Circulation: Heart Failure

ORIGINAL ARTICLE

Urinary cGMP (Cyclic Guanosine Monophosphate)/BNP (B-Type Natriuretic Peptide) Ratio, Sacubitril/Valsartan, and Outcomes in Heart Failure With Reduced Ejection Fraction: An Analysis of the PARADIGM-HF Trial

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BACKGROUND: The ratio of ucGMP (urinary cyclic guanosine monophosphate) to BNP (B-type natriuretic peptide) is thought to reflect the responsiveness of tissues to natriuretic peptides.

METHODS: We examined the relationship between ucGMP/BNP ratio and clinical outcomes, the effect of sacubitril/valsartan, compared with enalapril, on the ucGMP/BNP ratio, and the efficacy of sacubitril/valsartan on clinical outcomes according to baseline ucGMP/BNP ratio in PARADIGM-HF trial (Prospective Comparison of ARNI With ACEI to Determine Impact on Global Mortality and Morbidity in Heart Failure). ucGMP/BNP ratio was available at baseline (N=2031), 1 month (N=1959), and 8 months after randomization (N=1746). The primary outcome was a composite of heart failure hospitalization or cardiovascular death.

RESULTS: Compared with the lowest tertile of baseline ucGMP/BNP ratio, patients in the higher tertiles had a lower risk of the primary outcome (tertile 1, reference; tertile 2, hazard ratio 0.57 [95% CI, 0.45–0.71]; tertile 3, hazard ratio, 0.54 [0.43–0.67]). Compared with baseline, the ucGMP/BNP ratio at 1 month and 8 months after randomization was higher with sacubitril/valsartan than with enalapril: ratio of geometric mean ratios at 1 month, 1.38 (95% CI, 1.27–1.51) and 8 months, 1.32 (95% CI, 1.20–1.45), and this difference was consistent across tertiles of ucGMP/BNP ratio at baseline ($P_{interaction}$=0.19 and 0.91, respectively). The effect of sacubitril/valsartan, compared with enalapril, was consistent across tertiles of ucGMP/BNP ratio at baseline for all outcomes ($P_{interaction}$ ≥0.31).

CONCLUSIONS: In patients with heart failure and reduced ejection fraction, higher ucGMP/BNP ratio was associated with better outcomes. Sacubitril/valsartan increased the ucGMP/BNP ratio, compared with enalapril, and the effect of sacubitril/valsartan on clinical outcomes was not modified by baseline ucGMP/BNP ratio.

REGISTRATION: URL: https://www.clinicaltrials.gov; Unique Identifier: NCT01035255.

Key Words: angiotensin blocker-neprilysin inhibitor ■ B-type natriuretic peptide ■ clinical trial ■ cyclic guanosine monophosphate ■ heart failure

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WHAT IS NEW?

- In patients with heart failure and reduced ejection fraction, a higher ratio of ucGMP (urinary cyclic guanosine monophosphate) to plasma BNP (B-type natriuretic peptide) was associated with better outcomes.
- Compared with enalapril, sacubitril/valsartan increased the ucGMP/BNP ratio from baseline to 1 and 8 months after randomization.
- The effect of sacubitril/valsartan, compared with enalapril, on clinical outcomes was not modified by baseline ucGMP/BNP ratio.

WHAT ARE THE CLINICAL IMPLICATIONS?

- These findings suggest that the natriuretic peptide-cGMP (cyclic guanosine monophosphate) axis remains intact and responsive in patients with heart failure and reduced ejection fraction and that augmentation of natriuretic peptide–mediated cGMP release could be therapeutically beneficial.

Nonstandard Abbreviations and Acronyms

AF	atrial fibrillation
ANP	atrial natriuretic peptide
BMI	body mass index
BNP	B-type natriuretic peptide
cGMP	cyclic guanosine monophosphate
eGFR	estimated glomerular filtration rate
GC	guanylate cyclase
HF	heart failure
HFrEF	heart failure with reduced ejection fraction
NPR-A	natriuretic peptide A receptor
NT-proBNP	N-terminal pro-B-type natriuretic peptide
PARADIGM-HF	Prospective Comparison of ARNI With ACEI to Determine Impact on Global Mortality and Morbidity in Heart Failure trial
sGC	soluble guanylate cyclase
ucGMP	urinary cyclic guanosine monophosphate

cGMP (cyclic guanosine monophosphate) is a cyclic nucleotide second messenger leading to downstream effects such as relaxation of vascular smooth muscle.[1–3] cGMP synthesis is catalyzed by GC (guanylate cyclase) of which there are 2 major types. Membrane-bound particulate GC is activated by peptide hormones such as A-type (atrial) and BNP (B-type natriuretic peptide) and sGC (soluble guanylate cyclase) is classically activated by nitric oxide.[1–3] Acting through cGMP, natriuretic peptides are a key endogenous system protecting against pressure and volume overload, countering the actions of the renin-angiotensin-aldosterone system.[4] One mechanism responsible for the clearance of natriuretic peptides is the activity of the enzyme neprilysin or neutral endopeptidase. This has been exploited therapeutically, with the development of neprilysin inhibitors, which reduce the breakdown of natriuretic and other vasoactive peptides, a strategy shown to be beneficial in patients with heart failure (HF) with reduced ejection fraction (HFrEF).[5,6] Plasma cGMP reflects spill-over of intracellular cGMP and cGMP is filtered freely into the urine. ucGMP (urinary cGMP) levels reflect the activity of endogenous vasoactive substances such as ANP (atrial natriuretic peptide) and BNP, and the ratio of ucGMP to these natriuretic peptides may provide a measure of the responsiveness of the GC-cGMP axis to endogenous natriuretic peptide production and augmentation of natriuretic peptides by neprilysin inhibition.[7] The responsiveness of this axis may be attenuated by other changes in HFrEF such as natriuretic peptide receptor downregulation and increased intracellular degradation of cGMP.[8–11] To investigate this further, we have examined the relationship between the ratio of ucGMP to plasma BNP (ucGMP/BNP) and outcomes in the PARADIGM-HF trial (Prospective Comparison of ARNI With ACEI to Determine Impact on Global Mortality and Morbidity in Heart Failure) and the effect of sacubitril/valsartan, compared with enalapril, on ucGMP/BNP ratio. Since the ucGMP/BNP ratio is thought to reflect the responsiveness of tissues to natriuretic peptides and other vasoactive peptides and neprilysin inhibition increases BNP levels (and possibly natriuretic peptide-induced cGMP release), we hypothesized that a lower ratio is associated with worse clinical outcomes and that sacubitril/valsartan increases the ucGMP/BNP ratio.

