BACKGROUND: PENK (proenkephalin) is a stable surrogate for enkephalins, endogenous opioid peptides, which exert cardiodepressive effects and improve renal function. PENK has been associated with heart failure (HF) severity and renal dysfunction. We therefore hypothesized that PENK could be associated with deterioration of kidney function and could have a role as a novel renal marker in HF.

METHODS AND RESULTS: In 2180 patients with HF of a large multicenter cohort (BIOSTAT-CHF [A Systems Biology Study to Tailored Treatment in Chronic Heart Failure]), the relationship between PENK and clinical variables, plasma and urinary biomarkers, and clinical end points was established. Data were validated in a separate cohort of 1703 patients with HF. PENK was elevated (>80 pmol/L, 99th percentile) in 1245 (57%) patients. Higher PENK was associated with more advanced HF and glomerular and tubular dysfunction. The strongest independent predictor of PENK was estimated glomerular filtration rate. Others were plasma NGAL (neutrophil gelatinase–associated lipocalin) and NT-proBNP (N-terminal pro-B-type natriuretic peptide; all \( P < 0.001 \)). Using correlation heatmaps and hierarchical cluster analyses, PENK clustered with estimated glomerular filtration rate, creatinine, NGAL, galectin-3, and urea. Higher PENK was independently associated with increased risk of deterioration of kidney function between baseline and 9 months (odds ratio, 1.29 [1.02–1.65] per PENK doubling; \( P = 0.038 \); defined as >25% decrease in estimated glomerular filtration rate) and mortality (hazard ratio, 1.23 [1.07–1.43] per doubling; \( P = 0.004 \)). Analyses in the validation cohort yielded comparable findings.

CONCLUSIONS: Higher PENK levels are associated with more severe HF, with glomerular and tubular renal dysfunction, with incidence of a deterioration of kidney function, and with mortality. These findings suggest that the opioid system might be involved in deteriorating kidney function in HF.

Key Words: creatinine ■ glomerular filtration rate ■ heart failure ■ incidence ■ proenkephalin

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WHAT IS NEW?

- The opioid system, reflected by PENK (proenkephalin), is not only independently associated with glomerular dysfunction, but also with tubular damage in heart failure (HF).
- After subjection to meticulous positioning across a wide variety of clinical variables, plasma, and urinary biomarkers, PENK was repeatedly positioned in a panel of renal markers in HF.
- Our study confirms the possibility of the opioid system being a common pathway affecting both the heart and kidney in HF, a cardiorenal connector.
- PENK was strongly associated with increased mortality, but not with a higher risk of HF readmissions.

WHAT ARE THE CLINICAL IMPLICATIONS?

- PENK could become a novel, comprehensive renal marker in HF, reflecting both cardiac, glomerular, and tubular dysfunction.
- Higher PENK levels relate to an increased risk of mortality.

Enkephalins are endogenous opioid peptides that exert several cardiovascular effects by reducing myocardial contractility, blood pressure, and heart rate, while also inhibiting norepinephrine release and sympathetic vasoconstriction.1 Opioid peptides also play a role in ischemic preconditioning and cardiac hypertrophy.1 Besides cardiovascular effects, enkephalins exert renal effects predominantly by increasing renal blood flow and urinary output.2 By their established effects on cardiac contractility, hemodynamics, and renal function, enkephalins might play a pathophysiological role in the development and progression of cardiorenal failure.

However, endogenous enkephalins are unstable and difficult to measure in plasma. PENK (proenkephalin) is a stable and accurate surrogate marker of enkephalins that can be easily measured in plasma.3,4 A previous study showed that in patients with an acute myocardial infarction, higher PENK levels were associated with a higher risk for the development of heart failure (HF).5 In studies investigating PENK in HF populations, PENK levels were higher in patients with HF compared to healthy individuals,6 and higher levels of PENK were associated with worse(ning) renal function, HF severity, and adverse clinical events.6,7 Further validation and a deeper understanding of the role of PENK in HF is however required. Because of the profound cardiovascular and renal effects of enkephalins and previously reported associations of PENK with both disease severity and renal dysfunction in HF, we hypothesized that PENK, as a surrogate of the opioid system, could become a novel renal marker in HF, reflecting both cardiac, glomerular, and tubular dysfunction. To establish this, we investigated PENK in relation to a wide variety of clinical variables, plasma and urinary biomarkers, and clinical outcome, and the findings were subsequently validated in a separate patient cohort.

