent of target dose of beta-blockers (69 ± 29% vs. 71 ± 28%, P=0.278) and MRA (52 ± 19 vs. 53 ± 24%, P=0.352) nor to the loop diuretic dose at baseline and after six months of Sacubitril/Valsartan therapy.

The baseline characteristics of the 35 patients who completed the six months follow-up with Sacubitril/Valsartan were compared regarding they were above or below the median baseline CRP value (2.5 mg/L) in **Table 1**. Patients with baseline CRP values above the median had a significantly higher percentage of NYHA ≥ III (65% vs. 33%, P=0.028) and a reduced exercise time in CPET (361 ± 297 seconds vs. 575 ± 265 seconds, P=0.034). No other significant differences were found.

CRP analysis

The mean CRP value was 5.1 ± 6.7 mg/L baseline and 3.0 ± 2.7 mg/L at 6 months. There were 9 (26%) patients with ≥ 5 mg/L of CRP baseline and 7 (20%) after therapy.

After 6 months of Sacubitril-Valsartan therapy, 24 (69%) patients had an improvement in CRP values with a significantly reduction (median 2.5 mg/L (IQR 1.3-5.0) vs. 2.2 mg/L (IQR 0.9-4.0), P=0.014) as compared to baseline (**Figure 1**). Mean leukocytes values (7857 ± 2289 vs. 7324 ± 2106, P=0.021) were also significantly

Table 1. Baseline characteristics of the study population (n=35) with CPR above and below the median

	CPR ≤ 2.5 mg/L	CPR > 2.5 mg/L	P
Age (years)	56.6 ± 13.4	60.8 ± 7.9	0.265
Male gender	83%	82%	0.939
Ischemic etiology	56%	29%	0.118
Body Mass Index (kg/m^2)	27.3 ± 3.4	29.0 ± 4.1	0.193
NYHA ≥ III	33%	65%	0.028
HF hospitalization in the last year (%)	39%	47%	0.625
Diabetes mellitus	28%	35%	0.632
Atrial Fibrillation	33%	47%	0.407
Leukocytes (× 10^9/L)	7932 ± 2201	7778 ± 2445	0.846
Glomerular Filtration Rate (mL/min)	91 ± 31	97 ± 29	0.592
Brain Natriuretic Peptide (pg/ml)	270 (127-500)	321 (117-511)	0.882
Heart Rate (bpm)	71 ± 13	74 ± 13	0.449
Left Ventricular End-Diastolic Diameter	72 ± 9	71 ± 8	0.757
Left Ventricular Ejection Fraction (%)	29.6 ± 6.8	29.1 ± 6.2	0.822
Global Longitudinal Strain (%)	-6.6 ± 3.0	-7.4 ± 1.9	0.350
CPET duration (seconds)	575 ± 265	361 ± 297	0.034
Peak oxygen consumption (mL/kg/min)	15.9 ± 5.7	12.6 ± 6.4	0.126
VE/VCO$_2$ slope	36.5 ± 7.7	35.9 ± 7.3	0.838

Values are mean ± standard deviation; BNP values are expressed as median (Interquartile range).

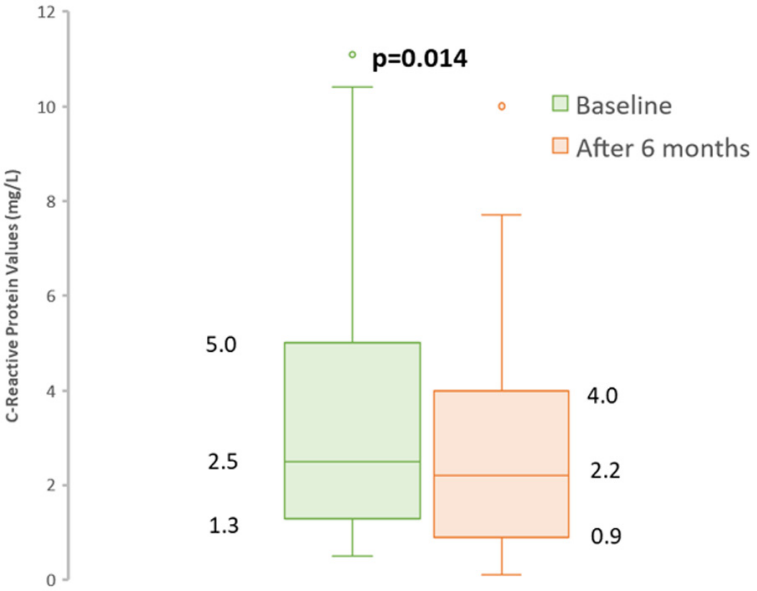

Figure 1. C-Reactive Protein significant reduction (P=0.014, Wilcoxon test) before (green chart) and 6 months after (orange chart) Sacubitril-Valsartan therapy.

reduced after 6 months of Sacubitril/Valsartan therapy (**Figure 2**).

