ORIGINAL RESEARCH ARTICLE

Neprilysin inhibition, endorphin dynamics, and early symptomatic improvement in heart failure: a pilot study

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Abstract

Aim Sacubitril/valsartan is a first-in-class angiotensin receptor-neprilysin inhibitor developed for the treatment of heart failure with reduced ejection fraction. Its benefits are achieved through the inhibition of neprilysin (NEP) and the specific blockade of the angiotensin receptor AT1. The many peptides metabolized by NEP suggest multifaceted potential consequences of its inhibition. We sought to evaluate the short-term changes in serum endorphin (EP) values and their relation with patients' physical functioning after initiation of sacubitril/valsartan treatment.

Methods and results A total of 105 patients with heart failure with reduced ejection fraction, who were candidates for sacubitril/valsartan treatment, were included in this prospective, observational, multicentre, and international study. In a first visit, and in agreement with current guidelines, treatment with angiotensin-converting enzyme inhibitors or angiotensin receptor blocker was replaced by sacubitril/valsartan because of clinical indication by the responsible physician. By protocol, patients were reevaluated at 30 days after the start of sacubitril/valsartan. Serum levels of α- (α-EP), γ-Endorphin (γ-EP), and soluble NEP (sNEP) were measured using enzyme-linked immunoassays. New York Heart Association (NYHA) functional class was used as an indicator of patient's functional status. Baseline median levels of circulating α-EP, γ-EP, and sNEP were 582 (160–772), 101 (37–287), and 222 pg/mL (124–820), respectively. There was not a significant increase in α-EP nor γ-EP serum values after sacubitril/valsartan treatment (P value = 0.194 and 0.102, respectively). There were no significant differences in sNEP values between 30 days and baseline (P value = 0.103). Medians (IQR) of Δα-EP, Δγ-EP, and ΔsNEP between 30 days and baseline were 9.3 ($-34 − 44$), -3.0 ($-46.0 − 18.9$), and 0 units ($-16.4 − 157.0$), respectively. In a pre–post sacubitril/valsartan treatment comparison, there was a significant improvement in NYHA class, with 36 (34.3%) patients experiencing improvement by at least one NYHA class category. Δα-EP and ΔsNEP showed to be significantly associated with NYHA class after 30 days of treatment ($P = 0.014$ and $P < 0.001$, respectively). Δα-EP was linear and significantly associated with NYHA class improvement after 30 days of sacubitril/valsartan treatment.

Conclusions These preliminary data suggest that beyond the haemodynamic benefits achieved with sacubitril/valsartan, the altered cleavage of endorphin peptides by NEP inhibition may participate in patients' symptoms improvement.

Keywords Heart failure; Neprilysin; Sacubitril/valsartan; Endorphins; α-Endorphin; γ-Endorphin

Introduction

Sacubitril/valsartan is a first-in-class angiotensin receptor neprilysin inhibitor developed for the treatment of heart failure (HF) with reduced ejection fraction (HFrEF). The PARADIGM-HF trial, which tested sacubitril/valsartan for treating HFrEF, showed that it was superior to enalapril in reducing the risk of cardiovascular death and HF hospitalization, as well as improving quality of life of surviving HFrEF patients.[1,2] The benefits of sacubitril/valsartan are achieved via two mechanisms: (i) the inhibition of neprilysin (NEP) causes an increase in the activity of its substrates, such as natriuretic peptides, and (ii) the specific blockade of the angiotensin receptor AT1 instead of the inhibition of the angiotensin-converting enzyme.[3]

Neprilysin is an abundant enzyme that can also be found in its circulating soluble form, which has emerged as a new biomarker with promising implications for prognosis and therapy in patients with HF.[4–6] Its ubiquity allows it to participate in the degradation path of multiple substrates, suggesting many multifaceted potential consequences of its inhibition beyond the natriuretic peptide system.[7] Neuropeptides, including endorphins (EPs), are also recognized substrates that NEP is capable of degrading. α-Endorphin (α-EP) and γ-Endorphin (γ-EP), well-known endogenous opioid peptides, have morphinomimetic behaviour, affect several physiologic activities, and are potential antipsychotic and analgesic drugs.[8] EPs are also capable of producing feelings of welfare and tolerance to pain.[9,10]

In the present work, we aimed to explore the dynamics of serum EPs values in patients with HFrEF upon treatment with sacubitril/valsartan and its relation to the improvement in functional class assessed by New York Heart Association (NYHA) class.