METHODS

Patient Populations

The data that support the findings of this study are available from the corresponding author upon reasonable request. For the present study, we used the index and validation cohort of BIOSTAT-CHF (A Systems Biology Study to Tailored Treatment in Chronic Heart Failure), which have been described in detail before.8 In brief, BIOSTAT-CHF was an investigator-driven multicenter clinical study consisting of 2516 patients in the index cohort with the aim to identify patients with a poor outcome despite currently recommended treatment. Patients were included after presentation with either new onset or worsening HF, which was defined as left ventricular ejection fraction (EF) ≤40% or BNP (brain natriuretic peptide) >400 pg/mL or NT-proBNP (N-terminal pro-B-type natriuretic peptide) >2000 pg/mL. Patients were expected and encouraged to be uptitrated to recommended treatment doses.9

Data were validated in the BIOSTAT-CHF validation cohort consisting of 1738 patients with HF from 6 centers in Scotland, United Kingdom.8 In summary, patients ≥18 years diagnosed with HF with a previous hospital admission for HF requiring diuretic treatment and current treatment with furosemide ≥20 mg/d or equivalent were included. They had previously not been treated or received ≤50% of target doses of ACE (angiotensin-converting enzyme) inhibitors/ARBs (angiotensin receptor blockers) and β-blockers as described in the 2008 European Society of Cardiology guidelines and treatment was expected to be initiated or uptitrated.

All patients enrolled in BIOSTAT-CHF provided written informed consent to participate in the study and BIOSTAT-CHF was conducted in concordance with the declaration of Helsinki, national ethics and legal requirements, as well as relevant EU legislation. The study was also approved by national and local ethics committees.

Study Design and Biomarker Measurements

PENK was measured in 2180 patients in the index cohort and in 1703 patients in the validation cohort. The study subsets used in this study did not differ greatly from the patients without PENK measurements in the BIOSTAT index and validation cohort (Tables I and II in the Data Supplement). PENK was measured using a sandwich immunoassay targeting PENK amino acids 119–159 (spingotest penKid, Sphingotec GmbH, Hennigsdorf, Germany) as described previously.3,5 The lower detection limit was 5.5 pmol/L and intra- and interassay coefficients of variation were 6.4% and 9.5%, respectively at 50 pmol/L and 4.0% and 6.5%, respectively at 150 pmol/L.3 The normal range of PENK has been established in a general population cohort (the Malmö Diet and Cancer Study), where PENK was measured in 1929 healthy individuals.10 The median (range) was 45 (9–518) pmol/L, and the 99th percentile was 80 pmol/L. The association between PENK and deterioration of kidney function was
analyzed with the latter defined as >25% decrease in estimated glomerular filtration rate (eGFR) between baseline and 9 months, with sensitivity analyses performed for >30% decrease and >40% decrease in eGFR. Furthermore, associations between PENK and change of creatinine over time as a continuous variable was analyzed in additional sensitivity analyses. After blood was drawn by venipuncture, samples were stored at −80°C. If possible, analyses were performed directly based on standardized international methods, otherwise, they were performed in a central laboratory. Urinary measurements were performed using standardized methods. All urinary measurements were corrected for urinary creatinine. Urinary KIM (kidney injury molecule)-1 and NGAL (neutrophil gelatinase–associated lipocalin) were measured using an in-house developed and validated multiplex immunoassay (xMAP; Lumixen, Austin, TX) as described previously. IL (interleukin)-6 and endothelin-1 were measured in frozen plasma by Singulex Inc (Alameda, CA) using high-sensitivity single molecule counting (SMC) technology (RUO, Erenna GmbH). Bio-ADM (bioactive adrenomedullin) was measured using an in-house developed and validated multiplex immunoassay (xMAP; Lumixen, Austin, TX) as described previously. IL (interleukin)-6 and endothelin-1 were measured in frozen plasma by Singulex Inc (Alameda, CA) using high-sensitivity single molecule counting (SMC) technology (RUO, Erenna Immunoassay System). Bio-ADM (bioactive adrenomedullin) was measured using an immunoassay developed by Sphingotec GmbH (Henningsdorf, Germany). High-sensitivity troponin T was measured using the Roche Elecsys assay on a Cobas e411 analyzer, using standard methods (Roche Diagnostics GmbH, Mannheim, Germany). Measurement of additional biomarkers was performed as previously described.

Study End Points

In the index cohort and the validation cohort, the relation of PENK with 3 clinical outcomes was evaluated: all-cause mortality, unscheduled HF hospitalization, and a composite outcome of all-cause mortality and HF hospitalization. The end points were adjusted for the BIOSTAT risk models that were created for each specific outcome in this cohort, which included age, log blood urea nitrogen, log NT-proBNP, hemoglobin, and β-blocker use at baseline for all-cause mortality; it included age, HF hospitalization in previous year, peripheral edema, systolic blood pressure, and eGFR for HF hospitalization; and it included age, HF hospitalization in previous year, systolic blood pressure, log NT-proBNP, hemoglobin, high-density lipoprotein, sodium, and β-blocker use at baseline for the combined end point.