METHODS

PARADIGM-HF was a randomized, double-blind, placebo-controlled trial in patients with chronic HFrEF, which evaluated the efficacy and safety of the angiotensin-receptor-neprilysin inhibitor (ARNI) sacubitril/valsartan compared with enalapril, in addition to standard to standard care. The design and primary results of PARADIGM-HF have been reported previously.[5,12] The institutional review boards of the 1043 participating institutions in 47 countries approved the protocol, and all patients gave written informed consent. The corresponding author had full access to all the trial data and takes responsibility for its integrity and the data analysis. Trial data will be made available by the sponsor, Novartis, in accordance with their data sharing policy.

Patients and Study Procedures

Key inclusion criteria included age of ≥18 years, New York Heart Association functional class II-IV, left ventricular

Table 1. Baseline Characteristics According to Tertiles of ucGMP/BNP Ratio at Baseline

	Tertile 1: <2.29, N=679	Tertile 2: 2.29–5.17, N=680	Tertile 3: ≥5.18, N=680	*P* value
Age, y, mean (SD)	70.0±9.7	67.6±9.5	64.2±10.2	<0.001
Male sex, N (%)	530 (78.1)	549 (80.7)	577 (84.9)	0.001
Geographic region, N (%)				<0.001
North America	142 (20.9)	102 (15.0)	82 (12.1)	
Western Europe and other	297 (43.7)	326 (47.9)	299 (44.0)	
Central Europe	240 (35.3)	252 (37.1)	299 (44.0)	
Race, N (%)				0.006
White	658 (96.9)	641 (94.3)	637 (93.7)	
Black	10 (1.5)	21 (3.1)	32 (4.7)	
Asian	3 (0.4)	1 (0.1)	3 (0.4)	
Other	8 (1.2)	17 (2.5)	8 (1.2)	
Physiological measures, mean (SD)				
Systolic blood pressure, mmHg	122.5±16.1	123.3±15.3	123.8±15.6	0.11
Heart rate, bpm	70.8±11.4	71.4±12.3	72.1±12.2	0.05
Body mass index, kg/m^2	28.6±5.1	29.6±5.4	30.2±5.6	<0.001
Creatinine, µmol/L	111±30	102±26	97±23	<0.001
eGFR, mL/min per 1.73 m^2	58±19	64±17	69±17	<0.001
BNP, ng/L, median (IQR)	347 (229–577)	200 (138–303)	119 (72–176)	<0.001
ucGMP, nmol/L, median (IQR)	458 (273–718)	706 (480–1019)	1086 (696–1646)	<0.001
ucGMP/BNP ratio, nmol/ng, median (IQR)	1.4 (0.9–1.8)	3.5 (2.9–4.2)	8.5 (6.4–13.3)	<0.001
NT-proBNP, ng/L, median (IQR)	2540 (1351–5355)	1427 (831–2426)	1071 (689–1785)	<0.001
Current smoker, N (%)	74 (10.9)	89 (13.1)	110 (16.2)	0.004
Ischemic cause of HF, N (%)	469 (69.1)	445 (65.4)	389 (57.2)	<0.001
Duration of HF, N (%)				0.08
<1 y	128 (18.9)	152 (22.4)	169 (24.9)	
1–5 y	244 (35.9)	239 (35.1)	242 (35.6)	
>5 y	307 (45.2)	289 (42.5)	269 (39.6)	
LVEF, mean (SD)	29.3±6.6	30.9±5.9	31.1±5.9	<0.001
NYHA class at randomization, N (%)				0.02
I–II	495 (72.9)	507 (74.7)	531 (78.2)	
III–IV	184 (27.1)	172 (25.3)	148 (21.8)	
KCCQ-OSS, median (IQR)	74.5 (56.8–88.0)	76.8 (61.5–88.4)	76.0 (60.2–89.6)	0.09
KCCQ-CSS, median (IQR)	77.1 (58.9–90.6)	78.4 (63.2–89.6)	79.7 (62.5–91.7)	0.05
Medical history, N (%)				
Hospitalization for HF	411 (60.5)	387 (56.9)	424 (62.4)	0.49
Hypertension	550 (81.0)	512 (75.3)	525 (77.2)	0.09
Diabetes	291 (42.9)	278 (40.9)	239 (35.1)	0.004
History of atrial fibrillation	351 (51.7)	302 (44.4)	336 (49.4)	0.40
Atrial fibrillation on ECG	180 (26.7)	189 (28.3)	235 (34.8)	0.001
Previous myocardial infarction	348 (51.3)	342 (50.3)	298 (43.8)	0.006
Previous stroke	71 (10.5)	82 (12.1)	58 (8.5)	0.24
Chronic obstructive pulmonary disease	123 (18.1)	106 (15.6)	128 (18.8)	0.73
Cancer	78 (11.5)	51 (7.5)	38 (5.6)	<0.001
Treatment, N (%)				
Beta-blocker	641 (94.4)	651 (95.7)	650 (95.6)	0.31
Mineralocorticoid-receptor antagonist	284 (41.8)	307 (45.1)	327 (48.1)	0.02
Diuretic	558 (82.2)	550 (80.9)	564 (82.9)	0.71
Digitalis	129 (19.0)	149 (21.9)	181 (26.6)	<0.001
Antiplatelet	394 (58.0)	401 (59.0)	379 (55.7)	0.39
Oral anticoagulant	291 (42.9)	283 (41.6)	305 (44.9)	0.46
Statin	431 (63.5)	458 (67.4)	420 (61.8)	0.51
Implantable cardioverter-defibrillator	196 (28.9)	199 (29.3)	180 (26.5)	0.33
Cardiac resynchronization therapy	99 (14.6)	68 (10.0)	61 (9.0)	0.001

BNP indicates B-type natriuretic peptide; eGFR, estimated glomerular filtration rate; HF, heart failure; IQR, interquartile range; KCCQ-CSS, Kansas City Cardiomyopathy Questionnaire clinical summary score; KCCQ-OSS, Kansas City Cardiomyopathy Questionnaire overall summary score; LVEF, left ventricular ejection fraction; NT-proBNP, N-terminal pro-B-type natriuretic peptide; NYHA, New York Heart Association; and ucGMP, urinary cyclic guanosine monophosphate.

ejection fraction of ≤35% (changed from ≤40% by a protocol amendment), elevated natriuretic peptide levels (plasma BNP ≥150 ng/L or NT-proBNP [N-terminal pro-B-type natriuretic peptide] ≥600 ng/L; BNP ≥100 ng/L or NT-proBNP ≥400 ng/L if hospitalized for HF within the previous 12 months), and treatment with a stable dose of an ACE (angiotensin-converting enzyme) inhibitor (ACE-i) or angiotensin receptor blocker equivalent to enalapril 10 mg/day for at least 4 weeks before the screening visit. Key exclusion criteria included symptomatic hypotension or systolic blood pressure <95 mmHg at randomization (100 mmHg at screening), estimated glomerular filtration rate (eGFR) <30 mL/min per 1.73 m^2 at randomization (or screening), potassium >5.4 mmol/L at randomization (>5.2 mmol/L at screening), a history of angioedema, and intolerance to ACE inhibitor or angiotensin receptor blocker.[12]

