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RESEARCH ARTICLE

Changes in mid-regional pro-adrenomedullin during treatment with sacubitril/valsartan

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Aims Adrenomedullin is a vasodilatory peptide with a role in microcirculatory and endothelial homeostasis. Adrenomedullin is a substrate for neprilysin and may therefore play a role in beneficial effects of sacubitril/valsartan (Sac/Val) treatment.

Methods and results Mid-regional pro-adrenomedullin (MR-proADM) was measured in 156 patients with heart failure with reduced ejection fraction (HFrEF) treated with Sac/Val and 264 patients with heart failure with preserved ejection fraction (HFpEF) randomized to treatment with Sac/Val or valsartan. Echocardiography and Kansas City Cardiomyopathy Questionnaire results were collected at baseline and after 6 and 12 months in the HFrEF cohort. Median (Q1–Q3) baseline MR-proADM concentrations were 0.80 (0.59–0.99) nmol/L in HFrEF and 0.88 (0.68–1.20) nmol/L in HFpEF. After 12 weeks of treatment with Sac/Val, MR-proADM increased by median 49% in HFrEF and 60% in HFpEF, while there were no significant changes in valsartan-treated patients (median 2%). Greater increases in MR-proADM were associated with higher Sac/Val doses. Changes in MR-proADM correlated weakly with changes in N-terminal pro-B-type natriuretic peptide, cardiac troponin T and urinary cyclic guanosine monophosphate. Increases in MR-proADM were associated with decreases in blood pressure, but not significantly associated with changes in echocardiographic parameters or health status.

Conclusions MR-proAD concentrations rise substantially following treatment with Sac/Val, in contrast to no change from valsartan. Change in MR-proADM from neprilysin inhibition did not correlate with improvements in cardiac structure and function or health status. More data are needed regarding the role of adrenomedullin and its related peptides in the treatment of heart failure.
Clinical Trial Registration: PROVE-HF ClinicalTrials.gov Identifier: NCT02887183, PARAMOUNT ClinicalTrials.gov Identifier: NCT00887588.

Keywords MR-proADM • Neprilysin • Angiotensin receptor–neprilysin inhibitors • Sacubitril/valsartan • Biomarker

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Introduction

Adrenomedullin is a 52-amino acid vasodilatory peptide with important actions in the microcirculation and endothelium.[1,2] Adrenomedullin plays a key role in vascular homeostasis in heart failure (HF), where higher circulating concentrations of the peptide are hypothesized to maintain endothelium integrity and reduce vascular leakage.[3] Infusion of synthetic human adrenomedullin increases left ventricular (LV) systolic function and cardiac index, reduces vascular resistance and improves atrial function.[4] Because of its role in HF pathophysiology, measurement of adrenomedullin has been pursued to better understand HF and its complications. However, measurement of adrenomedullin is complicated by several analytical issues, such as short half-life, fast metabolism, low concentrations, rapid degradation by proteases and binding to complement factor H. To circumvent these issues, attention has been focused on the measurement of a 48-amino acid mid-regional pro-peptide (MR-proADM) co-secreted with adrenomedullin. MR-proADM is biologically inactive, has a substantially longer half-life and is stable in human plasma.[5,6]

In patients with HF, concentrations of MR-proADM are frequently elevated.[7] Higher MR-proADM levels associate with worse LV structure and function in the general population[8] and concentrations of MR-proADM provide incremental prognostic information to natriuretic peptides in patients with HF.[9] Thus, measurement of MR-proADM has been suggested as an indirect means to assess adrenomedullin.

Although direct infusion of adrenomedullin has been explored as a treatment for HF,[4] other means by which to enhance its effects have not been specifically tested. Adrenomedullin is a substrate for neprilysin and small observational studies have reported increased levels of both biologically active adrenomedullin (bio-ADM) and MR-proADM after treatment with angiotensin receptor–neprilysin inhibitors (ARNI).[10] This rise might be partially associated with direct or indirect benefit from ARNI treatment. The trajectory of MR-proADM during treatment with ARNI and its association with changes in cardiac structure, function and health status is not known. We aimed to assess changes in MR-proADM following treatment with sacubitril/valsartan (Sac/Val) in patients with HF and reduced ejection fraction (HFrEF) from the Prospective Study of Biomarkers, Symptom Improvement and Ventricular Remodeling During Entresto Therapy for Heart Failure (PROVE-HF) study, and to correlate those changes with changes in cardiac structure and function, haemodynamics, and health status. We compared those results to MR-proADM changes in patients treated with Sac/Val in the Prospective Comparison of ARNI with ARB on Management of Heart Failure with Preserved Ejection Fraction (PARAMOUNT) trial in patients with HF and preserved ejection fraction (HFpEF) that included a control arm treated with valsartan.

Methods

Patient population

The design of the PROVE-HF study has previously been published.[11] It was a prospective, observational, multicentre, open-label, single-arm study that enrolled 794 patients ≥18 years with chronic HFrEF, an LV ejection fraction (LVEF) ≤40% and New York Heart Association (NYHA) class II–IV who were initiated on Sac/Val at the baseline visit. Follow-up study visits in PROVE-HF, that included biomarker sampling, were scheduled for 2, 4, 6, 8, 12, 26, 36, and 52 weeks. Patients were initiated on Sac/Val according to the US Prescribing Information and study drug was titrated approximately every 2 weeks through day 60, with a goal dose of Sac/Val of 97/103 mg twice daily (or highest tolerated dose). The PROVE HF labile biomarker substudy was performed at sites with access to equipment to ensure optimal blood samples without degradation and included 144 (18%) study participants. Patients who were included in the biomarker substudy had comparable baseline characteristics to the total PROVE-HF participants, as previously described.[12]

PARAMOUNT was a phase II, randomized, double-blind, parallel group, active control trial in 290 patients ≥18 years with a documented history of HF with associated signs or symptoms and an LVEF ≥45%, as previously described.[13] Patients were randomized 1:1 to Sac/Val titrated to 97/103 mg twice daily versus valsartan titrated to 160 mg twice daily and followed for 12 weeks in the core study. Biomarkers were collected at baseline and 12 weeks and MR-proADM was analysed in 264 (88%) PARAMOUNT participants at baseline.

The PROVE-HF and PARAMOUNT study protocols were approved by the relevant institutional review boards. All participants were required to sign informed consent prior to enrolment.