Statistical Analysis

Descriptive statistics were used to examine the relationship of quartiles of PENK with clinical variables. Data are presented as means±SD when normally distributed, as medians (interquartile range) for skewed variables and as frequencies (percentage) for categorical variables. Continuous normally distributed variables were tested with the Student independent t test or ANOVA, whereas skewed variables were tested using the Kruskal-Wallis H test. Categorical variables were tested with χ² tests. Trends over quartiles of PENK were statistically tested with the Cochran-Armitage trend test, Jonckheere-Terpstra test, or a linear regression model for categorical variables, non-normally distributed continuous variables, and normally distributed continuous variables, respectively. Predictors of PENK were analyzed using univariable and multivariable regression analyses, in which all variables with P<0.10 in univariable analysis were included in multivariable analysis and subjected to the backward elimination method. Before linear regression analysis, the assumption of normal distribution was checked. If necessary, variables were log transformed (using natural logarithm). Histograms of log-transformed PENK in the index and validation cohort are illustrated in Figures I and II in the Data Supplement, respectively. The correlation heatmaps and dendrograms were constructed using the ggplot2, reshape2, and Hmisc packages and additionally the packages foreign, lm.beta, pROC, psych, ModelGood, survival, and survminer were used in R. The dendrograms were constructed using the hierarchical clustering method within the Hmisc package in R, which, in short, performs a hierarchical cluster analysis using a set of dissimilarities for the n objects being clustered (more details are provided in Expanded Methods in the Data Supplement). Cox proportional hazard models were constructed for all 3 end points to evaluate the prognostic predictability of PENK and adjusted for their respective BIOSTAT risk models. Transformations were performed as appropriate with multifrational polynomials used to assess linearity of associations. The proportional hazard assumption was checked by inspection of log-log plots. Results are expressed as hazard ratios (HRs) with their corresponding 95% CIs. Extensive additional adjustment for renal markers and markers known to be predictors of poor outcome in HF was consequently performed. A 2-tailed P value <0.05 was considered statistically significant. Statistical analyses were performed with IBM SPSS Statistics version 23 and R (version 1.1.383, R Foundation for Statistical Computing, Vienna, Austria) at http://www.r-project.org.

RESULTS

Baseline Characteristics According to Plasma PENK Levels

In the BIOSTAT-CHF index cohort, mean left ventricular EF was 31%, 49% of patients were in New York Heart Association class III, and 7% of patients had HF with preserved EF (HFpEF). Baseline characteristics in relation to quartiles of PENK are displayed in Table 1. Median plasma PENK concentration was 86.2 (63.7–120.2) pmol/L, and 1245 (57%) patients had PENK levels >80 pmol/L (99th percentile of the normal range of PENK). Higher levels of PENK were found in older patients and in patients with more severe HF (higher New York Heart Association classification, higher natriuretic peptides) lower systolic blood pressure, poorer renal function (higher creatinine, lower eGFR, higher plasma NGAL, higher urea), a higher degree of albuminuria (urine albumin-to-creatinine ratio [UACR]), and a higher degree of tubular damage (higher urinary KIM-1, higher urinary NGAL; all P for trend ≤0.001).

Association Between PENK Levels and Clinical Variables

Table 2 shows that the strongest associations of higher levels of log plasma PENK concentrations were with lower eGFR, higher plasma NGAL, higher NT-proBNP, lower ALAT (alanine transaminase), and lower diastolic blood pressure. The adjusted R² for the model was 0.561 (Table 2).
### Table 1. Baseline Characteristics of BIOSTAT-CHF Index Cohort in Relation to Quartiles of PENK