On trial entry, ongoing therapy with ACE inhibitor or angiotensin receptor blocker was stopped, and patients received enalapril 10 mg twice daily for 2 weeks (single-blind) followed by sacubitril/valsartan, uptitrated from 100 mg twice daily to 200 mg twice daily, for additional 4 to 6 weeks (single-blind run-in period). Patients tolerating both drugs at the target doses were then randomly assigned to double-blind therapy with sacubitril/valsartan or enalapril in a 1:1 ratio.[12]

BNP and ucGMP Measurements

As part of the biomarker sub-study of PARADIGM-HF, blood and urine samples were collected at baseline, that is, immediately before the single-blind run-in period; at the end of the run-in period, that is, while taking sacubitril/valsartan immediately before randomization; 1 month after randomization; and 8 months after randomization. The samples were collected on the same day, but at different time points, that is, the collected urine sample was first morning void urine (patients received instructions and urine collection containers prior to the visits), and blood samples were collected at the study sites. These samples were analyzed in a central laboratory using frozen plasma (for BNP and NT-proBNP) and first morning void urine (for ucGMP). Plasma BNP was measured by the Advia Centaur chemiluminescent immunoassay (Siemens Healthcare Diagnostics, Tarrytown, New York) with a reporting range of 2.7 to 4590 ng/L. Plasma NT-proBNP was measured by the Roche Elecsys proBNP chemiluminescent immunoassay assay (Roche Diagnostics GmbH, Penzberg, Germany) with a reporting range of 8

Table 2. Outcomes According to Tertiles of ucGMP/BNP Ratio at Baseline

	Tertile 1: <2.29, N=679	Tertile 2: 2.29–5.17, N=680	Tertile 3: ≥5.18, N=680
HF hospitalization or cardiovascular death			
N (%)	211 (31.1)	128 (18.8)	127 (18.7)
Event rate per 100 person-years (95% CI)	14.9 (13.0–17.0)	8.3 (6.9–9.8)	7.9 (6.7–9.4)
HR (95% CI)*	Reference	0.57 (0.45–0.71)	0.54 (0.43–0.67)
HR (95% CI)†	Reference	0.62 (0.49–0.77)	0.62 (0.49–0.79)
HR (95% CI)‡	Reference	0.75 (0.59–0.95)	0.82 (0.64–1.06)
HF hospitalization			
N (%)	140 (20.6)	82 (12.1)	79 (11.6)
Event rate per 100 person-years (95% CI)	9.9 (8.4–11.7)	5.3 (4.3–6.6)	4.9 (4.0–6.1)
HR (95% CI)*	Reference	0.56 (0.42–0.73)	0.52 (0.40–0.69)
HR (95% CI)†	Reference	0.62 (0.47–0.83)	0.62 (0.46–0.83)
HR (95% CI)‡	Reference	0.78 (0.58–1.04)	0.85 (0.62–1.17)
Cardiovascular death			
N (%)	115 (16.9)	67 (9.9)	64 (9.4)
Event rate per 100 person-years (95% CI)	7.2 (6.0–8.7)	4.0 (3.2–5.1)	3.7 (2.9–4.8)
HR (95% CI)*	Reference	0.55 (0.41–0.75)	0.50 (0.37–0.69)
HR (95% CI)†	Reference	0.60 (0.44–0.82)	0.60 (0.43–0.83)
HR (95% CI)‡	Reference	0.72 (0.52–1.00)	0.77 (0.54–1.09)
All-cause death			
N (%)	146 (21.5)	106 (15.6)	95 (14.0)
Event rate per 100 person-years (95% CI)	9.2 (7.8–10.8)	6.4 (5.3–7.7)	5.6 (4.5–6.8)
HR (95% CI)*	Reference	0.69 (0.54–0.89)	0.60 (0.46–0.78)
HR (95% CI)†	Reference	0.77 (0.59–0.99)	0.74 (0.56–0.97)
HR (95% CI)‡	Reference	0.89 (0.68–1.17)	0.91 (0.68–1.22)

BNP indicates B-type natriuretic peptide; HF, heart failure; HR, hazard ratio; and ucGMP, urinary cyclic guanosine monophosphate.

*The Cox models are adjusted for treatment assignment and region.

†The Cox models are adjusted for treatment assignment, region, age, sex, New York Heart Association functional class, HF duration, prior HF hospitalization, HF etiology, diabetes, atrial fibrillation, systolic blood pressure, heart rate, estimated glomerular filtration rate, ejection fraction, and body mass index.

‡The Cox models are adjusted for the above and log of NT-proBNP (N-terminal pro-B-type natriuretic peptide).

to 35 000 pg/mL, as previously described.[13] ucGMP was measured by the Parameter enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, MN) with a reporting range of 6.4 to 500 pmol/mL.[14]

Outcomes

The primary outcome in PARADIGM-HF was the composite of HF hospitalization or cardiovascular death. In the present analysis, we also examined each of the components of the primary outcome and death from any cause.

Statistical Analyses

In the present analysis, patients were divided into 3 subgroups, based on the tertiles of baseline ucGMP/BNP ratio.

Baseline characteristics were summarized as frequencies with percentages, means with SD, or medians with interquartile ranges. Differences in baseline characteristics across tertiles of ucGMP/BNP ratio at baseline were tested using the Cochran-Armitage trend test for binary variables, the Cochran-Mantel-Haenszel test for categorical variables, and the Jonckheere-Terpstra test and linear regression for non-normal and normally distributed continuous variables, respectively.

The association between tertiles of ucGMP/BNP ratio at baseline and clinical outcomes was evaluated using the Kaplan-Meier estimator (all-cause death), the Aalen-Johansen estimator (taken the competing risk of death into account for all outcomes except all-cause death), and Cox proportional-hazards models, with treatment group assignment and geographic region as fixed-effect factors to calculate hazard ratios with 95% CIs. In addition, HRs adjusted for treatment-group assignment, geographic region, age, sex, systolic blood pressure, heart rate, eGFR, left ventricular ejection fraction, body mass index (BMI), NT-proBNP (log-transformed), New York Heart Association functional class, duration of HF, prior HF hospitalization, HF etiology, and a history of diabetes and atrial fibrillation (AF) were reported. The association between ucGMP/BNP ratio at baseline as a continuous variable and outcomes was also examined in adjusted restricted cubic spline analyses.

The relationship between the change in ucGMP/BNP ratio from baseline to randomization (examined both as an absolute and relative change) and outcomes was assessed in restricted cubic spline analyses.