Laboratory analysis

In PROVE-HF blood samples were collected at baseline and after 2, 4, 6, 8, 12, 26, 36, and 52 weeks and in PARAMOUNT blood samples were collected at baseline and after 52 weeks, by venipuncture with different collection tubes and sample handling used in the two studies. In PROVE-HF, blood for MR-proADM testing was collected in tubes containing EDTA and a proprietary protease inhibitor cocktail (P100, BD Biosciences, Franklin Lakes, NJ, USA) that is known to inhibit proteolytic degradation and modification during and after blood collection. Samples were centrifuged cold, and plasma was immediately shipped on dry ice to the central laboratory for storage at −70°C until batch analysed (Clinical Reference Laboratory, Lenexa, KS, USA). In PARAMOUNT, blood for MR-proADM testing was collected in EDTA tubes, centrifuged at room temperature, stored at −20°C for 2–4 weeks, then shipped on dry ice to the central laboratory for storage at −70°C until batch analysed (Quest Diagnostics, Valencia, CA, USA).

In both PROVE-HF and PARAMOUNT, MR-proADM was measured by the B.R.A.H.M.S. KRYPTOR assay (Thermo Fisher Scientific Inc., Hennigsdorf, Germany) with a measuring range (with automatic dilution) of 0.05 nmol/L to 100 nmol/L and inter-run coefficient of variation <3.8%. N-terminal pro-B-type natriuretic peptide (NT-proBNP) and high-sensitivity troponin T (hsTnT) were measured with the Cobas proBNP II and Troponin T immunoassays, respectively (Roche Diagnostics, Indianapolis, IN, USA), as previously described.[14] Atrial natriuretic peptide (ANP) was measured with testing performed at the Mayo Clinic in Rochester, MN, by a sensitive radioimmunoassay (Phoenix Pharmaceuticals, Mountain View, CA, USA), with a measuring range of 0.8–1250 pg/ml as previously described.[12] Cyclic guanosine monophosphate (cGMP) was measured in spot urine using the R&D Systems Parameter immunoassay (Minneapolis, MN, USA) with a measuring range of 3.2 and 500 pmol/ml. cGMP concentrations were indexed to creatinine (CREP2 enzymatic assay, Roche Diagnostics).

Echocardiography and Kansas City Cardiomyopathy Questionnaire

In PROVE-HF, measurements of cardiac structure and function were performed by echocardiography at baseline and again at 6 and 12 months of follow-up. The echocardiograms were analysed at a core laboratory after study procedures had completed by readers who were blinded to study drug and biomarker data and were blinded temporally to the study visit. Quantification and indexation of parameters were performed according to current recommendations.[15] The Kansas City Cardiomyopathy Questionnaire (KCCQ) was used in PROVE-HF to collect self-reported health status at baseline and after 6 and 12 months of follow-up. Participant responses were scaled into the overall summary score and clinical summary score.

Statistical analysis

Continuous variables are presented as mean ± standard deviation for parametric variables and median (Q1–Q3) for non-parametric variables such as MR-proADM concentrations. Relative changes in MR-proADM were calculated as ratio by dividing the follow-up concentration with the baseline concentration, and absolute changes by subtracting the baseline sample from the follow-up sample. Quartiles of MR-proADM at baseline and quartiles of change from baseline were generated, and baseline characteristics and outcome variables (echocardiographic measures of cardiac structure/function, blood pressure and change in KCCQ scores) are presented across these quartiles with *p* for trend using regression and Cuzick's test. We also used linear mixed models and latent growth curve modelling (a longitudinal approach allowing for assessment of intercept and slope of change) of trajectories of MR-proADM over time. MR-proADM was analysed continuously using linear regression analyses and restricted cubic spline with log-transformed concentrations as the dependent variable in unadjusted and adjusted models including the following *a priori* selected covariates: age, sex, race, NYHA class, body mass index (BMI), LVEF, hypertension, diabetes mellitus, myocardial infarction (MI), coronary artery disease, coronary revascularization, atrial fibrillation, ischaemic HF aetiology, months since HF diagnosis, estimated glomerular filtration rate (eGFR), systolic blood pressure and heart rate, in addition to baseline MR-proADM. The association between MR-proADM, NT-proBNP, ANP and urinary cGMP (baseline and changes) were analysed using Spearman correlation and linear regression analyses with log-transformed values. *P*-values are 2-sided, and with values less than 0.05 considered significant. All analysis were performed using R version 3.5 (R Foundation for Statistical Computing, Vienna, Austria).

Results

Clinical characteristics by baseline MR-proADM concentrations

In the PROVE-HF study of patients with HFrEF, a total of 156 biomarker substudy patients had available MR-proADM concentrations at baseline; these patients were aged mean 64 ± 12 years, 75% were men and the median NT-proBNP concentration was 548 (Q1–Q3: 246–1367) pg/ml. The median concentration of MR-proADM was 0.80 (Q1–Q3: 0.59–0.99) nmol/L, and patients in the higher quartiles of MR-proADM were older, with lower eGFR, lower diastolic blood pressure and more frequently had hypertension, atrial fibrillation/flutter and an ischaemic HF aetiology (*Table 1*). Higher quartiles of MR-proADM were associated with the presence of peripheral oedema, but not with jugular venous distension, paroxysmal nocturnal dyspnoea, pulmonary rales, third heart sound or NYHA functional class.

In the PARAMOUNT study of patients with HFpEF, a total of 264 patients had available MR-proADM at baseline. The patients were aged mean 71 ± 9 years, 42% were men and the median NT-proBNP concentration was 833 (Q1–Q3: 485–1377) pg/ml. The median concentration of MR-proADM was 0.88 (Q1–Q3: 0.68–1.20) nmol/L. Similar to PROVE-HF, patients in the higher quartiles of MR-proADM were older, with lower eGFR, lower diastolic blood pressure and higher BMI (online supplementary *Table S1*).

Cross-sectional association between MR-proADM and cardiac structure, function and health status at baseline

At baseline in PROVE-HF, the median (Q1–Q3) LVEF was 31 (26–34) %, LV end-diastolic volume index (LVEDVi) was 83.1 (75.5–94.6) ml/m^2 and the KCCQ overall summary score was 67.2 (46.7–81.9) points. E/e' was higher in the higher quartiles of MR-proADM, while there was a U-shaped association with LVEF and no association with indexed LV volume, indexed LV mass, indexed left atrial volume or mitral regurgitation severity (*Table 2*). Patients in the higher quartiles of MR-proADM had worse health status as measured by KCCQ overall summary score and clinical summary score.

At baseline in PARAMOUNT, higher quartiles of MR-proADM were associated with higher LVEF and lower LV mass index, while there were no significant association with LV volume, left atrial volume or E/e' (online supplementary *Table S1*).