| Variables                                      | PENK, pmol/L | Q1, n=545 | Q2, n=545 | Q3, n=545 | Q4, n=545 | P for Trend |
|------------------------------------------------|--------------|-----------|-----------|-----------|-----------|-------------|
| **Clinical characteristics**                   |              |           |           |           |           |             |
| Age, y                                         | 62±11        | 68±11     | 71±11     | 75±11     | <0.001†   |             |
| Sex (male), n (%)                              | 459 (84)     | 405 (74)  | 375 (69)  | 356 (65)  | <0.001†   |             |
| NYHA classification (III/IV), n (%)            | 292 (55)     | 304 (57)  | 325 (61)  | 384 (73)  | <0.001†   |             |
| Systolic blood pressure, mm Hg                 | 128±22       | 123±21    | 124±22    | 123±22    | 0.001†    |             |
| Diastolic blood pressure, mm Hg                | 79±13        | 75±13     | 74±13     | 71±12     | 0.049†    |             |
| Heart rate, beats per minute                   | 80 (67–98)   | 78 (69–97) | 78 (67–94) | 78 (67–94) | <0.001†   |             |
| LVEF, %                                        | 30 (24–35)   | 30 (25–35) | 30 (25–37) | 31 (25–40) | <0.001†   |             |
| HFpEF,* n (%)                                  | 17 (3)       | 33 (6)    | 42 (8)    | 61 (11)   | <0.001†   |             |
| **Primary heart failure cause, n (%)**         |              |           |           |           |           |             |
| Ischemic heart disease                         | 205 (38)     | 218 (41)  | 250 (47)  | 284 (53)  | <0.001†   |             |
| Hypertension                                   | 55 (10)      | 58 (11)   | 53 (10)   | 62 (11)   | 0.662     |             |
| Cardiomyopathy                                 | 173 (32)     | 157 (29)  | 131 (25)  | 85 (16)   | <0.001†   |             |
| Valvular disease                               | 37 (7)       | 39 (7)    | 41 (8)    | 51 (9)    | 0.127     |             |
| **Medication, n (%)**                          |              |           |           |           |           |             |
| ACE inhibitor/angiotensin receptor blocker      | 433 (79)     | 410 (75)  | 389 (71)  | 332 (61)  | <0.001†   |             |
| β-blocker                                      | 469 (86)     | 456 (84)  | 450 (83)  | 437 (80)  | 0.009†    |             |
| Loop diuretics                                 | 541 (99)     | 542 (99)  | 542 (99)  | 544 (99)  | 0.224     |             |
| Aldosterone antagonist                         | 323 (59)     | 301 (55)  | 282 (52)  | 236 (43)  | <0.001†   |             |
| Diabetes mellitus, n (%)                       | 152 (28)     | 159 (29)  | 182 (33)  | 210 (39)  | <0.001†   |             |
| Smoking (past or current), n (%)               | 381 (70)     | 348 (64)  | 339 (62)  | 317 (58)  | <0.001†   |             |
| **Laboratory values**                          |              |           |           |           |           |             |
| Hemoglobin, g/dL                               | 14.0 (12.9–15.1) | 13.7 (12.5–14.7) | 13.1 (11.8–14.2) | 12.1 (10.8–13.4) | <0.001†   |             |
| Sodium, mmol/L                                 | 140 (138–142) | 140 (137–142) | 139 (137–142) | 139 (136–142) | 0.002†    |             |
| Potassium, mmol/L                              | 4.2 (3.9–4.5) | 4.2 (3.9–4.5) | 4.2 (3.9–4.6) | 4.2 (3.9–4.7) | 0.067     |             |
| NT-proBNP, pg/mL                               | 1652 (735–3628) | 2298 (1053–4591) | 2977 (1442–5765) | 5270 (2432–11113) | <0.001†   |             |
| Glucose, mmol/L                                | 6.3 (5.7–7.8) | 6.2 (5.4–7.5) | 6.2 (5.3–8.0) | 6.5 (5.3–8.5) | 0.702     |             |
| ASAT, U/L                                      | 27 (21–37)   | 25 (20–36) | 25 (19–34) | 24 (18–33) | <0.001†   |             |
| ALAT, U/L                                      | 30 (21–47)   | 27 (18–44) | 23 (16–34) | 20 (14–29) | <0.001†   |             |
| BNP, pg/mL                                     | 147 (65–333) | 210 (86–443) | 260 (107–498) | 333 (152–652) | <0.001†   |             |
| **Renal function**                             |              |           |           |           |           |             |
| Aldosterone, pg/mL                             | 93 (44–180)  | 92 (42–200) | 93 (44–187) | 93 (41–199) | 0.556     |             |
| Creatinine, μmol/L                             | 87 (72–98)   | 96 (80–112) | 106 (88–126) | 149 (145–186) | <0.001†   |             |
| eGFR, ml/min per 1.73 m²                       | 79±18        | 67±18     | 57±17     | 39±17     | <0.001†   |             |
| Plasma NGAL, ng/mL                             | 42 (28–64)   | 49 (33–74) | 66 (42–97) | 103 (67–166) | <0.001†   |             |
| Renin, μU/mL                                   | 66 (20–198)  | 77 (29–240) | 97 (30–285) | 116 (41–333) | <0.001†   |             |
| Urea, mmol/L                                   | 8.1 (5.9–12.9) | 9.4 (7.0–14.9) | 11.5 (8.3–18.2) | 16.5 (11.0–26.2) | <0.001†   |             |
| Urinary creatinine, mmol/L                     | 6.5 (3.3–11.3) | 5.7 (1.9–10.1) | 4.8 (2.5–9.3) | 4.1 (2.4–6.6) | <0.001†   |             |
| Urinary KIM-1, ng/gCr                           | 1509 (643–2574) | 1557 (791–2903) | 1947 (1003–3622) | 2863 (1533–5056) | <0.001†   |             |
| Urinary NAG, μg/gCr                            | 24 (11–47)   | 27 (14–62) | 36 (18–84) | 54 (23–16) | <0.001†   |             |
| UAACR, mg/gCr                                   | 15 (5–55)    | 16 (6–54)  | 30 (9–104) | 57 (12–199) | <0.001†   |             |
| **Biomarkers**                                  |              |           |           |           |           |             |
| CRP, ng/mL                                     | 13 145 (5740–26 208) | 11 933 (5006–24 660) | 13 536 (6149–26 146) | 16 350 (7014–30 250) | 0.002†    |             |
| FGF-23, RU/mL                                  | 137 (91–267) | 182 (109–388) | 237 (130–582) | 491 (242–1243) | <0.001†   |             |