Methods

Study design and participants

A prospective, observational, multicentre, international study was conducted between 1 November 2016 and 1 December 2017. Patients were eligible if they had stable HF with left ventricular ejection fraction ≤40% and were under treatment with angiotensin-converting enzyme inhibitor or angiotensin receptor blocker (*Figure 1*).

In a first visit, and in agreement with current guidelines,[11,12] treatment with angiotensin-converting enzyme inhibitor or angiotensin receptor blocker was replaced by sacubitril/valsartan because of clinical indication by the responsible physician. Doses of sacubitril/valsartan treatment were individualized. By protocol, patients were reevaluated at 30 days after the start of sacubitril/valsartan, and no other therapeutic modifications were performed between both visits. Demographic information, medical history, vital signs, 12-leads electrocardiogram, standard laboratory data, and pharmacological treatments were routinely assessed at visits. A trained cardiologist assessed the NYHA association class in the study visits. The estimated glomerular filtration rate (eGFR) was estimated by the Modification of Diet in Renal Disease Study equation, based on creatinine and other characteristics of the patients. Institutional committees of each participating centre approved the study protocol, local ethics committees approved the study, and all patients provided written informed consent prior to enrolment. This study was conducted in accordance with the Declaration of Helsinki. Patients with presence of extracardiac disease with an estimated life expectancy of less than 1 year, treated with narcotic analgesics, with moderate to severe alcoholism, severe renal (eGFR $<$ 30 mL/min/1.73 m^2) or severe hepatic insufficiency (Child-Pugh C classification), hyperkalaemia ($>$5.4 mmol/L), systolic blood pressure $<$ 100 mmHg, prior history of angioedema, pregnant women, or participating in randomized clinical trial, were excluded.

Measurement of serum endorphins levels

Blood samples were collected and serum was obtained by centrifugation for 10 min at 3000 rpm and stored at −80°C. All samples were obtained between 09:00 am and 12:00 pm using the same protocol at the first visit and at 30 days.

α-EP was measured by Human α-Endorphin ELISA kit (BlueGene Biotech; Shanghai, China; Code No. E01A3095; Lot No. 20161219). Serum aliquots were diluted 1:2 in PBS (pH 7.0–7.2). This assay has high sensitivity and excellent specificity for detecting α-EP, and the manufacturer observed no significant cross-reactivity or interference between α-EP and analogues. The range of the assay is between 0 and 2500 pg/mL. The sensitivity in this assay is 1.0 pg/mL.

γ-EP was analysed by a competitive binding enzyme-linked immunosorbent assay technology, Human γ-Endorphin ELISA kit (Abbexa Ltd.; Cambridge Science Park, Cambridge, UK; Code No. abx258229; Lot No. L201701A884). Assay's range is between 12.35 and 1000 pg/mL, and assay's sensitivity is 5.47 pg/mL.

Measurement of sNEP

Soluble neprilysin was measured by a sandwich immunoassay human neprilysin/CD10 ELISA kit (Aviscera Bioscience; Santa Clara, CA; Code No. SK00724-01; Lot No. 20113187). Serum aliquots were diluted one quarter in dilution buffer provided by the manufacturer before incubation. Standard range and

Figure 1 Patients flow diagram. ACEi, angiotensin-converting-enzyme inhibitor; ARB, angiotensin II receptor blockers; ARNI, angiotensin receptor-neprilysin inhibitor; HF, heart failure; LVEF, left ventricular ejection fraction.
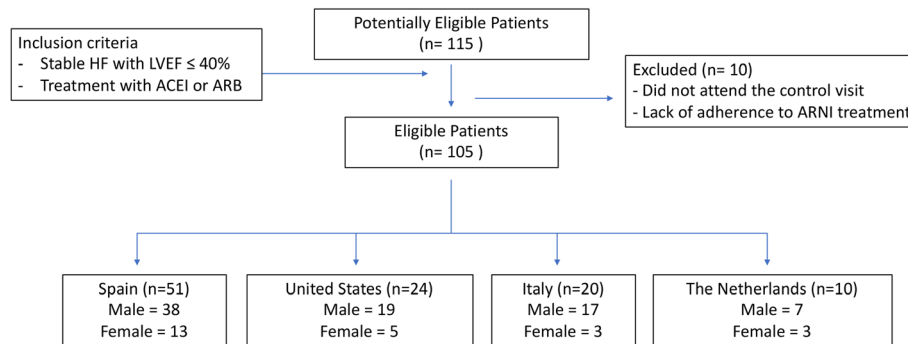
sensitivity are between 62.5–8000 and 30 pg/mL, respectively. Physicians in charge of clinical assessment were blinded to endorphins and sNEP levels.