Trajectories of MR-proADM concentrations after initiation of sacubitril/valsartan

Mid-regional pro-adrenomedullin increased rapidly after initiation of Sac/Val in PROVE-HF (*Figure 1*; online supplementary *Table S2*). After 2 weeks, the median MR-proADM concentration was 1.06 (0.11–1.43) nmol/L, corresponding to a 37% increase from baseline. The highest median concentration was 1.21 (0.87–1.62) nmol/L, observed at week 36, that was 57% greater than baseline. Eighty-five (57%) patients that reached the target dose of Sac/Val (97/103 mg bid) at 12 weeks had greater increases in MR-proADM than patients who remained on the lowest dose (24/26 mg bid) (median 56% [39–74] vs. 35% [26–52], $p = 0.003$) (online supplementary *Table S3*). The distribution of change in MR-proADM through the full follow-up (from baseline to 12 months) is presented in online supplementary Figure *S1*. There were no significant differences in age, sex, race, BMI, baseline LVEF, renal function, comorbidities or medical therapy at baseline across quartiles of change in MR-proADM from baseline to 12 months (online supplementary *Table S4*).

Table 1 Baseline characteristics of the PROVE-HF study population by quartiles of baseline mid-regional pro-adrenomedullin concentrations

	MR-proADM				*p*-value for trend
	Quartile 1 (*n* = 40)	Quartile 2 (*n* = 39)	Quartile 3 (*n* = 38)	Quartile 4 (*n* = 39)	
MR-proADM range, nmol/L	0.25–0.58	0.59–0.80	0.81–0.99	0.99–2.49	
Age, years	55.3 (13.1)	62.7 (9.4)	67.5 (9.7)	72.1 (11.3)	<0.001
Male sex	29 (72.5)	34 (87.2)	26 (68.4)	28 (71.8)	0.23
Race					0.06
White	22 (55.0)	30 (76.9)	30 (78.9)	32 (82.1)	
Black	13 (32.5)	9 (23.1)	8 (21.1)	6 (15.4)	
Other	5 (12.5)	0 (0.0)	0 (0.0)	1 (2.6)	
NYHA class III or IV	6 (15.0)	10 (25.6)	10 (26.3)	14 (35.9)	0.21
BMI, kg/m^2	29.0 (5.4)	31.0 (6.4)	31.4 (5.8)	32.9 (7.4)	0.06
eGFR, ml/min/1.73 m^2	78.9 (20.9)	68.2 (12.3)	57.6 (14.3)	54.4 (16.1)	<0.001
Systolic blood pressure, mmHg	123 (12)	128 (15)	124 (14)	126 (13)	0.51
Diastolic blood pressure, mmHg	80 (9)	78 (7)	76 (13)	71 (9)	0.005
Pulse, bpm	73 (12)	73 (10)	71 (15)	69 (13)	0.42
Oedema	5 (12.5)	13 (33.3)	13 (34.2)	17 (43.6)	0.022
Jugular venous distension	1 (2.5)	3 (7.7)	1 (2.6)	6 (15.4)	0.143
Paroxysmal nocturnal dyspnoea	4 (13.8)	4 (12.9)	5 (14.7)	6 (16.2)	0.98
Past medical history, *n* (%)					
Hypertension	30 (75.0)	30 (76.9)	36 (94.7)	36 (92.3)	0.025
Coronary revascularization	15 (37.5)	18 (46.2)	19 (50.0)	22 (56.4)	0.40
Diabetes mellitus	12 (30.0)	20 (51.3)	19 (50.0)	21 (53.8)	0.12
Myocardial infarction	12 (30.0)	15 (38.5)	16 (42.1)	17 (43.6)	0.60
Coronary artery disease	15 (37.5)	18 (46.2)	18 (47.4)	15 (38.5)	0.74
Atrial fibrillation/flutter	8 (20.0)	13 (33.3)	18 (47.4)	18 (46.2)	0.039
Non-ischaemic HF aetiology	25 (62.5)	16 (41.0)	14 (36.8)	13 (33.3)	0.04
Months since HF diagnosis	37 [11–82]	50 [15–100]	58 [17–138]	53 [11–159]	0.53
Baseline medications, *n* (%)					
Beta-blocker	36 (90.0)	36 (92.3)	35 (92.1)	37 (94.9)	0.88
ACEi/ARB	33 (82.5)	32 (82.1)	34 (89.5)	27 (69.2)	0.15
MRA	14 (35.0)	16 (41.0)	10 (26.3)	10 (25.6)	0.41

Values are given as *n* (%), mean (standard deviation), or median [Q1–Q3].
ACEi, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker; BMI, body mass index; eGFR, estimated glomerular filtration rate; HF, heart failure; MRA, mineralocorticoid receptor antagonist; MR-proADM, mid-regional pro-adrenomedullin; NYHA, New York Heart Association.

In PARAMOUNT, concentrations of MR-proADM after 12 weeks were available in 247 patients (94% of those at baseline). In the Sac/Val group the relative increase in MR-proADM was median (Q1–Q3) 60% (32–81%) from baseline to 12 weeks, while it was 2% (−10% to 13%) in the valsartan group.

Changes in MR-proADM in association with changes in established cardiovascular biomarkers

In PROVE-HF, there were significant and direct associations between concentrations of MR-proADM and NT-proBNP, ANP and hsTnT at baseline (online supplementary *Table S5*). Changes from baseline to 52 weeks in MR-proADM correlated weakly, but significantly, with changes in NT-proBNP (rho = 0.20) and hsTnT (rho = 0.25), while correlation with ANP was not statistically significant (rho = 0.18). There were also significant associations between changes in MR-proADM and changes in urinary cGMP (rho = 0.27).

In PARAMOUNT, changes in MR-proADM from baseline to week 12 correlated weakly with changes in NT-proBNP (rho = 0.09), troponin (rho = 0.15) and urinary cGMP (rho = 0.11).