(Continued)


Heatmap and Dendrogram Demonstrating That PENK Is a Cardiorenal Marker

Figure 1 shows the correlation heatmap including PENK, clinical variables, and plasma and urinary biomarkers. This heatmap demonstrates that PENK levels show the strongest correlation with eGFR (Spearman $\rho=-0.68$; $P<0.001$), creatinine (Spearman $\rho=0.57$; $P<0.001$), NGAL (Spearman $\rho=0.49$; $P<0.001$), FGF-23 (Spearman $\rho=0.42$; $P<0.001$), and urea (Spearman $\rho=0.41$; $P<0.001$). In a hierarchical cluster analysis (Figure 2), PENK clustered with renal markers eGFR, plasma NGAL, urea, and creatinine, and with galectin-3.

PENK as a Predictor of Deterioration of Kidney Function

The relationship between PENK and a deterioration of kidney function is displayed in Table 3. Based on the definition of >25% decrease in eGFR, the incidence of deterioration of kidney function was 222 (19.0%) in 1170 patients with available measurements in this study population. Doubling of PENK was a significant predictor for deterioration of kidney function, even after adjustment for plasma NGAL, urinary KIM-1, urinary NGAL, and UACR (odds ratio, 1.29 [95% CI, 1.02–1.65]; $P=0.038$). Sensitivity analyses with >30% decrease and 40% decrease in eGFR as the definition for deterioration of kidney function provided comparable results. Doubling of baseline eGFR was univariately not predictive of deterioration of kidney function and showed a significant interaction with PENK, which led to exclusion from the adjusted model. Doubling of PENK remained a significant and strong predictor when regression analysis was repeated with serum creatinine change as a continuous variable and as an end point in receiver operating characteristic curves (Table III and Figure III in the Data Supplement).

Plasma PENK Levels Predict Outcome

In total, 583 (26.7%) patients died, 553 (25.4%) were hospitalized for HF, and 914 (41.9%) either died or were hospitalized for HF during a median follow-up of 21 (15–27) months. In univariable Cox regression analysis (Table 4), doubling of PENK was significantly associated with all 3 outcomes (HR=1.87 [95% CI, 1.72–2.03]; $P<0.001$ for all-cause mortality; HR=1.52 [95% CI, 1.38–1.68]; $P<0.001$ for HF hospitalization; and HR=1.66 [95% CI, 1.55–1.79]; $P<0.001$ for the composite end point). After adjustment for the previously described risk models developed for each specific end point with addition of NT-proBNP, eGFR, and log UACR, higher doubling of PENK was significantly associated with a higher risk of all-cause mor-