Statistical analysis

Continuous variables were expressed as mean (±standard deviation) or median (interquartile range) as appropriate. Discrete variables were expressed as percentages. The primary outcome was the association between absolute changes in plasma $\Delta\alpha$ and $\Delta\gamma$-endorphins (visit posttreatment − visit pretreatment) with the NYHA class at 30 days ($NYHA_{30\text{-}d}$ class). Secondary outcome included the association between $\Delta sNEP$ (visit posttreatment − visit pretreatment) and $NYHA_{30\text{-}d}$ class. The independent association among these markers and $NYHA_{30\text{-}d}$ class was tested with analysis of covariance. $NYHA_{baseline}$ class was included as a covariate in all models. Other covariates were selected by means of multivariable fractional polynomial method, which combines backward elimination with the selection of a fractional polynomial function. This ensures the right match between the functional form of continuous covariates and the outcome. A two-sided P value of <0.05 was set as alpha for all analyses. All analyses were performed using Stata 14.1.

Results

Baseline characteristics of HF patients treated with sacubitril/valsartan are shown in *Table 1*. A total of 115 patients admitted in six sites in four countries (Spain, Italy, the Netherlands, and United States) were enrolled. However, we excluded 10 patients because they did not attend the follow-up visits, or they admitted they did not adhere to treatment. The median (IQR) age was 68 years (60–75), and 81 (77.1%) were male. The most frequent aetiology was

ischaemic heart disease (62.9%). Baseline medians of left ventricular ejection fraction, NT-proBNP, and eGFR were 30% (25–35), 925 ng/L (374–1700), and 64 mL/min/1.73 m^2 (50–82), respectively. The starting dose of sacubitril/valsartan was 24/26 mg in 67 (63.8%) and 49/51 mg in 38 (36.2%) patients according to their clinical status.

Baseline median levels of circulating α-EP, γ-EP, and sNEP were 582 (160–772), 101 (37–287), and 222 pg/mL (124–820), respectively. There was not a significant increase of α-EP nor γ-EP serum values after sacubitril/valsartan treatment (P value = 0.194 and 0.102, respectively). There were no significant differences in sNEP values between 30 days and baseline (P value = 0.103). Medians (IQR) of $\Delta\alpha$-EP, $\Delta\gamma$-EP, and $\Delta sNEP$ between 30 days and baseline were 9.3 ($-34 - 44$), -3.0 ($-46.0 - 18.9$), and 0 units ($-16.4 - 157.0$), respectively (*Table 1*). Baseline NYHA functional class is shown in *Table 1*. After 30 days treatment with sacubitril/valsartan, a significant improvement in NYHA class was found (*Figure 2*), with 36 (34.3%) patients experiencing improvement by at least one NYHA class category. Before sacubitril/valsartan treatment 8.6% of the patients were in NYHA class I, while after the treatment the percentage of patients in NYHA class I was 32.4%. A multivariable analysis revealed that $NYHA_{baseline}$ class (*Figure 3A*), $NT\text{-}proBNP_{baseline}$ (*Figure 3B*), and $eGFR_{baseline}$ (*Figure 3C*) were significant predictors of $NYHA_{30\text{-}d}$ class.