Changes in MR-proADM in association with changes in cardiac structure, function and health status in PROVE-HF

After 12 months of treatment with Sac/Val in PROVE-HF, there were improvements in measures of cardiac structure and function, including a median (Q1–Q3) overall absolute increase in LVEF of 9.9 (6.4–14.8) % and a decrease in LVEDVi of 12.9 (7.7–17.8) ml/m^2. KCCQ scores also improved overall to 12 months, from

Table 2 **Measures of cardiac structure and function (by echocardiography) and measures of health status (by Kansas City Cardiomyopathy Questionnaire) by quartiles of mid-regional pro-adrenomedullin concentrations at baseline in the PROVE-HF study**

	MR-proADM				*p*-value
	Quartile 1 ($n = 40$)	Quartile 2 ($n = 39$)	Quartile 3 ($n = 38$)	Quartile 4 ($n = 39$)	
Echocardiography					
LVEF, %	32.1 [28.9–36.3]	27.5 [25.5–33.5]	28.4 [24.5–31.8]	30.8 [22.6–33.3]	0.033
LVEDVi, ml/m^2	81.5 [74.0–93.0]	87.5 [74.8–102.6]	80.3 [75.5–94.4]	83.4 [77.6–93.2]	0.64
LVESVi, ml/m^2	55.4 [49.2–63.9]	60.5 [52.0–75.0]	54.8 [51.2–69.8]	59.2 [53.0–67.7]	0.33
LAVi, ml/m^2	33.0 [26.8–40.8]	36.0 [28.9–44.9]	37.6 [29.3–46.9]	36.9 [32.8–44.9]	0.17
E/e' ratio	9.1 [7.0–12.7]	9.4 [7.6–13.1]	10.6 [9.0–13.9]	13.0 [10.4–17.2]	0.015
LVMi, g/m^2	113.2 [89.9–132.1]	122.5 [104.1–141.4]	106.5 [98.7–128.0]	119.6 [110.7–133.7]	0.28
MR severity 3–4+	2 (5.9)	2 (5.3)	2 (5.4)	4 (11.4)	0.69
Health status					
KCCQ overall summary score	73.6 [64.3–84.1]	67.7 [50.9–82.9]	57.9 [45.2–83.8]	58.9 [27.2–72.9]	0.02
KCCQ clinical summary score	78.9 [66.2–90.7]	72.4 [59.9–89.1]	67.2 [50.5–84.4]	65.2 [41.6–76.0]	0.01

Values are given as median [Q1–Q3] and *p*-value is for trend across quartiles.
KCCQ, Kansas City Cardiomyopathy Questionnaire; LAVi, left atrial volume index; LVEDVi, left ventricular end-diastolic volume index; LVEF, left ventricular ejection fraction; LVESVi, left ventricular end-systolic volume index; LVMi, left ventricular mass index; MR, mitral regurgitation; MR-proADM, mid-regional pro-adrenomedullin.

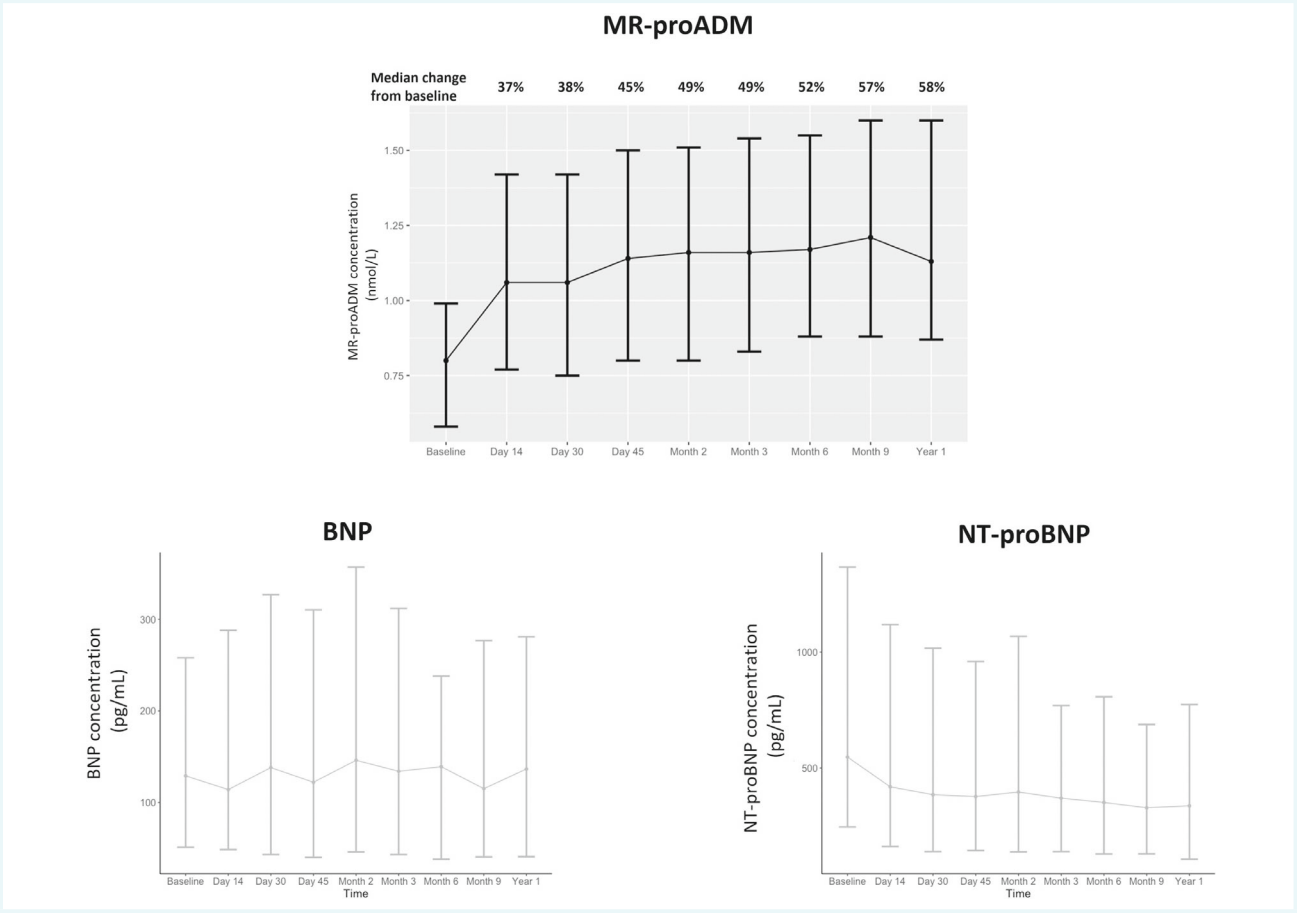

Figure 1 Trajectory of mid-regional pro-adrenomedullin (MR-proADM), B-type natriuretic peptide (BNP), and N-terminal pro-B-type natriuretic peptide (NT-proBNP) concentrations before (baseline) and during exposure to sacubitril/valsartan in PROVE-HF. Presented as median (Q1–Q3) of the concentration at each time point.