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**Table 1. Continued**

| Variables                  | PENK, pmol/L | Q1, n=545 | Q2, n=545 | Q3, n=545 | Q4, n=545 | $P$ for Trend |
|----------------------------|--------------|-----------|-----------|-----------|-----------|---------------|
| Bio-ADM, pg/mL             | 27 (19–41)   | 30 (21–45)| 36 (24–55)| 47 (31–74)| $<0.001^†$ |               |
| ANP, ng/mL                 | 15 (10–24)   | 19 (12–29)| 23 (14–32)| 29 (19–39)| $<0.001^†$ |               |
| Galectin-3, ng/mL          | 16 (12–22)   | 18 (14–25)| 22 (17–29)| 30 (22–41)| $<0.001^†$ |               |
| GDF-15, pg/mL              | 1803 (1236–2900) | 2228 (1563–3599) | 2992 (2019–4647) | 400 (3043–7657) | $<0.001^†$ |               |
| Osteopontin, ng/mL         | 195 (164–232)| 206 (170–245)| 220 (183–259)| 254 (209–297)| $<0.001^†$ |               |
| Pentraxin-3, ng/mL         | 1.9 (1.1–3.2)| 2.0 (1.1–3.6)| 2.1 (1.3–3.3)| 2.4 (1.5–4.1)| $<0.001^†$ |               |
| Peristin, ng/mL            | 4.9 (3.0–8.4)| 6.1 (3.5–9.5)| 6.8 (3.8–10.5)| 7.4 (4.4–11.7)| $<0.001^†$ |               |
| RAGE, ng/mL                | 2.5 (1.7–3.5)| 2.6 (1.9–4.0)| 2.8 (2.0–4.1)| 3.4 (2.3–4.6)| $<0.001^†$ |               |
| ST-2, ng/mL                | 7.2 (3.4–15.3)| 8.1 (3.6–18.0)| 9.0 (3.8–21.2)| 12.2 (5.6–27.3)| $<0.001^†$ |               |
| Syndecan-1, ng/mL          | 1.8 (0.9–3.1)| 1.9 (1.0–3.4)| 2.1 (1.3–3.9)| 3.3 (2.0–5.2)| $<0.001^†$ |               |
| TNFR-1A, ng/mL             | 0.7 (0.4–1.1)| 0.9 (0.5–1.4)| 1.1 (0.7–1.7)| 2.0 (1.2–3.0)| $<0.001^†$ |               |
| ET-1, pg/mL                | 5.0 (3.8–6.8)| 5.2 (3.9–7.0)| 5.4 (4.1–7.3)| 5.7 (4.4–7.5)| $<0.001^†$ |               |
| IL-6, pg/mL                | 4.3 (2.6–8.0)| 4.5 (2.3–9.0)| 5.2 (3.0–9.8)| 7.4 (4.2–15.0)| $<0.001^†$ |               |
| High-sensitivity TnT       | 21 (14–36)   | 26 (17–43) | 32 (22–52) | 50 (33–83) | $<0.001^†$ |               |

PENK concentration, median (range): Q1 52.1 (44.2–58.4), min=5.5, max=63.7; Q2, 75.5 (69.4–81.0), min=63.8, max=86.2; Q3, 100.0 (92.7–108.9), min=86.2, max=120.1; Q4, 159.9 (135.1–207.1), min=120.3, max=1853.1. ACE indicates angiotensin-converting enzyme; AT1, angiotensin type 1 receptor; TnT, troponin T; and UACR, urine albumin-to-creatinine ratio.

*Defined as LVEF >45%.

†$P$ value <0.05.

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tality (HR=1.23 [95% CI, 1.07–1.43]; P=0.004). The association with HF hospitalization and the composite end point was, however, attenuated in multivariable analysis. After adjustment for an even more extensive model, doubling of PENK remained associated with a higher risk of all-cause mortality (HR=1.22 [95% CI, 1.05–1.42]; P=0.010). The association of PENK with outcome was also analyzed per quartile of PENK (Tables IV through VI in the Data Supplement), yielding comparable findings. Kaplan-Meier curves for the combined end point per quartile of PENK are shown in Figure 3, illustrating an increasing risk with higher quartiles of PENK (log-rank P<0.001).

Results in the Validation Cohort
The above analyses were subsequently validated in 1703 patients of the BIOSTAT-CHF validation cohort. In the BIOSTAT-CHF validation cohort, mean left ventricular EF was 41%, 44% of patients were in New York Heart Association class III, and 34% of patients had HFP EF. Baseline characteristics of quartiles of PENK in the BIOSTAT-CHF validation cohort are displayed in Table VII in the Data Supplement. An association between higher PENK levels and, among others, higher New York Heart Association classification, lower hemoglobin, higher NT-proBNP, higher creatinine, lower eGFR, higher urea, and higher UACR (all P for trend <0.001) was confirmed.

Linear Regression Analysis for PENK
The strongest predictors for log PENK were eGFR, log glucose, log urea, log NT-proBNP, and log ALAT, adjusted R² for the model=0.639 (Table 2).

Biomarker Position of PENK
As a sensitivity analysis, we repeated these analyses in the validation cohort, including only variables available in the BIOSTAT-CHF validation cohort. This resulted in a comparable correlation heatmap (Figure IV in the Data Supplement) and hierarchical cluster analysis (Figure V in the Data Supplement).

Relation of PENK With Outcome
In the validation cohort, a total of 518 (30.4%) patients died, 427 (25.1%) were hospitalized for HF, and 714 (41.9%) either died or were hospitalized for HF during a median follow-up of 21 (11–32) months. Cox regression analysis of doubling of PENK with outcome (Table 5) yielded comparable results to the index cohort, also when assessed over quartiles of PENK (Tables IV through VI in the Data Supplement).