In the group of 17 (49%) patients with at least 25% improvement in CRP values with Sacubitril/Valsartan therapy, the benefit of several clinical, CPET and echocardiographic parameters were not significantly different from the benefit of patients with no improvement or an improvement inferior to 25% in CRP values (**Tables 2** and **3**), since CPET duration, pVO$_2$, VE/VCO$_2$ slope, left ventricular end-diastolic diameter and global longitudinal Strain significantly improved in both groups in a similar numerical way.

Sacubitril/Valsartan dose and CRP analysis

Sacubitril/Valsartan therapy was started 24/26 mg twice a day in 18 (51%) patients and 49/51 mg twice a day in 17 (49%) patients. At six months the dose was 24/26 mg twice a day in 10 (29%) patients, 49/51 mg twice a day in 11 (31%) patients and 97/103 mg twice a day in 14 (40%) patients.

The difference in CRP values between baseline and 6 months were significantly improved in patients at the 24/26 mg (median 2.1 mg/L

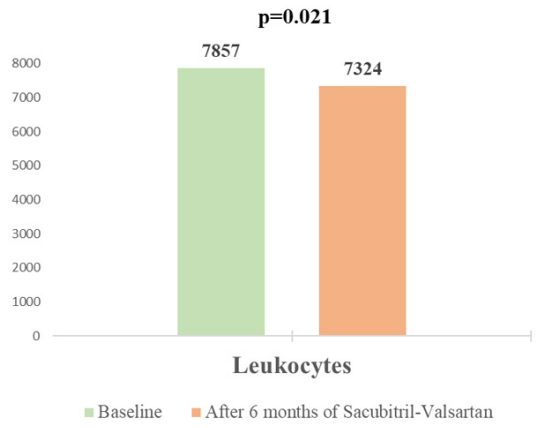

Figure 2. Leukocytes reduction (P=0.021, paired samples t-Test) before (green chart) and 6 months after (orange chart) Sacubitril-Valsartan therapy.

(IQR 0.3-4.7), P=0.005) and 49/51 mg (median 0.6 mg/L (IQR 0.2-1.2), P=0.047) but not at the 97/103 mg Sacubitril/Valsartan dose (median -0.3 mg/L (IQR-0.8-0.2), P=0.258).

Discussion

Systemic inflammation is a common feature of HF. However, recent trial with anti-inflammatory therapies in HF patients have been mostly unsuccessful. These trials included the use of Colchicine [10], Methotrexate [9], Anakinra [8], Etanercept [7, 20] and Infliximab [6], showing that the causal link between inflammation and HF is not well understood and that the direct inhibition of biomarkers of inflammation that are elevated in patients with HF could not be enough to translate into a prognosis benefit [1].

CRP is increased in patients with HF [11-13]. However, if this elevation is a factor contributing to the HF development or solely a consequence of the inflammatory process is not well explained [21]. Patients with baseline CRP values above the median in our trial had a significantly higher percentage of NYHA ≥ III and a reduced exercise time in CPET, showing signs of worse prognosis despite no other significant differences were found in other known prognosis markers of HF such as pVO_2 and left ventricular ejection fraction.

To the best of our knowledge, this was the first prospective study evaluating the CRP changes with Sacubitril/Valsartan therapy. These patients had optimal HF medical therapy, as shown by a numerically higher percentage of patients treated baseline with beta-blockers (100% VS 93.1%), MRA (94.3% VS 52.2%), ICD (85.6% VS 14.9%) and CRT (20% VS 7%) when compared to the PARADIGM-HF trial [15]. However, this was a high-risk group since 51% had NYHA class ≥ III and 43% had at least one hospitalization for worsening HF in the year previously to the study.

The median CRP value before Sacubitril/Valsartan treatment was 2.5 mg/L. This value is similar to the METIS (2.8 mg/L) and the VALHEFT (3.2 mg/L) trials [9, 21]. A numerically higher median value (6.6 mg/L) was found in the TIME-CHF trial [3], while even higher CRP values (12.6 mg/L) can be found in patients with acute HF [4].

Despite the initial CRP median value of our trial was not especially high in comparison with other HF treatments, Sacubitril-Valsartan therapy was able to significantly improve CRP (P=0.014) values after 6 months of treatment (**Figure 1**). Mean leukocytes values were also significantly reduced (**Figure 2**). This information is in line with a significant reduction as compared to Valsartan of proinflammatory cytokines (matrix metalloproteinase-8, IL-6, and monocyte chemoattractant protein-1) with Sacubitril/Valsartan in an animal study [17].

Whether this reduction was only a consequence of clinical improvement with Sacubitril/Valsartan or an anti-inflammatory effect was also present cannot be evaluated from our study, since in the group with the higher improvement in CRP values, the benefit in CPET duration, pVO_2, VE/VCO_2 slope, left ventricular end-diastolic diameter and global longitudinal Strain were not significantly different from the benefit of the other patients with less improvement in CRP values.