Table 3 Changes in measures of cardiac structure and function (by echocardiography), blood pressure and measures of health status (by Kansas City Cardiomyopathy Questionnaire) and side effects by quartiles of relative change in mid-regional pro-adrenomedullin concentrations from baseline to 52 weeks in the PROVE-HF study

	MR-proADM change				*p*-value
	Quartile 1 ($n = 32$)	Quartile 2 ($n = 32$)	Quartile 3 ($n = 32$)	Quartile 4 ($n = 31$)	
Change in MR-proADM (range)	−18% to 32%	32–58%	58–82%	82–279%	
Changes in echocardiography from baseline to 12 months					
LVEDVi, ml/m^2	−11.7 [−7.6, −15.9]	−15.5 [−9.1, −17.4]	−10.6 [−5.3, −13.4]	−16.7 [−9.3, −21.2]	0.03
LVMi, g/m^2	−12.4 [−8.9, −21.5]	−12.5 [2.7, −29.5]	−6.4 [8.5, −27.5]	−22.0 [−5.8, −30.4]	0.51
LVEF, %	9.3 [4.7, 14.7]	10.4 [7.3, 15.5]	10.7 [6.8, 15.8]	8.6 [4.5, 15.7]	0.72
LAVi, ml/m^2	−6.1 [−3.3, −12.3]	−8.5 [−3.8, −12.4]	−5.8 [−4.3, −9.7]	−6.9 [−3.7, −8.7]	0.53
E/e', ratio	−0.3 [−3.4, 1.7]	−1.1 [−2.1, 3.9]	0.0 [−1.9, 1.4]	−2.0 [−3.0, −0.7]	0.14
Changes in blood pressure from baseline to 12 months					
Systolic blood pressure	−2.3 (15.8)	−5.1 (17.1)	−4.3 (15.4)	−16.2 (16.3)	0.004
Diastolic blood pressure	−2.8 (9.9)	−3.7 (11.8)	−4.6 (11.0)	−8.5 (8.5)	0.16
Changes in KCCQ from baseline to 12 months					
KCCQ overall summary score	11.0 [3.0, 23.3]	3.1 [−2.0, 12.5]	9.0 [0.4, 16.7]	2.6 [−7.8, 16.7]	0.10
KCCQ clinical summary score	5.7 [0, 18.9]	6.3 [−5.2, 16.1]	3.9 [−0.9, 14.2]	1.5 [−9.2, 15.6]	0.36
Adverse events					
Dizziness	6 (18.8)	10 (31.2)	4 (12.5)	7 (22.6)	0.32
Hypotension	4 (12.5)	3 (9.4)	3 (9.4)	6 (19.4)	0.60
Hyperkalaemia	1 (3.1)	4 (12.5)	3 (9.4)	5 (16.1)	0.37
Worsening renal function	1 (3.1)	0 (0.0)	1 (3.1)	2 (6.5)	0.54

Values are given as median (Q1, Q3) and *p*-value is for trend across quartiles.
KCCQ, Kansas City Cardiomyopathy Questionnaire; LAVi, left atrial volume index; LVEDVi, left ventricular end-diastolic volume index; LVEF, left ventricular ejection fraction; LVMi, left ventricular mass index; MR-proADM, mid-regional pro-adrenomedullin.

median 67.2 (46.7–81.9) points to 75.7 (58.8–90.3) for KCCQ overall summary score. There was no significant association between changes in MR-proADM and changes in cardiac structure and function, i.e. the median increase in LVEF was 9.3%, 10.4%, 10.7% and 8.6% in quartiles 1–4 of change in MR-proADM, respectively ($p = 0.72$ by quartiles, *Table 3*; $p = 1.00$ continuously, *Figure 2*). Of note, there was an association between change in LVEDVi and quartiles of change in MR-proADM ($p = 0.03$), but this was not present in the continuous analysis ($p = 0.31$ unadjusted and $p = 0.49$ adjusted). There was no significant association between changes in MR-proADM and changes in KCCQ scores, that is, the median change in overall summary score was 11.0, 3.1, 9.0 and 2.6 points in quartiles 1–4 of change in MR-proADM, respectively ($p = 0.10$ by quartiles, *Table 3*; $p = 0.31$ continuously, *Figure 2*). Changes in MR-proADM were significantly associated with changes in systolic blood pressure, that is, mean reduction of 2.3, 5.1, 4.3 and 16.2 mmHg in quartiles 1–4 of change in MR-proADM, respectively ($p = 0.004$ by quartiles, *Table 3*; $p < 0.001$ continuously, *Figure 2*). The association between changes in MR-proADM and systolic blood pressure remained significant after adjusting for potential confounders ($p = 0.014$; online supplementary *Figure S2*).

At 6 months, the changes in these parameters were intermediate to that of 12 months, with similar associations to changes in MR-proADM.

There were no differences in the frequency of known side-effects from Sac/Val across quartiles of change in MR-proADM, including hypotension, worsening renal function and dizziness (*Table 3*).

These results were consistent when assessing absolute changes (as opposed to relative changes used in the primary analysis) in MR-proADM (online supplementary *Table S6*). The results were also consistent when assessing latent growth trajectories of MR-proADM (online supplementary *Table S7*), except that there were no association between changes in blood pressure and trajectories.

Discussion

In this analysis of two studies examining the role of Sac/Val for treatment of HFrEF and HFpEF, ARNI therapy was shown to cause a statically significant increase in MR-proADM in both patients with HFrEF and patients with HFpEF. Moreover, we made several observations that may improve our understanding of MR-proADM and the mechanism of action of Sac/Val. First, we demonstrate that MR-proADM concentrations prior to treatment with Sac/Val associate with worse cardiac function and health status in cross-sectional analysis. Second, we show that levels of MR-proADM increase substantially (50–60%) following initiation of Sac/Val in both patients with HFrEF and HFpEF, with an association between higher Sac/Val dose and greater increase in MR-proADM. In contrast, there were no significant changes in MR-proADM from treatment with valsartan. Third, there was an association between greater increases in MR-proADM and greater reductions in blood pressure. Finally, we demonstrate that despite substantial increases in MR-proADM owing to neprilysin inhibition, we found