The difference between randomized treatment groups in the change in ucGMP/BNP ratio from baseline to 1 month and 8 months after randomization, respectively, was analyzed using analysis of covariance models, adjusted for baseline ucGMP/BNP ratio, and results were reported as the ratio of geometric means with 95% CIs. The absolute and relative difference between randomized treatment groups in the change in ucGMP/BNP ratio from baseline to 1 month and 8 months was also analyzed according to certain thresholds (eg, 5-unit increase) with logistic regression models, and results were reported as odds ratios with 95% CIs. These models were not adjusted. Since BMI, AF, and eGFR are associated with BNP levels, the difference between randomized treatment groups in

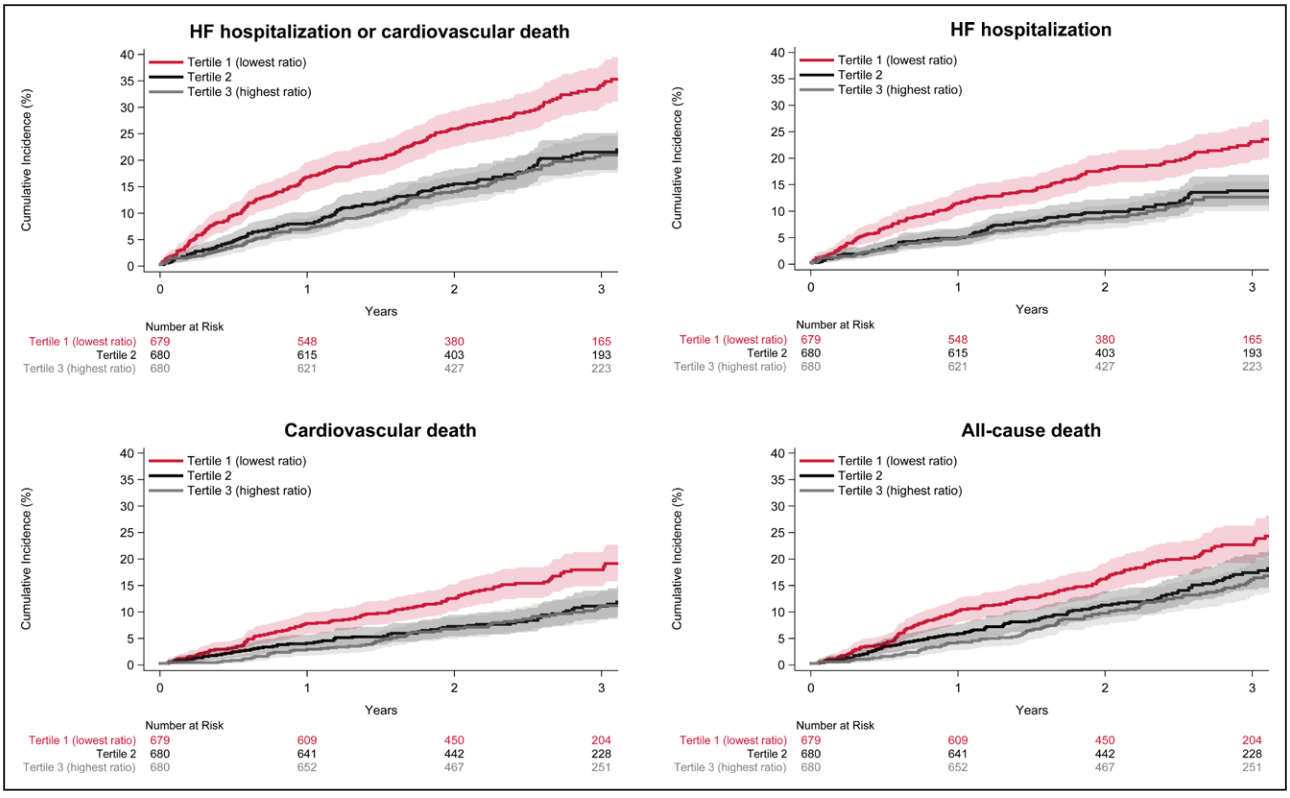

Figure 1. Cumulative incidence of outcomes according to tertiles of ucGMP/BNP ratio at baseline.
This figure shows the cumulative incidence of clinical outcomes according to tertiles of ucGMP/BNP ratio at baseline. The shaded area represents 95% CIs. BNP indicates B-type natriuretic peptide; HF, heart failure; and ucGMP, urinary cyclic guanosine monophosphate.

the change in ucGMP/BNP ratio from baseline to 1 month and 8 months after randomization was also examined in these subgroups (ie, BMI <30 kg/m^2; BMI ≥30 kg/m^2; no history of AF; history of AF; eGFR <60 mL/min per 1.73 m^2; eGFR ≥60 mL/min per 1.73 m^2).

The effects of sacubitril/valsartan versus enalapril on clinical outcomes according to tertiles of ucGMP/BNP ratio at baseline were evaluated with Cox proportional-hazards models, adjusted for geographic region. The treatment effect was also examined according to continuous baseline ucGMP/BNP ratio. Finally, the effects of sacubitril/valsartan, compared with valsartan, on the primary outcome according to the change in ucGMP/BNP ratio from baseline to 1 month after randomization was analyzed. In this landmark analysis, patients who experienced the primary outcome within 1 month after randomization were excluded (N=15). All analyses were conducted using SAS version 9.4 (SAS Institute, Cary, NC) and STATA version 17.0 (College Station, TX). A *P* value of 0.05 was considered statistically significant.

RESULTS

In total, 2039, 2031, 1959, and 1746 patients had both ucGMP and BNP measurements available at baseline, randomization, 1 month after randomization, and 8 months after randomization, respectively. The median ucGMP/BNP ratio at baseline was 3.50 (25th–75th percentile, 1.81–6.42). The 3 groups defined by tertiles of ucGMP/BNP ratio at baseline were (1) <2.29, (2) 2.29 to 5.17, and (3) ≥5.18.

Patient Characteristics

Baseline characteristics of the PARADIGM-HF population according to availability of ucGMP/BNP ratio at baseline are shown in Table S1. Patients with an ucGMP/BNP measurement at baseline differed from those without a measurement in a number of ways. They