After forcing the three markers in the statistical model, $\Delta\alpha$-EP and $\Delta sNEP$ showed to be significantly associated with NYHA class after 30 days of treatment ($NYHA_{30\text{-}d}$ class) ($P = 0.014$ and $P < 0.001$, respectively). For $\Delta\alpha$-EP, the association was linear and negative (*Figure 4A*), where higher $\Delta\alpha$-EP values are indicative of lower $NYHA_{30\text{-}d}$ class. For $\Delta\gamma$-EP, the association was not significant (*Figure 4B*). On the other hand, $\Delta sNEP$ was positive and linearly associated with higher $NYHA_{30\text{-}d}$ class (*Figure 4C*). In a sensitivity analysis, the sacubitril/valsartan dose $[(24/26)/(49/51)]$ was not significant as predictor of $NYHA_{30\text{-}d}$ class, either as main

Table 1 Baseline characteristics

Age (years)	66.7 ± 11.4
Men, n (%)	81 (77.1)
Aetiology, n (%)	
CM ischaemic	66 (62.9)
CM hypertensive	4 (3.8)
CM dilated	35 (33.3)
NYHA functional class, n (%)	
I	9 (8.6)
II	77 (73.3)
III	18 (17.1)
IV	1 (1)
Hypertension, n (%)	72 (68.6)
Dyslipidaemia, n (%)	72 (68.6)
Prior admission for acute heart failure, n (%)	54 (51.4)
Left ventricular ejection fraction (%)	30 (25–35) (25–35)
Heart rate (bpm)	65 (60–73)
Systolic blood pressure (mmHg)	115 (107–130)
Diastolic blood pressure (mmHg)	70 (62–78)
Urea (mg/dL)	45 (32–64))
Creatinine, mg/dL (IQR)	1.15 (0.95–1.38)
Sodium, mmol/L (IQR)	139 (137–141)
Haemoglobin, g/dl (IQR)	13.8 (12.4–14.8)
NT-proBNP, ng/L (IQR)	925 (374–1700)
eGFR, m/min/m^2 (IQR)	64 (50–82)
α-EP, pg/mL (IQR)	582 (160–772)
γ-EP, pg/mL (IQR)	101 (37–287)
sNEP, pg/mL (IQR)	222 (124–820)
Δα-EP, units (IQR)	9.3 (−34–44)
Δγ-EP, units (IQR)	−3 (−46–18.9)
ΔsNEP, units (IQR)	0 (−16.4–157)
Treatments	
Furosemide, n (%)	67 (63.8)
Torasemide, n (%)	15 (14.3)
Antialdosteronic, n (%)	91 (86.7)
Beta blockers, n (%)	101 (96.2)
Digoxin, n (%)	13 (12.4)
Statin, n (%)	71 (67.6)
Acetylsalicylic acid, n (%)	55 (52.4)
Nitrates, n (%)	15 (14.3)
Anticoagulants, n (%)	55 (52.4)
Initial daily dose Sac/Val 24/26 mg, n (%)	67 (63.8)
Initial daily dose Sac/Val 49/51 mg, n (%)	38 (36.2)

Values are expressed as mean ± SE or median (IQR).
CM, cardiomyopathy; EP, endorphin; eGFR, glomerular filtration rate; NEP, neprilysin; NT-proBNP, N-terminal pro-brain natriuretic peptides; NYHA, New York Heart Association; sNEP, soluble neprilysin.

effect or as interacting with any of the markers (*P* value for interactions for α-EP, γ-EP, and sNEP were 0.201, 0.808, and 0.233, respectively).

Discussion

In this contemporary cohort of patients with HFrEF, we found an important association between Δα-EP and the early clinical improvement after the start of sacubitril/valsartan. These findings suggest that the altered cleavage of endorphin peptides by NEP inhibition may participate in patients' symptoms improvement beyond the haemodynamic benefits achieved with sacubitril/valsartan.

Heart failure is a chronic and progressive disease with multiple hospital readmissions and worse prognosis. Symptoms of HF worsen patients' quality of life substantially. Thus, changes in one NYHA functional class category lead to positive improvements in patient's quality of life.[13,14] Sacubitril/valsartan has proven to be a superior alternative to angiotensin-converting enzyme inhibitor/angiotensin receptor blocker to treat HF, reducing the risk of death and improving the condition of patients with HErEF.[1,2,15] However, because of the large variety of substrates degraded by NEP, its pharmacological inhibition could lead to a significant increase in its substrates deriving in unknown effects.