However, these results can complement the background regarding the beneficial effects of Sacubitril/Valsartan in the HF population, considering that other treatments with prognosis benefit in HF patients, such as ACEI, ARB, beta-blockers and MRA have not consistently demonstrated to reduce CRP values in HF patients [1, 21].

Surprisingly, patients with the 24/26 mg and 49/51 mg dose at 6 months of sacubitril-val-

Table 2. Differences before and after six months of Sacubitril-Valsartan therapy in patients with at least 25% improvement in CRP

	Time 0	6 months	P
Heart Rate (bpm)	78 ± 13	70 ± 13	0.054
CPET duration (seconds)	543 ± 306	683 ± 256	0.001
Peak oxygen consumption (mL/kg/min)	14.7 ± 6.7	18.9 ± 5.7	0.026
VE/VCO_2 slope	36.5 ± 8.3	30.0 ± 3.6	0.107
Left Ventricular End-Diastolic Diameter	70 ± 7	65 ± 5	0.008
Left Ventricular Ejection Fraction (%)	29.7 ± 6.6	35.1 ± 6.2	0.266
Global Longitudinal Strain (%)	-7.0 ± 2.2	-8.8 ± 2.3	0.786
Glomerular Filtration Rate (mL/min)	93 ± 28	83 ± 25	< 0.001

Values are mean ± standard deviation.

Table 3. Differences before and after six months of Sacubitril-Valsartan therapy in patients without at least 25% improvement in CRP

	Time 0	6 months	P
Heart Rate (bpm)	70 ± 8	66 ± 8	0.502
CPET duration (seconds)	432 ± 270	597 ± 284	< 0.001
Peak oxygen consumption (ml/kg/min)	14.0 ± 5.74	17.7 ± 4.2	0.006
VE/VCO_2 slope	36.8 ± 6.2	32.2 ± 7.3	0.026
Left Ventricular End-Diastolic Diameter	73 ± 9	69 ± 9	0.008
Left Ventricular Ejection Fraction (%)	29.0 ± 6.3	35.4 ± 8.6	0.133
Global Longitudinal Strain (%)	-6.9 ± 3.0	-9.0 ± 3.2	0.009
Glomerular Filtration Rate (mL/min)	91 ± 29	84 ± 17	0.002

Values are mean ± standard deviation.

sartan therapy had significantly improved CRP values but not at the 97/103 mg Sacubitril/Valsartan dose, revealing a reduction in CRP values with sacubitril-valsartan as long as the highest tolerated dose was used. The highest benefit without the 97/103 mg dose is not easy to explain. However, this could be a bias since patients that tolerated the highest Sacubitril-Valsartan dose had lower median CRP baseline values (2.5 mg/L vs. 4.2 mg/L) than patients who cannot tolerate the 97/103 mg dose, possibly making this group of patients less prone to a higher reduction in CRP values with the therapy.

Study limitations

This is a single-centre study and the results were compared between baseline and after six months of Sacubitril/Valsartan therapy without a control group that would continue ACEI or ARB therapy. Nevertheless, after the results of the PARADIGM-HF trial [15], it would not be ethical to leave some patients without a therapy that showed to improve survival.

A strategy to reduce bias by concomitant improvement caused by other therapies other than Sacubitril/Valsartan was attempted by choosing for the study patients with previously optimized standard of care therapy (except for Sacubitril/Valsartan) for more than six months and non-recent major cardiovascular procedure. As so, there were no differences regarding beta-blockers and MRA dosage after six months of therapy or new cardiac surgery or percutaneous coronary intervention during follow-up.

Despite the small sample size, a significant reduction in CRP values may help to explain some of the beneficial effects associated with Sacubitril/Valsartan therapy. However, the study does not explain the potential anti-inflammatory mechanism of action of Sacubitril/Valsartan and therefore these results are hypothesis generating rather than conclusive statements about the anti-inflammatory effect of this therapy.

Median CRP values are even higher in acute HF [4]. Whether this reduction should be higher in this group of patients was not evaluated in our trial, since we only selected chronic ambulatory HF patients.

Conclusions

Systemic inflammation has been linked to disease development, progression and worsening prognosis in HF patients. CRP is a product of inflammation with higher levels independently associated with higher mortality and morbidity in HF patients too. To the best of our knowledge, this was the first study showing that Sacubitril/Valsartan therapy was able to significantly reduce CRP values in a chronic HF population. Whether this reduction was only a consequence of clinical improvement with Sacu-

bitril/Valsartan or an anti-inflammatory effect is also present should be further evaluated in future studies.

These results can complement the background regarding the beneficial effects of Sacubitril/Valsartan in the HF population.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. António Valentim Gonçalves, Department of Cardiology, Hospital de Santa Marta, Rua de Santa Marta, nº 50, 1169-024, Lisbon, Portugal. Tel: +351-961156697; E-mail: antonio.a.goncalves.14@gmail.com

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