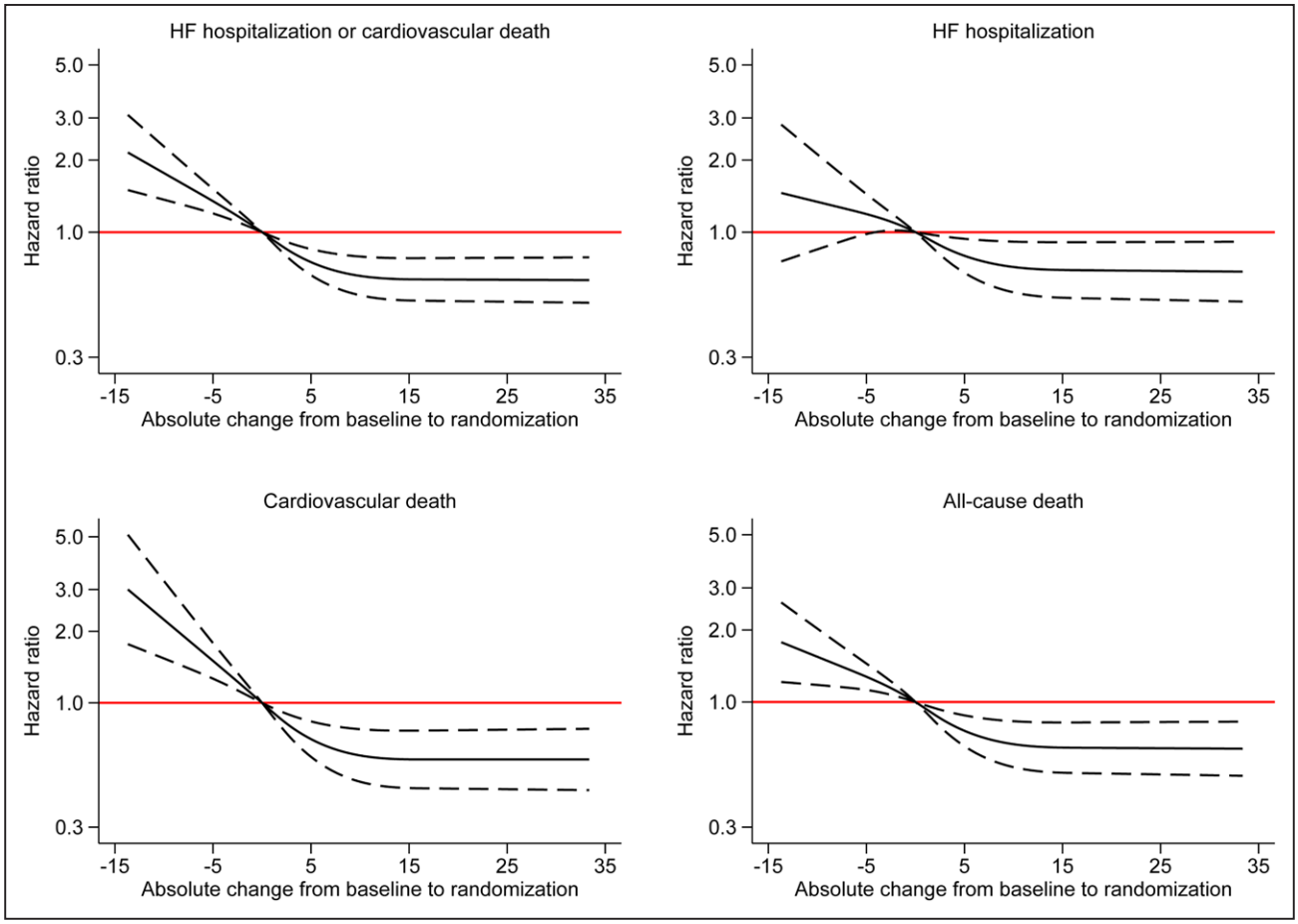

Figure 2. Change in ucGMP/BNP ratio from baseline to randomization and outcomes.
This figure shows the change in the ratio of ucGMP to BNP from the baseline visit (at which time all patients were receiving a renin-angiotensin system blocker) to the randomization visit (at which time all patients had completed 4- to 6-week treatment with sacubitril/valsartan titrated to 97/103 mg twice daily). The *x* axis shows the change in units (minus values mean decrease, positive values mean increase) and the *y* axis the hazard ratio for the outcomes of interest according to change in ucGMP/BNP ratio. An increase in ucGMP/BNP ratio was associated with a lower risk of all outcomes examined. The restricted cubic spline models are adjusted for baseline ucGMP/BNP ratio, treatment assignment, and region. The dashed lines represent 95% confidence intervals. The reference is 0 (no change). Figures have been restricted to the 2.5th to 97.5th percentile of the change in cGMP/BNP ratio from baseline to randomization, but the results are derived from models based on the entire spectrum of a change from baseline to randomization. BNP indicates B-type natriuretic peptide; and ucGMP, urinary cyclic guanosine monophosphate.

were older, more often men and White, and had more comorbidities. They were also more likely to have ischemic etiology, a longer duration of HF, and a higher left ventricular ejection fraction, but had lower NT-proBNP.

Baseline characteristics according to tertiles of ucGMP/BNP ratio at baseline are presented in Table 1. Compared with patients with a lower ucGMP/BNP ratio, those with a higher ucGMP/BNP ratio were younger and more often male. Patients with a higher ucGMP/BNP ratio had lower BNP and NT-proBNP and higher ucGMP, eGFR, and BMI. They were also less likely to have an ischemic etiology, prior myocardial infarction, and diabetes, but more often had AF on their ECG. Regarding background HF therapy, patients with a higher ucGMP/BNP ratio were more often treated with a mineralocorticoid receptor antagonist and digoxin, and less frequently with cardiac resynchronization therapy.

Outcomes According to Baseline ucGMP/BNP Ratio

Compared with the lowest tertile of ucGMP/BNP ratio at baseline, patients in the higher tertiles had a lower risk of all clinical outcomes (Table 2; Figure 1). After adjustment for NT-proBNP, this association remained statistically significant only for the composite of HF hospitalization or cardiovascular death, although there was a trend toward lower risk of the other outcomes in patients in the higher tertiles of ucGMP/BNP ratio. However, when examining ucGMP/BNP ratio as a continuous variable, the adjusted risk of all outcomes (except all-cause death) was significantly higher in patients with a ucGMP/BNP ratio below the median (ie, 3.50; Figure S1).

Outcomes According to Change in ucGMP/BNP Ratio From Baseline to Randomization

The associations between the absolute and relative change in ucGMP/BNP ratio from baseline to randomization (i.e., the period during which all patients were given sacubitril/valsartan), analyzed as a continuous variable, and clinical outcomes are shown in Figure 2 and Figure S2, respectively. Compared with no increase, patients with any increase (either an absolute or relative increase) in ucGMP/BNP ratio from baseline to randomization had a significantly lower risk of outcomes (Figure 2; Figure S2).

Effect of Sacubitril/Valsartan on ucGMP/BNP Ratio

During the active run-in period during which all patients were treated with sacubitril/valsartan titrated to a dose of 97/103 mg twice daily, the ucGMP/BNP ratio increased (Figure 3; Table S2). At randomization, the ucGMP/BNP ratio remained elevated in patients who continued to receive sacubitril/valsartan but declined in the half of patients who switched to enalapril. Consequently, compared with baseline, the ucGMP/BNP ratio at 1 month and 8 months after randomization was higher with sacubitril/valsartan than with enalapril: ratio of geometric mean ratios at 1 month, 1.38 (95% CI, 1.27–1.51) and 8 months, 1.32 (95% CI, 1.20–1.45; Figure 3; Table S2).