Endogenous opioid peptides play an important role in the management of pain and several physiological activities.[16,17] They are known to be released with states of pleasure including emotions brought upon by laughter, love, sex, and sports. They are also involved in the development of cardiovascular diseases such as hypertension and congestive HF, although the mechanisms of action are not completely known.[18,19] Endogenous opioid peptides may modulate cardiac function at the hormonal level via coupling to mu opiate receptors, which have impact in the heart's performance and the vasculature.[20] Endorphins such as α-EP and γ-EP act as functionally antagonistic neuropeptides in the brain, and their balanced expression is very important for behavioural homeostasis.[21] Its implication in diverse physiological activities determines its importance to maintain a correct balance between them.

α-EP and γ-EP are both originated from the same precursor[22,23]; the proteolytic activity cleaves β-EP into γ-EP and convert it into α-EP.[24] However, our results show that an increase in α-EP was associated with an early clinical improvement as determined by NYHA functional class. In spite that α-EP and γ-EP are equivalent substrates for NEP,[25] they seem to evolve differently. The baseline values of α-EP were higher than the basal values of γ-EP (582 vs. 101 pg/mL). This difference may be one of the reasons why there is an association between NYHA class and α-EP and not with γ-EP. Serum values of β-EP are well known in congestive[26] and acute[27] HF, but α-EP and γ-EP pathways are little studied in HF patients.

Opioid peptides are susceptible to rapid enzymatic degradation,[28] and it is known that a lot of peptidases are involved in their degradation, such as aminopeptidases, angiotensin-converting enzyme, insulin-degrading enzyme, and dipeptidyl peptidases, among others.[29–31] Previous studies reported a significant decrease in NHYA class after sacubitril/valsartan treatment,[31] and our data provide preliminary evidence of the association between Δα-EP and the early clinical improvement after sacubitril/valsartan treatment.

We did not find significant differences between baseline and posttreatment α-EP levels in the whole sample. However,