DISCUSSION
In 2 large independent cohorts of patients with HF, we demonstrated that plasma PENK levels were elevated

| Variable | Standardized β | T   | P Value | Variable | Standardized β | T   | P Value |
|----------|----------------|-----|---------|----------|----------------|-----|---------|
| eGFR     | −0.432         | −18.083 | <0.001† | eGFR     | −0.479         | −16.718 | <0.001† |
| Log plasma NGAL | 0.171 | 7.607 | <0.001† | Log glucose | −0.157 | −8.602 | <0.001† |
| Log NT-proBNP   | 0.120 | 4.867 | <0.001† | Log urea | 0.235 | 8.139 | <0.001† |
| Log ALAT      | −0.091 | −4.496 | <0.001† | Log NT-proBNP | 0.115 | 5.512 | <0.001† |
| Diastolic BP  | −0.082 | −3.980 | <0.001† | Log ALAT | −0.091 | −5.059 | <0.001† |
| Log LVEF      | 0.067 | 3.221 | 0.001† | Diastolic BP | −0.091 | −5.059 | <0.001† |
| Log UACR     | 0.075 | 3.195 | 0.001† | Female sex | 0.077 | 4.309 | <0.001† |
| Log KIM-1     | 0.069 | 3.076 | 0.002† | Log sodium | −0.074 | −4.104 | <0.001† |
| Log renin     | 0.064 | 3.031 | 0.002† | NYHA class IV | 0.067 | 3.507 | <0.001† |
| Log troponin T | 0.065 | 2.837 | 0.005† | Current smoker | 0.061 | 3.498 | <0.001† |
| Female sex    | 0.051 | 2.545 | 0.011† | Log potassium | −0.043 | −2.281 | 0.023† |
| Presence of diabetes mellitus | −0.043 | −2.152 | 0.032† | NYHA class II | −0.040 | −2.122 | 0.034† |
| Absence of valvular disease | 0.042 | 2.135 | 0.033† | Log UACR | 0.041 | 2.100 | 0.036† |
| Log CRP       | −0.042 | −2.091 | 0.037† | NYHA class IV | −0.039 | −1.990 | 0.047† |

ALAT indicates alanine transaminase; BP, blood pressure; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; KIM-1, kidney injury molecule-1; LVEF, left ventricular ejection fraction; NGAL, neutrophil gelatinase–associated lipocalin; NT-proBNP, N-terminal pro-B-type natriuretic peptide; NYHA, New York Heart Association; PENK, proenkephalin; and UACR, urine albumin-to-creatinine ratio.

*N=1231, R²=0.561.
†N=1209, R²=0.639.
‡P value <0.05.
and related to more severe HF, and worse renal function, both reflected by glomerular and tubular renal markers. These findings were validated in an independent cohort of patients with HF. Remarkably, higher PENK levels were a predictor of deterioration of kidney function independent of UACR and tubular markers. Finally, higher PENK levels were associated with increased mortality, but not with a higher risk of HF readmissions.

The role of PENK in HF, and thereby the possible role of the opioid system, might be defined by (1) its prominent association with renal dysfunction, which might lead to cardiac dysfunction, (2) cardiac depression, which might lead to renal dysfunction, or (3) a factor that both reflects cardiac and renal dysfunction. We suggest that the opioid system can be such a common pathway affecting both the heart and kidney in HF, a cardiorenal connector, which can be defined as “factors that are modulated by either heart or kidney failure, affect both organs, interact, and are associated with functional or structural, renal or cardiac consequences”, in addition to previously described common denominators. Based on our results, the way the opioid system affects both the heart and kidney could be either of a beneficial

Figure 1. Biomarker position of PENK (proenkephalin) depicted in a correlation heatmap. Negative correlations are expressed in green, neutral associations in yellow, and positive associations in red. Correlations are based on Spearman ρ as a correlation coefficient. ALAT indicates alanine transaminase; ASAT, aspartate aminotransferase; BNP, brain natriuretic peptide; CRP, C-reactive protein; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; FGF-23, fibroblast growth factor-23; HR, heart rate; KIM-1, kidney injury molecule-1; LVEF, left ventricular ejection fraction; NGAL, neutrophil gelatinase-associated lipocalin; NT-proBNP, N-terminal pro-B-type natriuretic peptide; SBP, systolic blood pressure; and UACR, urine albumin-to-creatinine ratio.
nature, or of a disadvantageous, damaging nature, as explained further on.

**Higher PENK Levels Are Associated With More Advanced HF**

Higher PENK levels were associated with more advanced HF, lower blood pressure, and lower heart rate, which can be explained by the direct cardiodepressive effects of PENK.

Alternatively, higher PENK levels with more advanced HF could have beneficial effects by influencing neurohumoral activity, which is a key mechanism in HF pathophysiology with sympathetic nervous system activation being one of the responses. The opioid system acts as an inhibitor of sympathetic activation; it inhibits norepinephrine release and sympathetic vascular constriction. Enkephalins are even coreleased with catecholamines in the heart. The opioid system could therefore be an important mediator in opposing neurohumoral activity in HF, which is supported by our finding of a strong correlation between higher levels of PENK and higher levels of renin. Similarly, natriuretic peptides are activated, and a relationship between the two has been demonstrated before. With sympathetic stimulation—having deleterious effects—being in overdrive in HF, it is possible that increased PENK levels are, in the end, a beneficial, adaptive mechanism limiting this overdrive, somewhat in similar vein as natriuretic peptides oppose activation of the renin-angiotensin system.