Examination of threshold increases in ucGMP/BNP ratio from baseline to 1 month after randomization showed that patients treated with sacubitril/valsartan

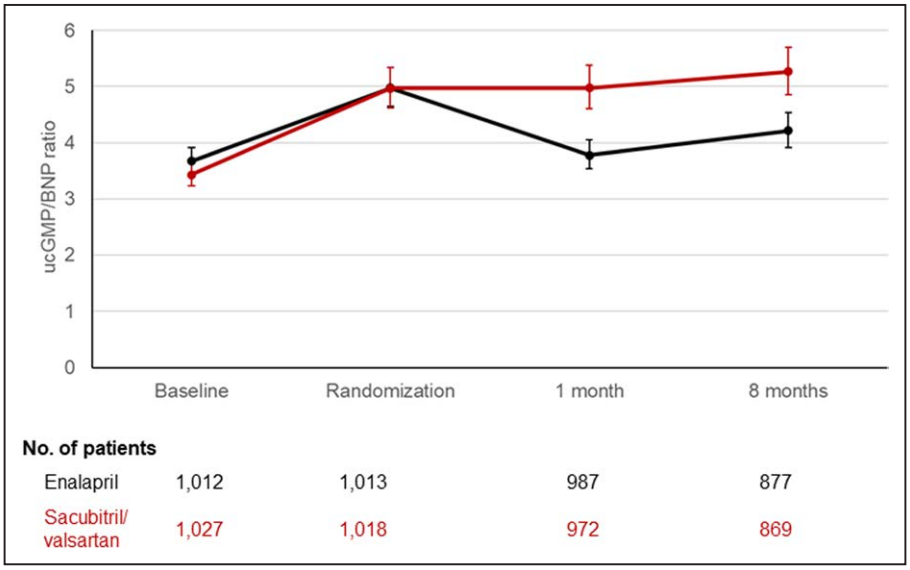

Figure 3. Change in ucGMP/BNP ratio during follow-up according to treatment assignment.
This figure shows the change in ucGMP/BNP ratio during follow-up according to treatment assignment. Dots represent geometric means, and error bars represent 95% CIs. BNP indicates B-type natriuretic peptide; HF, heart failure; and ucGMP, urinary cyclic guanosine monophosphate.

Table 3. Absolute Change in ucGMP/BNP Ratio From Baseline to 1 Month After Randomization

	Enalapril, N=949	Sacubitril/valsartan, N=943	Sacubitril/valsartan vs enalapril odds ratio (95% CI)
Increase, N (%)			
Any increase	486 (51.2)	611 (64.8)	1.75 (1.46–2.11)
>2.5-unit increase	189 (19.9)	334 (35.4)	2.21 (1.79–2.71)
>5-unit increase	96 (10.1)	205 (21.7)	2.47 (1.90–3.21)
>7.5-unit increase	56 (5.9)	150 (15.9)	3.02 (2.19–4.16)
>10-unit increase	44 (4.6)	107 (11.3)	2.63 (1.83–3.79)
>15-unit increase	26 (2.7)	67 (7.1)	2.72 (1.71–4.31)
>20-unit increase	19 (2.0)	54 (5.7)	2.97 (1.75–5.05)
Decrease, N (%)			
Any decrease	463 (48.8)	332 (35.2)	0.57 (0.47–0.69)
>5-unit decrease	100 (10.5)	65 (6.9)	0.63 (0.45–0.87)
>10-unit decrease	45 (4.7)	26 (2.8)	0.57 (0.35–0.93)
>15-unit decrease	33 (3.5)	9 (1.0)	0.27 (0.13–0.56)

Patients were included in this analysis if they had an available ucGMP/BNP ratio at baseline and 1 month after randomization. BNP indicates B-type natriuretic peptide; and ucGMP, urinary cyclic guanosine monophosphate.

were more likely to have larger increases in ucGMP/BNP ratio than those treated with enalapril (odds ratio for a 15-unit increase, 2.72 [95% CI, 1.71–4.31]; Table 3). Conversely, patients treated with sacubitril/valsartan were less likely to have large decreases in ucGMP/BNP ratio between baseline and 1 month than those treated with enalapril (odds ratio for a 15-unit decrease, 0.27 [95% CI, 0.13–0.56]; Table 3). Findings were similar when examining the change in ucGMP/BNP ratio from baseline to 8 months after randomization (Table S3) and if relative (rather than absolute) change in ucGMP/BNP ratio from baseline to 1 and 8 months after randomization was analyzed (Table S4).

The ucGMP/BNP ratio from baseline to 1 month and 8 months was consistently higher with sacubitril/valsartan, compared with enalapril, across tertiles of ucGMP/BNP ratio at baseline ($P_{interaction}$=0.19 and 0.91, respectively; Table S2).

Subgroups

Obese patients (defined as a BMI ≥30 kg/m^2) had a significantly lower BNP and higher ucGMP/BNP ratio than nonobese individuals (Table S5). In contrast, patients with AF, compared with those without, had a significantly higher BNP and lower ucGMP, but there was no significant difference in the ucGMP/BNP ratio between the groups. Patients with chronic kidney disease (defined as an eGFR <60 mL/min per 1.73 m^2) had significantly higher BNP, but lower ucGMP and ucGMP/BNP ratio, than those without chronic kidney disease (Table S5).

The ucGMP/BNP ratio from baseline to 1 month and 8 months was consistently higher with sacubitril/valsartan, compared with enalapril, regardless of BMI, AF, or eGFR at baseline (1 month, $P_{interaction}$ for all subgroups ≥0.52 at 1 month and ≥0.25 at 8 months; Table S6).

Effect of Sacubitril/Valsartan on Clinical Outcomes According to Baseline ucGMP/BNP Ratio

The effect of sacubitril/valsartan, compared with enalapril, was consistent across tertiles of ucGMP/BNP ratio at baseline for HF hospitalization or cardiovascular death ($P_{interaction}$=0.61), HF hospitalization ($P_{interaction}$=0.69), cardiovascular death ($P_{interaction}$=0.31), and all-cause death ($P_{interaction}$=0.32; Figure 4). The effect of sacubitril/valsartan, compared with enalapril, on clinical outcomes was also consistent when ucGMP/BNP ratio at baseline was examined as a continuous variable (Figure 5).

In a landmark analysis, the effect of sacubitril/valsartan, compared with valsartan, on the primary outcome was consistent, irrespective of the change in ucGMP/BNP ratio from baseline to 1 month after randomization (Figure S3).