Figure 2 NYHA class changes after sacubitril/valsartan initiation. NYHA, New York Hear Association.
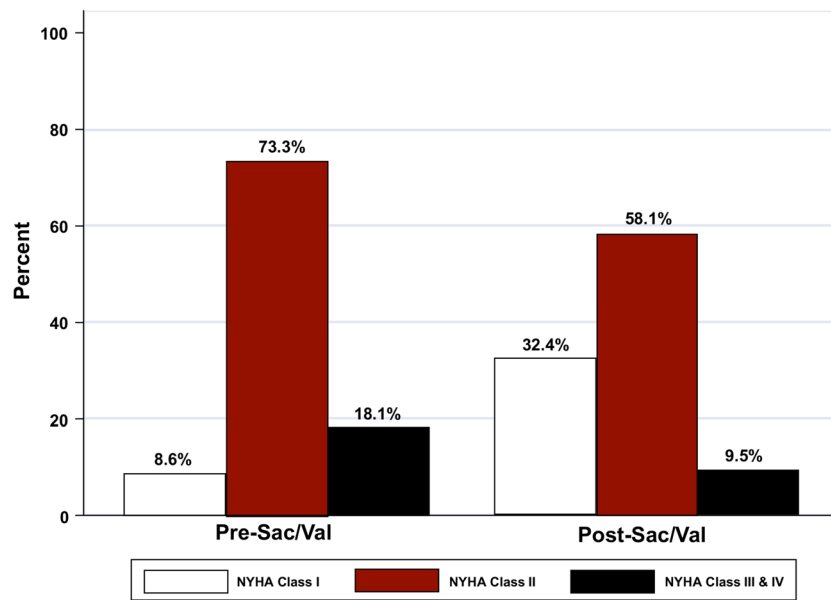
Figure 3 NYHA$_{baseline}$ class (A), NT-proBNP$_{baseline}$ (B), and eGFR$_{baseline}$ (C) were significantly predictors of the outcome. (A) Analysis adjusted by baseline values of eGFR-MDRD method, and NT-proBNP, and α-endorphin, -Log--endorphin, and -sNEP. (B) Analysis adjusted by baseline values of NYHA class and eGFR-MDRD method, and α-endorphin, -Log--endorphin, and -sNEP. (C) Analysis adjusted by baseline values of NYHA class, and NT-proBNP, and α-endorphin, -Log--endorphin, and -sNEP. eGFR, estimated glomerular filtration index; NT-proBNP, N-terminal pro-brain natriuretic peptides; NYHA, New York Heart Association; sNEP, soluble neprilysin; , 30-day minus baseline.
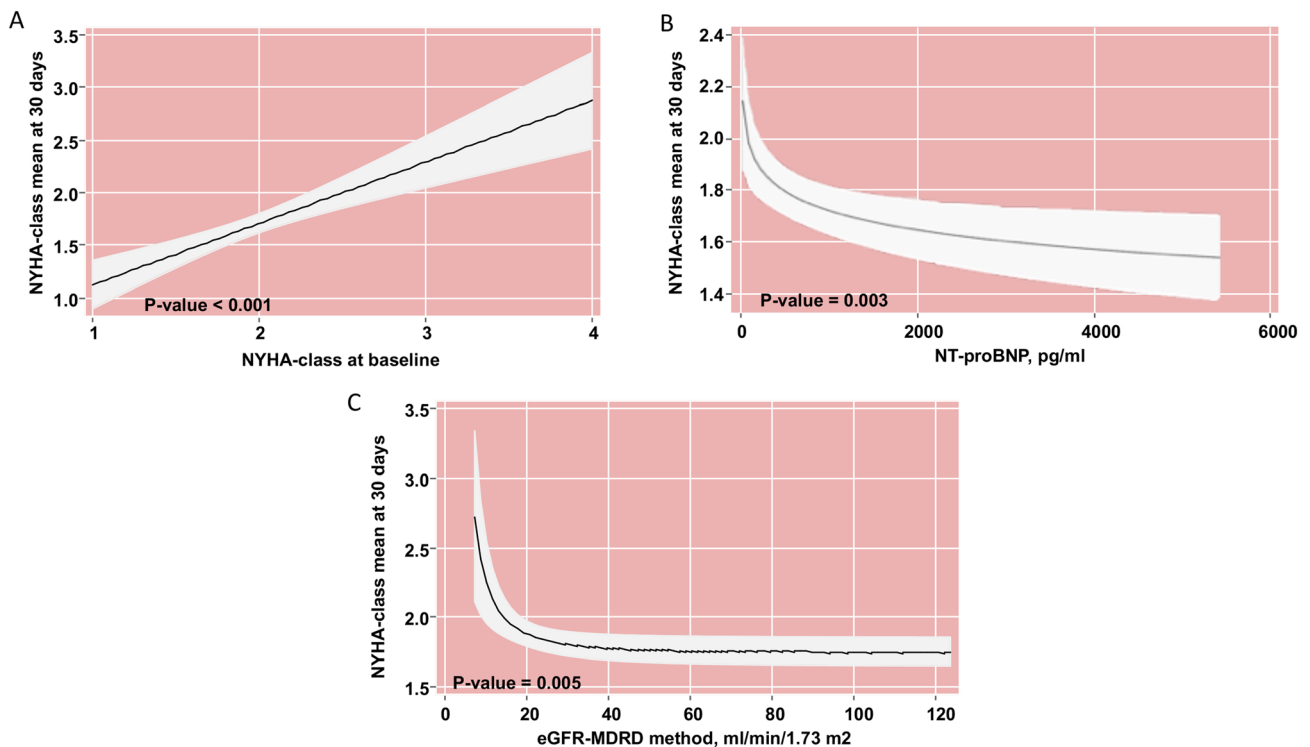
Figure 4 (A) α-endorphin dynamics and NYHA class at 30 days; (B) -endorphin dynamics and NYHA class at 30 days; (C) sNEP dynamics and NYHA class at 30 days. *All estimates adjusted by NYHA class, eGFR, NT-proBNP, α-endorphin, -endorphin, and -sNEP. eGFR, estimated glomerular filtration index; NYHA, New York Heart Association; sNEP, soluble neprilysin; , 30-day minus baseline.
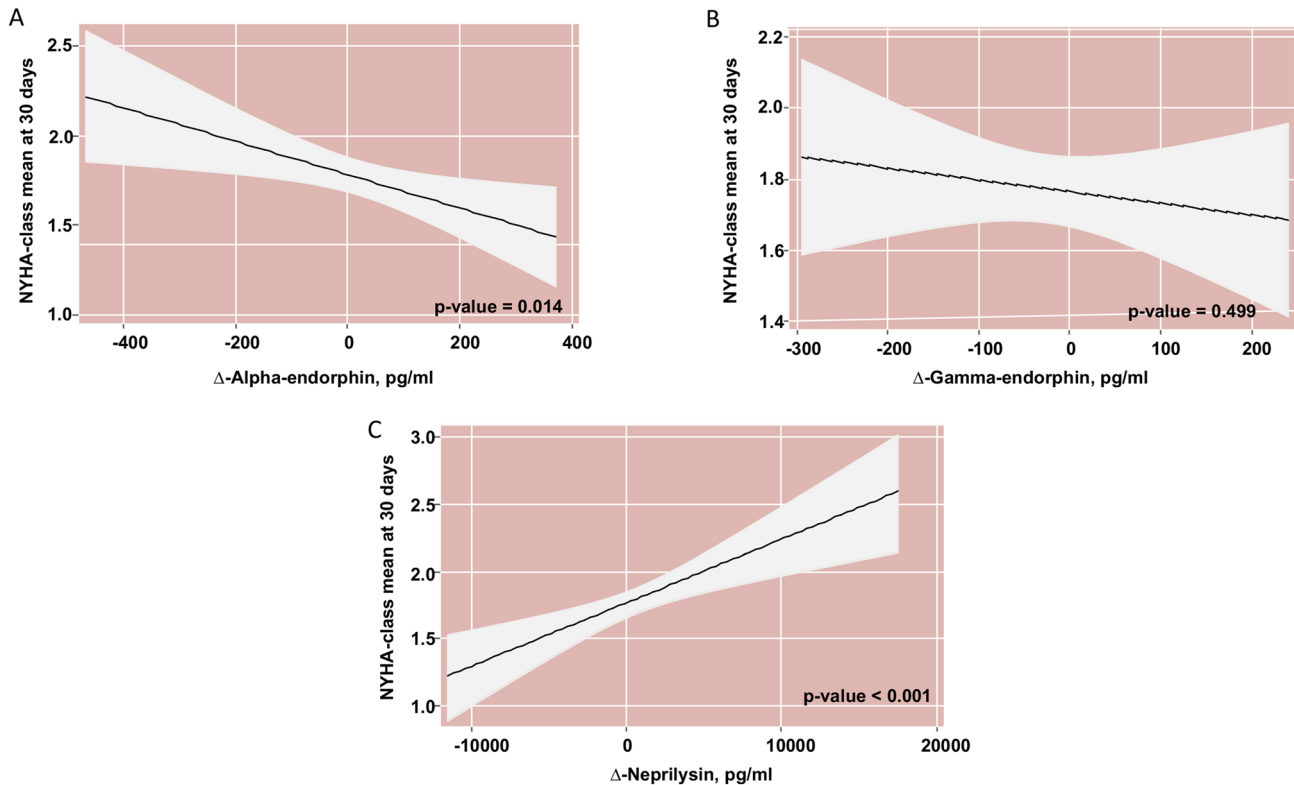
we found a significant relationship between Δα-EP and NYHA improvement. These findings suggest a heterogeneous and idiosyncratic endorphins response to sacubitril/valsartan that may partially explain the short-term clinical improvement found in some patients.[31–35] The absence of increased α-EP and γ-EP after treatment with sacubitril/valsartan in all patients may be due to the existence of these degradation pathways, because of the importance of maintaining its equilibrium for behavioural homeostasis.

This study has several limitations. The NYHA is based on the subjective assessment made by the physician and a more objective determination of the patients' functional status may be of interest. However, we should point out that NYHA evaluation was blinded to clinicians. In addition, because of the importance of the balance between α-EP and γ-EP, the homeostatic mechanisms for this maintenance could be the reason for not finding significant increases of these substrates after the treatment with sacubitril/valsartan; however, to corroborate this theory, further studies are necessary.

In conclusion, from an HF standpoint, endorphins and their effects and interactions are yet partially understood and studied. It is likely that endorphin elevation after NEP inhibition may have autocrine, paracrine, or endocrine functions; however, the duration and effects of endorphin elevation with sacubitril/valsartan is unclear. Our data suggest that beyond the haemodynamic benefits achieved with sacubitril/valsartan, the altered cleavage of endorphin peptides by NEP inhibition may participate in patients' symptoms improvement.

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Conflict of interest

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