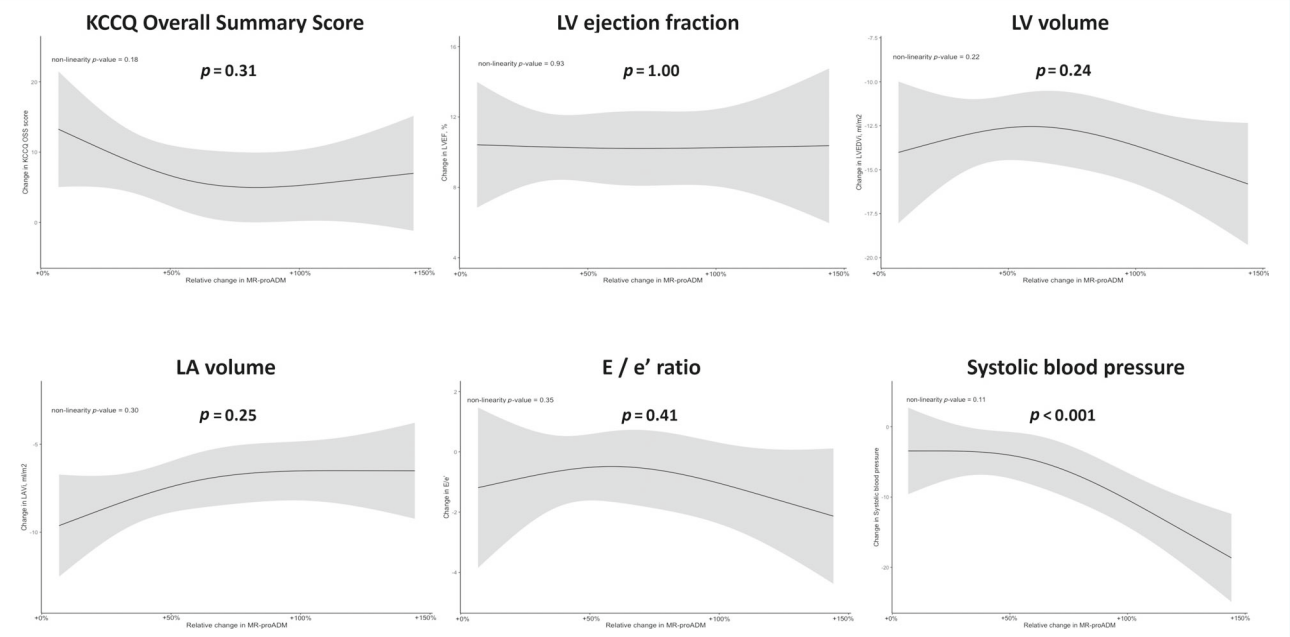

Figure 2 Association between changes in mid-regional pro-adrenomedullin (MR-proADM) with changes in measures of cardiac structure and function, health status and blood pressure from baseline to 12 months using unadjusted restricted cubic splines in PROVE-HF. The changes in MR-proADM are calculated as ratios and the changes in echocardiography parameters and Kansas City Cardiomyopathy Questionnaire (KCCQ) are calculated as delta values. LA, left atrial; LAVi, left atrial volume index; LV, left ventricular; LVEDVi, left ventricular end-diastolic volume index; LVEF, left ventricular ejection fraction; OSS, overall summary score.

no significant association between changes in MR-proADM and changes in cardiac structure and function or health status during treatment, suggesting that MR-proADM does not mediate the clinical benefits from ARNI in HF.

Neprilysin is a circulating endopeptidase that is responsible for the degradation of several vasoactive peptides. Through inhibition from Sac/Val, the biological effects of these peptides are augmented. In PARADIGM-HF, treatment with Sac/Val, as compared to enalapril, markedly improved outcomes in patients with HFrEF,[16] but the exact pathways mediating the effect are only now being clarified. Substantial increase in ANP (>100%) may explain a substantial amount of the reverse cardiac remodelling and health status benefits of Sac/Val[12]; notably, B-type natriuretic peptide remains close to unchanged after initiation of Sac/Val.[12,17,18] This reflects the difference in affinity to neprilysin and thus importance in mediating the treatment effect from the drug. As it is not degraded by neprilysin, reduction in NT-proBNP after treatment with Sac/Val is a strong marker of benefit, reflecting changes in disease status, and correlating with improvements in cardiac function and health status.[11]

MR-proADM is elevated in HF and higher levels associate with comorbidity burden and worse outcome.[7,9] In our study, we found comparable levels of baseline MR-proADM in chronic HFrEF (median 0.80 nmol/L) and HFpEF (median 0.88 nmol/L). These concentrations were higher than in a study of post-MI patients with asymptomatic LV dysfunction (median 0.54 nmol/L) who also had lower NT-proBNP.[19] Interestingly, MR-proADM levels in our study were similar to a cohort of patients with decompensated HFrEF (median 0.78 nmol/L), despite that the decompensated patients had substantially higher NT-proBNP.[20] In agreement with these previous studies, higher baseline concentrations of MR-proADM were associated with older age, worse renal function and lower blood pressure. In HFrEF higher MR-proADM was also associated with atrial fibrillation/flutter and a non-ischaemic HF aetiology, while in HFpEF it was inversely associated with previous MI. Thus, the aetiology of HF may play a role in the expression of MR-proADM with seemingly lower levels in coronary artery disease-associated HF. Among echocardiographic parameters, higher E/e' correlated with higher MR-proADM in HFrEF, while higher LVEF correlated with higher MR-proADM in HFpEF. Whether this addresses different biological processes for MR-proADM up-regulation related to haemodynamics or a consequence of how we categorize HF (by LVEF) is uncertain.

Higher baseline MR-proADM levels were associated with worse health status in patients with HFrEF, which might imply an association with more severe HF. Interestingly, however, there was only an association with peripheral oedema at baseline, and not with other signs of fluid overload such as jugular venous distension and paroxysmal nocturnal dyspnoea. Moreover, changes in MR-proADM did not correlate with changes in filling pressures (E/e') or KCCQ clinical summary score. Despite that patients in this study had chronic HF and were well-treated, this contradicts studies suggesting bio-ADM as a marker of congestion.[21] The adrenomedullin gene encodes a preprohormone, which

after cleavage generates a pro-ADM peptide. In contrast to MR-proADM, pro-ADM becomes glycine-extended and enzymatically converted to bio-ADM. These different molecules derived from adrenomedullin and various methods for quantification may impact what they reflect clinically. For instance, adrenomedullin as measured by the proximity extension assay technology, were one of few biomarkers associated with E/e', peak oxygen consumption and treatment effect of spironolactone in the Aldo-DHF trial proteomics study of patients with HFpEF.[22] In contrast, we found no association between MR-proADM and E/e' in our HFpEF cohort, and mineralocorticoid receptor antagonist use was not associated with higher MR-proADM at baseline or changes in MR-proADM in neither of our studies.