DISCUSSION

The ratio of ucGMP/BNP is thought to reflect the responsiveness of tissues to natriuretic peptides and other vasoactive peptides.[10] We found that patients with a higher ucGMP/BNP ratio had better outcomes than those with a lower ucGMP/BNP ratio, and ucGMP/BNP ratio remained associated with outcomes, independently of other prognostic variables. Sacubitril/valsartan

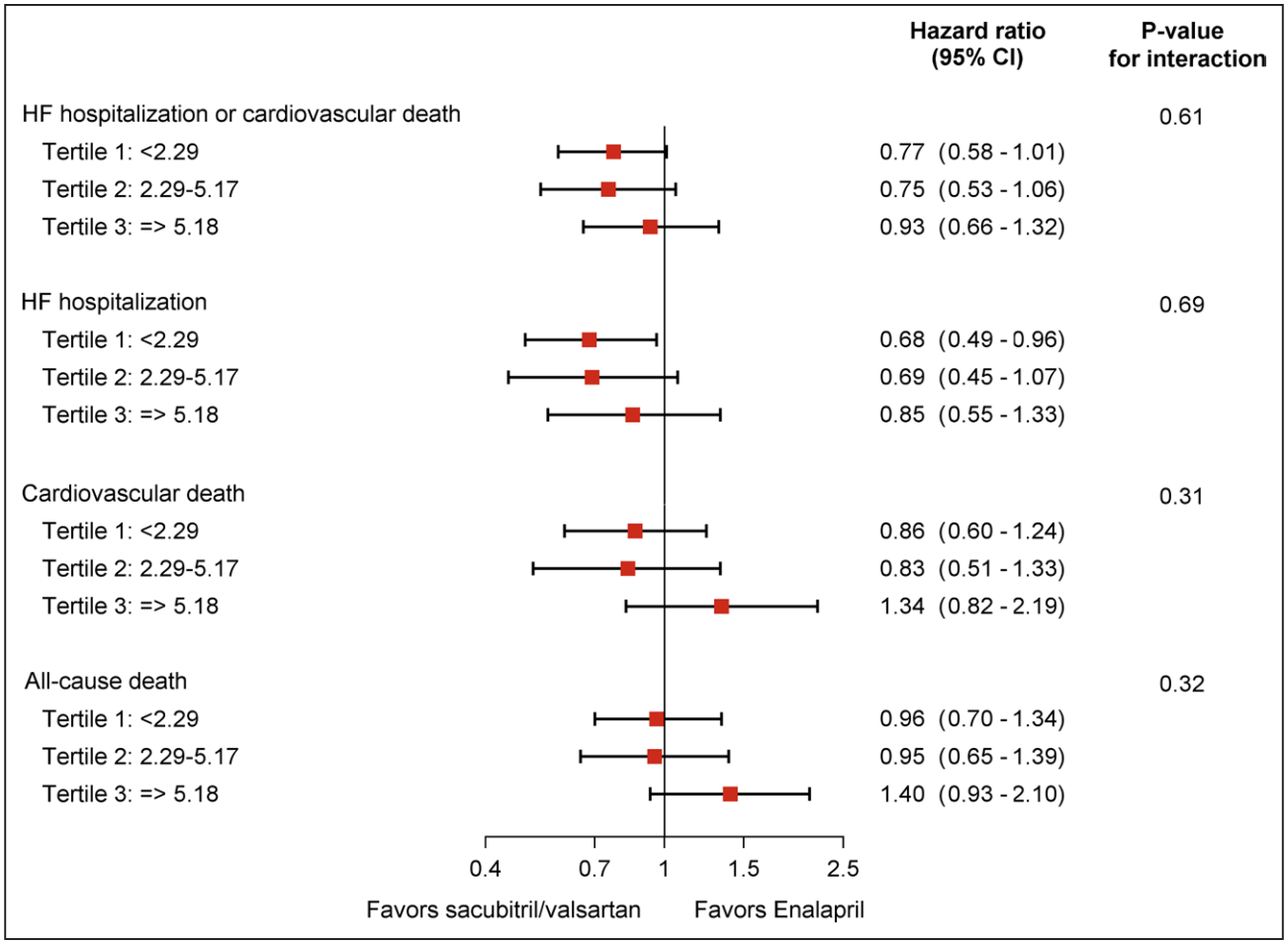

Figure 4. Effects of sacubitril/valsartan compared with enalapril according to tertiles of ucGMP/BNP ratio at baseline. This figure shows the effect of sacubitril/valsartan, compared with enalapril, on clinical outcomes according to tertiles of ucGMP/BNP ratio at baseline. The Cox models are adjusted for region. BNP indicates B-type natriuretic peptide; HF, heart failure; and ucGMP, urinary cyclic guanosine monophosphate.

increased ucGMP/BNP ratio, compared with enalapril, and the effect of sacubitril/valsartan on clinical outcomes was not modified by ucGMP/BNP ratio at baseline.

Induction of HF in experimental animals increases the secretion of natriuretic peptides. Although the circulating levels of ANP and BNP remain elevated once HF is established, the ratio of plasma and urinary cGMP to plasma natriuretic peptides decreases to as much as half of that in control animals.[10,15–18] Surprising, little is known about ucGMP/BNP ratio as a potential index of activation of the natriuretic peptide-cGMP axis in people with HF. A few studies have reported both natriuretic peptide and cGMP levels in patients with HF and controls and although a ratio was not formally calculated, the data provided suggest a reduction in cGMP relative to ANP or BNP in patients, compared to controls.[8,9,19–22] We know of only 1 other study, which reported ucGMP/BNP ratio in participants with HFrEF and in that study participants with severe HFrEF (n=31) had a significantly lower ratio than patients with less advanced HF.[23] These findings are consistent with our analysis of ucGMP/BNP tertiles which showed patients with the lowest ratio had an overall worse clinical profile. More importantly, we found that a low ucGMP/BNP ratio is associated with significantly worse clinical outcomes, including both HF hospitalization and cardiovascular mortality. These data are consistent with the possibility that more advanced HF is a state of relative cGMP deficiency (due to reduced natriuretic peptide-induced cGMP release) and that greater cGMP deficiency is associated with higher morbidity and mortality. If correct, this suggests that augmentation of natriuretic peptide-mediated cGMP release could be therapeutically beneficial in patients with HFrEF.

Several explanations for the reduced cGMP/BNP ratio in experimental models of HF have been proposed, mainly reflecting "downstream" adaptions such as NP receptor downregulation and uncoupling or upregulation of phosphodiesterases that degrade cGMP.[1,3,24] Our finding that sacubitril/valsartan increased ucGMP/BNP ratio, compared with enalapril, suggests that each

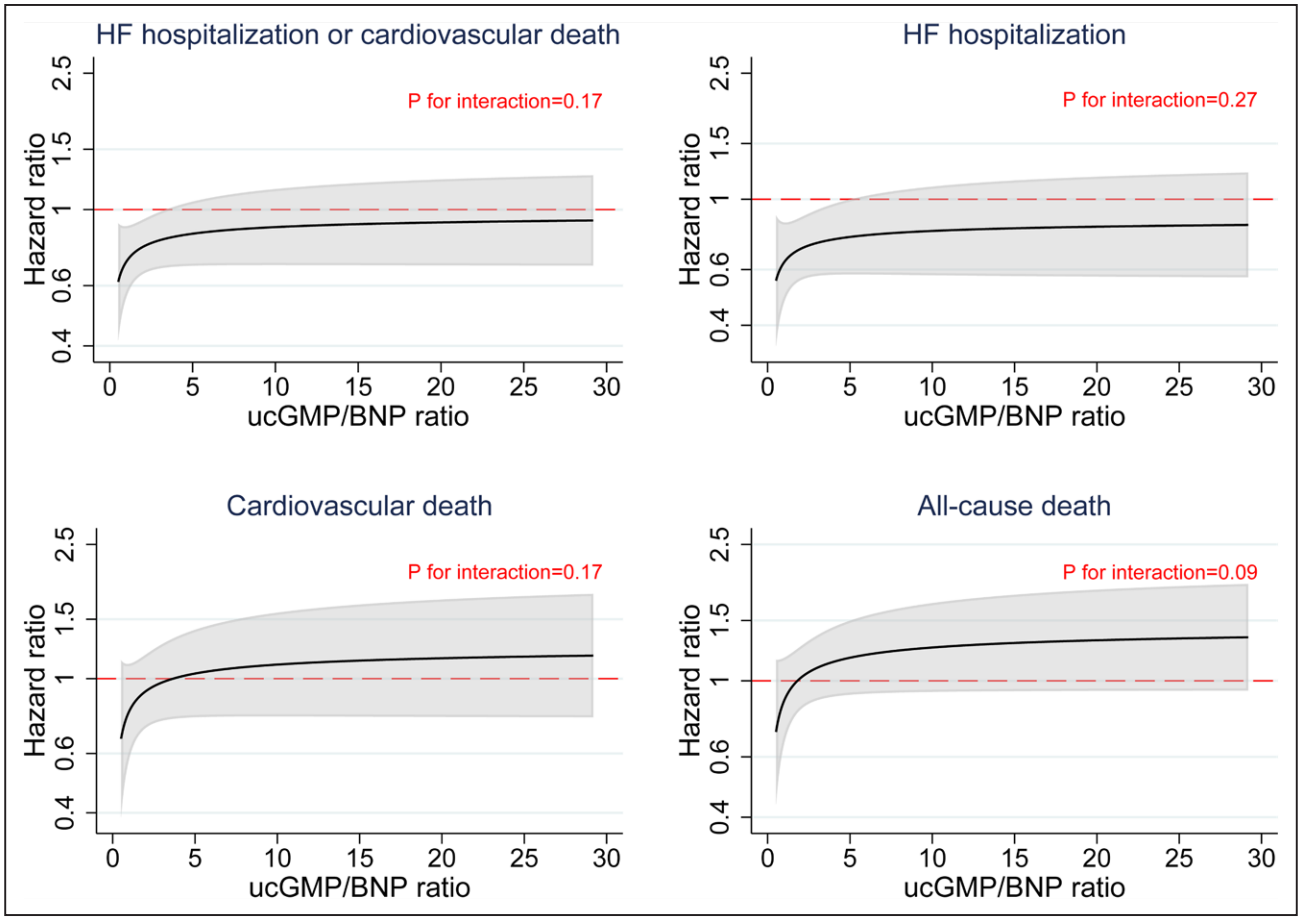

Figure 5. Effects of sacubitril/valsartan compared with enalapril according to continuous ucGMP/BNP ratio at baseline. This figure shows the effect of sacubitril/valsartan, compared with enalapril, on clinical outcomes according to continuous ucGMP/BNP ratio at baseline. The shaded area represents 95% CI. Median ucGMP/BNP ratio is 3.5. The *P* value reflects the interaction between the ucGMP/BNP ratio at baseline and the treatment effect. A *P* value >0.05 indicates a nonsignificant interaction, that is, that ucGMP/BNP ratio at baseline does not modify the effect of sacubitril/valsartan vs enalapril. The fractional polynomial model is adjusted for region. BNP indicates B-type natriuretic peptide; HF, heart failure; and ucGMP, urinary cyclic guanosine monophosphate.

of these downstream adaptions can be overcome by augmenting natriuretic peptide levels through neprilysin inhibition, although we cannot exclude the possibility that other vasoactive peptides augmented by neprilysin inhibition such as bradykinin and adrenomedullin might directly or indirectly stimulate cGMP production. Our findings also raise the possibility that alternative or additional therapeutic approaches to increasing cGMP production related to particulate guanylate cyclase activity may be useful in HFrEF.[1,3] One of these is inhibition of the phosphodiesterases degrading cGMP produced by particulate guanylate cyclase, an approach that might even be coupled with neprilysin inhibition.[25,26] Another would be to use a synthetic agonist that directly stimulates the NPR-A (natriuretic peptide A receptor).[27,28]

Recently, a possible "upstream" mechanism that could cause reduced cGMP production has also been proposed. It has been reported that as HF advances, a higher proportion of measured circulating BNP is the inactive prohormone, which has not been processed to active mature BNP (the prohormone is not distinguished from mature BNP by conventional assays).[29,30] However, neprilysin inhibition would still be expected to increase levels of mature BNP in these patients, and thus ucGMP/BNP ratio, if there is downstream tissue responsiveness (or if the increase in this ratio reflects the action of other vasoactive peptides augmented by neprilysin inhibition).

Importantly, we found that not only does neprilysin inhibition increase ucGMP/BNP ratio, suggesting that the natriuretic peptide-cGMP axis remains intact and responsive in HFrEF, but that responsiveness was seen even in patients with the lowest baseline ucGMP/BNP ratio, and neprilysin inhibition consistently improved clinical outcomes across the range of ucGMP/BNP ratios at baseline.

Limitations

As with all clinical studies of this type there are limitations. A key assumption of this study is that ucGMP reflects

systemic as opposed to mainly renal production. We think that this assumption is reasonable as mice genetically modified not to express NPR-A have reduced levels of circulating and urinary cGMP.[31] In human studies, infusion of natriuretic peptides leads to a direct increase in ucGMP.[32,33] Moreover, in patients with HF, ucGMP correlates with right atrial pressure.[19] Importantly, the primary natriuretic peptide produced in the kidney, urodilatin, is not thought to be a substrate for neprilysin.[34] However, we cannot exclude a contribution from intrarenal cGMP production in response to neprilysin inhibition. While we measured ucGMP/BNP ratio, the cGMP response to ANP is greater than to BNP and ucGMP/ANP ratio might have been more informative (but ANP is less stable, and its measurement is impractical in a large multinational trial). Finally, the characteristics of the patients enrolled in the biomarker sub-study were significantly different from those who were not.

Conclusions

In patients with HFrEF, a higher ucGMP/BNP ratio was associated with better outcomes. Sacubitril/valsartan increased the ucGMP/BNP ratio, compared with enalapril, irrespective of the baseline ucGMP/BNP ratio, and the effect of sacubitril/valsartan on clinical outcomes was not modified by ucGMP/BNP ratio at baseline. These findings suggest that the natriuretic peptide-cGMP axis remains intact and responsive in HFrEF and that augmentation of natriuretic peptide-mediated cGMP release could be therapeutically beneficial.

ARTICLE INFORMATION

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Supplemental Material

Tables S1–S6
Figures S1–S3

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