Although not biologically active, MR-proADM appears to be a substrate for neprilysin. Concentrations of the peptide increased significantly after treatment with Sac/Val and did so in a dose-dependent fashion. We demonstrate that it is sacubitril that causes the increase in MR-proADM, as patients treated with valsartan in PARAMOUNT had stable levels. Interestingly, we did not identify any baseline characteristic that was associated with greater increases in MR-proADM, including demographics, comorbidities and baseline medication. The magnitude of MR-proADM increase from Sac/Val was also remarkably consistent between the studies (median 49% increase in PROVE-HF and 60% increase in PARAMOUNT, both after 12 weeks). In contrast there was a non-significant 2% increase in the valsartan-treated group in PARAMOUNT, emphasizing that the changes in MR-proADM we observed were mainly from inhibition of neprilysin. This is consistent with data from post-MI patients without HF, but with LVEF ≤40%, who increased in MR-proADM from 0.54 nmol/L to 0.89 nmol/L during 52 weeks of treatment with Sac/Val.[19] Although the rise in MR-proADM was less pronounced than that for ANP in HF, the increase in MR-proADM was nonetheless substantial, and changes were independent of other biomarkers. In fact, changes in MR-proADM from Sac/Val correlated poorly with changes in other biomarkers, including the natriuretic peptides. The effects on MR-proADM represents a different biological pathway than the natriuretic peptides: adrenomedullin functions as a protective factor for blood vessels, exerting various vascular actions against vascular damage and remodelling.[3] In PROVE-HF, higher MR-proADM at baseline was associated with the presence of ischaemic HF aetiology, suggesting an up-regulation from ischaemia. Interestingly, in post-MI patients with asymptomatic LV dysfunction, the increase in MR-proADM was more pronounced than that for ANP (which is opposite to that observed in PROVE-HF).[19] In the Prospective ARNI versus ACE Inhibitor Trial to Determine Superiority in Reducing Heart Failure Events after Myocardial Infarction (PARADISE-MI), Sac/Val did not significantly reduce the risk of incident HF or cardiovascular mortality compared to ramipril.[23] However, Sac/Val significantly reduced the risk of new coronary events.[24] Thus, augmentation of adrenomedullin from Sac/Val treatment may improve the microcirculation and endothelial function, both of which are important pathophysiologic aspects of MI and HF.[25]

The fact that we found no association between the rise in MR-proADM after Sac/Val treatment and mechanistic benefits from the drug leaves more questions than answers. Interestingly, greater increases in MR-proADM associated with a greater reduction in blood pressure, which is in line with our biological understanding of adrenomedullin as a potent vasodilator.[4] Despite this there was no significant association with changes in cardiac structure, function, or health status. It may be that MR-proADM does not mediate the effect of Sac/Val in HF, despite considerable increases and established cardioprotective effects. But it may also be that baseline elevations – linked to risk and worse cardiac status – obscures benefit from pharmacologic increase of MR-proADM after Sac/Val therapy. We previously reported that ANP is more elevated in higher risk individuals but the early increase in ANP from Sac/Val treatment was nonetheless predictive of reverse cardiac remodelling.[14] More data, including whether bio-ADM mediates any benefit of Sac/Val, are needed to better understand the findings of the current analyses.

Limitations

In contrast to the PARAMOUNT trial, there was no control group treated with valsartan in PROVE-HF. However, as the baseline levels of MR-proADM and the Sac/Val-related increases in MR-proADM were similar between the studies, it seems reasonable to assume that valsartan would have a neutral effect on MR-proADM levels also in HFrEF. This was also demonstrated in asymptomatic post-MI patients with LV dysfunction.[19] There is a risk of selection bias, as this biomarker substudy was planned to only include a subset of the total PROVE-HF population. However, sites were selected based on equipment availability and not patient clinical characteristics, and we have previously shown that the substudy patients had comparable baseline characteristics as the full population of PROVE-HF.[12] Biomarker measurements were not available at all visits in all patients, although a high proportion (81%) had MR-proADM measured at the last visit. We did not measure bio-ADM, but concentrations of MR-proADM are accepted as a surrogate for this peptide; it is possible that a differential impact of Sac/Val might be present between MR-proADM and bio-ADM.

Conclusion

Greater concentrations of MR-proADM associate with more structural heart disease and worse health status, confirming the role of this biomarker to severity of HF. MR-proADM concentrations rise substantially from treatment with Sac/Val in HFrEF and HFpEF. These increases associated with decreases in blood pressure, but not with changes in echocardiographic parameters or KCCQ scores. Although this suggests that MR-proADM does not mediate the clinical benefits from neprilysin inhibition, more data are needed to better understand whether increases in adrenomedullin after Sac/Val treatment participate in the benefit of this drug.

Supplementary Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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References

1. Eto T. A review of the biological properties and clinical implications of adrenomedullin and proadrenomedullin N-terminal 20 peptide (PAMP), hypotensive and vasodilating peptides. *Peptides*. 2001;**22**:1693–1711. https://doi.org/10.1016/s0196-9781(01)00513-7
2. Hinson JP, Kapas S, Smith DM. Adrenomedullin, a multifunctional regulatory peptide. *Endocr Rev*. 2000;**21**:138–167. https://doi.org/10.1210/edrv.21.2.0396
3. Kato J, Tsuruda T, Kita T, Kitamura K, Eto T. Adrenomedullin. *Arterioscler Thromb Vasc Biol*. 2005;**25**:2480–2487. https://doi.org/10.1161/01.ATV.0000184759.91369.f8
4. Bene RD, Lazzeri C, Barletta G, Vecchiarino S, Guerra CT, Franchi F, *et al.* Effects of low-dose adrenomedullin on cardiac function and systemic haemodynamics in man. *Clin Physiol*. 2000;**20**:457–465. https://doi.org/10.1046/j.1365-2281.2000.00284.x
5. Morgenthaler NG, Struck J, Alonso C, Bergmann A. Measurement of midregional proadrenomedullin in plasma with an immunoluminometric assay. *Clin Chem*. 2005;**51**:1823–1829. https://doi.org/10.1373/clinchem.2005.051110
6. Struck J, Tao C, Morgenthaler NG, Bergmann A. Identification of an adrenomedullin precursor fragment in plasma of sepsis patients. *Peptides*. 2004;**25**:1369–1372. https://doi.org/10.1016/j.peptides.2004.06.019
7. Caruhel P, Mazier C, Kunde J, Morgenthaler NG, Darbouret B. Homogeneous time-resolved fluoroimmunoassay for the measurement of midregional proadrenomedullin in plasma on the fully automated system B.R.A.H.M.S KRYPTOR®. *Clin Biochem*. 2009;**42**:725–728. https://doi.org/10.1016/j.clinbiochem.2009.01.002
8. Neumann JT, Tzikas S, Funke-Kaiser A, Wilde S, Appelbaum S, Keller T, *et al.* Association of MR-proadrenomedullin with cardiovascular risk factors and subclinical cardiovascular disease. *Atherosclerosis*. 2013;**228**:451–459. https://doi.org/10.1016/j.atherosclerosis.2013.03.006
9. Maisel A, Mueller C, Nowak RM, Peacock WF, Ponikowski P, Mockel M, *et al.* Midregion prohormone adrenomedullin and prognosis in patients presenting with acute dyspnea: Results from the BACH (Biomarkers in Acute Heart Failure) trial. *J Am Coll Cardiol*. 2011;**58**:1057–1067. https://doi.org/10.1016/j.jacc.2011.06.006
10. Arfsten H, Goliasch G, Bartko PE, Prausmüller S, Spinka G, Cho A, *et al.* Increased concentrations of bioactive adrenomedullin subsequently to angiotensin-receptor/neprilysin-inhibitor treatment in chronic systolic heart failure. *Br J Clin Pharmacol*. 2021;**87**:916–924. https://doi.org/10.1111/bcp.14442
11. Januzzi JL Jr, Prescott MF, Butler J, Felker GM, Maisel AS, McCague K, *et al.* Association of change in N-terminal pro-B-type natriuretic peptide following initiation of sacubitril-valsartan treatment with cardiac structure and function in patients with heart failure with reduced ejection fraction. *JAMA*. 2019;**322**:1085–1095. https://doi.org/10.1001/jama.2019.12821
12. Murphy SP, Prescott MF, Camacho A, Iyer SR, Maisel AS, Felker GM, *et al.* Atrial natriuretic peptide and treatment with sacubitril/valsartan in heart failure with reduced ejection fraction. *JACC Heart Fail*. 2021;**9**:127–136. https://doi.org/10.1016/j.jchf.2020.09.013
13. Solomon SD, Zile M, Pieske B, Voors A, Shah A, Kraigher-Krainer E, *et al.*; Prospective comparison of ARNI with ARB on Management Of heart failUre with preserved ejectioN fracTion (PARAMOUNT) Investigators. The angiotensin receptor neprilysin inhibitor LCZ696 in heart failure with preserved ejection fraction: A phase 2 double-blind randomised controlled trial. *Lancet*. 2012;**380**:1387–1395. https://doi.org/10.1016/S0140-6736(12)61227-6
14. Murphy SP, Prescott MF, Maisel AS, Butler J, Piña IL, Felker GM, *et al.* Association between angiotensin receptor-neprilysin inhibition, cardiovascular biomarkers, and cardiac remodeling in heart failure with reduced ejection fraction. *Circ Heart Fail*. 2021;**14**:e008410. https://doi.org/10.1161/CIRCHEARTFAILURE.120.008410
15. Lang RM, Bierig M, Devereux RB, Flachskampf FA, Foster E, Pellikka PA, *et al.*; Chamber Quantification Writing Group; American Society of Echocardiography's Guidelines and Standards Committee; European Association of Echocardiography. Recommendations for chamber quantification: A report from the American Society of Echocardiography's Guidelines and Standards Committee and the Chamber Quantification Writing Group, developed in conjunction with the European Association of Echocardiography, a branch of the European Society of Cardiology. *J Am Soc Echocardiogr*. 2005;**18**:1440–1463. https://doi.org/10.1016/j.echo.2005.10.005
16. McMurray JJ, Packer M, Desai AS, Gong J, Lefkowitz MP, Rizkala AR, *et al.*; PARADIGM-HF Investigators and Committees. Angiotensin-neprilysin inhibition versus enalapril in heart failure. *N Engl J Med*. 2014;**371**:993–1004. https://doi.org/10.1056/NEJMoa1409077
17. Myhre PL, Vaduganathan M, Claggett B, Packer M, Desai AS, Rouleau JL, *et al.* B-type natriuretic peptide during treatment with sacubitril/valsartan: The PARADIGM-HF trial. *J Am Coll Cardiol*. 2019;**73**:1264–1272. https://doi.org/10.1016/j.jacc.2019.01.018
18. Myhre PL, Prescott MF, Murphy SP, Fang JC, Mitchell GF, Ward JH, *et al.* Early B-type natriuretic peptide change in HFrEF patients treated with sacubitril/valsartan. *JACC Heart Fail*. 2022;**10**:119–128. https://doi.org/10.1016/j.jchf.2021.09.007

19. Docherty KF, Campbell RT, Brooksbank KJM, Dreisbach JG, Forsyth P, Godeseth RL, *et al.* Effect of neprilysin inhibition on left ventricular remodeling in patients with asymptomatic left ventricular systolic dysfunction late after myocardial infarction. *Circulation*. 2021;**144**:199–209. https://doi.org/10.1161/circulationaha.121.054892
20. Morbach C, Marx A, Kaspar M, Güder G, Brenner S, Feldmann C, *et al.* Prognostic potential of midregional pro-adrenomedullin following decompensation for systolic heart failure: Comparison with cardiac natriuretic peptides. *Eur J Heart Fail*. 2017;**19**:1166–1175. https://doi.org/10.1002/ejhf.859
21. Ter Maaten JM, Kremer D, Demissei BG, Struck J, Bergmann A, Anker SD, *et al.* Bio-adrenomedullin as a marker of congestion in patients with new-onset and worsening heart failure. *Eur J Heart Fail*. 2019;**21**:732–743. https://doi.org/10.1002/ejhf.1437
22. Schnelle M, Leha A, Eidizadeh A, Fuhlrott K, Trippel TD, Hashemi D, *et al.* Plasma biomarker profiling in heart failure patients with preserved ejection fraction before and after spironolactone treatment: Results from the Aldo-DHF trial. *Cell*. 2021;**10**:2796. https://doi.org/10.3390/cells10102796
23. Pfeffer MA, Claggett B, Lewis EF, Granger CB, Køber L, Maggioni AP, *et al.*; PARADISE-MI Investigators and Committees. Angiotensin receptor-neprilysin inhibition in acute myocardial infarction. *N Engl J Med*. 2021;**385**:1845–1855. https://doi.org/10.1056/NEJMoa2104508
24. Mehran R, Steg PG, Pfeffer MA, Jering K, Claggett B, Lewis EF, *et al.* The effects of angiotensin receptor-neprilysin inhibition on major coronary events in patients with acute myocardial infarction: Insights from the PARADISE-MI trial. *Circulation*. 2022;**146**:1749–1757. https://doi.org/10.1161/CIRCULATIONAHA.122.060841
25. De Boer RA, Pinto YM, Van Veldhuisen DJ. The imbalance between oxygen demand and supply as a potential mechanism in the pathophysiology of heart failure: The role of microvascular growth and abnormalities. *Microcirculation*. 2003;**10**:113–126. https://doi.org/10.1038/sj.mn.7800188