**Table 3. Logistic Regression Analyses of Deterioration of Kidney Function* Using PENK and Other Renal Markers**

| Variable† | Odds Ratio | 95% CI | P Value |
|-----------|------------|--------|---------|
| Halving of baseline eGFR‡ | 0.87 | 0.68–1.11 | 0.270 |
| Doubling of baseline PENK‡ | 1.52 | 1.24–1.87 | <0.001§ |
| PENK adjusted for doubling of baseline plasma NGAL | 1.53 | 1.21–1.94 | <0.001§ |
| PENK adjusted for above+double baseline urinary KIM-1 | 1.43 | 1.13–1.82 | 0.003§ |
| PENK adjusted for above+double baseline urinary NGAL | 1.34 | 1.05–1.70 | 0.018§ |
| PENK adjusted for above+double baseline UACR | 1.29 | 1.02–1.65 | 0.038§ |

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eGFR indicates estimated glomerular filtration rate; KIM-1, kidney injury molecule-1; NGAL, neutrophil gelatinase-associated lipocalin; PENK, proenkephalin; and UACR, urine albumin-to-creatinine ratio.

*Defined as >25% decrease in eGFR between baseline and 9 mo; 1170 available measurements.
†Variables were base 2 log-transformed.
‡Univariate. P for interaction eGFR and PENK: 0.028.
system.1,2,4 The presence of a relationship between higher PENK levels and more advanced HF is further supported by the fact that the highest proportion of enkephalins in the heart originate from cardiomyocytes themselves,5,6 and the strong association between higher PENK levels and poor outcome in our study, especially the highest quartile of PENK. This was with the exception of HF hospitalization and the combined end point after adjustment, from which one could hypothesize that the association with all-cause mortality may be largely through renal dysfunction. Interestingly, the association with all-cause mortality remained after extensive adjustment including multiple renal and other disease severity markers and the BIOSTAT risk model, which also includes several markers of HF severity and renal function, suggesting that PENK provides additional prognostic information.

In baseline analyses, higher PENK also associated with a higher degree of cardiomyocyte stretch (ANP), remodeling (eg, galectin-3 and ST-2 [suppression of tumorigenicity-2]), and inflammation (eg, CRP [C-reactive protein] and IL-6). Because these are all related to more severe disease, this further strengthens the relationship between higher PENK and more advanced HF. A higher degree of inflammation, another key component in HF pathophysiology,7 could furthermore be explained by the presence of endogenous opioid peptides at inflammatory sites through immune cells to provide analgesia.27

Higher PENK Levels Are Associated With Worse Renal Function

In this study, PENK showed particularly strong clustering with renal markers. PENK was strongly associated with creatinine in both cohorts and similarly, in hierarchical cluster analysis and correlation heatmap analyses, PENK clustered with creatinine, eGFR, plasma NGAL, and urea, but also with galectin-3, which also has strong relation to renal function.28 Higher PENK levels furthermore asso-

| Outcomes                      | Univariable | Adjusted Model 1† | Adjusted Model 2† |
|-------------------------------|-------------|-------------------|-------------------|
|                               | HR (95% CI) | P Value           | HR (95% CI)       | P Value           |
| All-cause mortality           | 1.87 (1.72–2.03) | <0.001§           | 1.23 (1.07–1.43) | 0.004§           |
| HF hospitalization            | 1.52 (1.38–1.68) | <0.001§           | 1.03 (0.89–1.20) | 0.673            |
| All-cause mortality or HF hospitalization | 1.66 (1.55–1.79) | <0.001§           | 1.12 (0.99–1.26) | 0.065            |

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| Outcomes                      | Univariable | Adjusted Model 1† | Adjusted Model 2† |
|-------------------------------|-------------|-------------------|-------------------|
|                               | HR (95% CI) | P Value           | HR (95% CI)       | P Value           |
| All-cause mortality           | 1.93 (1.74–2.14) | <0.001§           | 1.22 (1.01–1.47) | 0.038§           |
| HF hospitalization            | 1.61 (1.43–1.82) | <0.001§           | 0.97 (0.79–1.19) | 0.764            |
| All-cause mortality or HF hospitalization | 1.76 (1.60–1.93) | <0.001§           | 0.99 (0.84–1.16) | 0.900            |

CRP indicates C-reactive protein; eGFR, estimated glomerular filtration rate; GDF-15, growth differentiation factor-15; HF, heart failure; HR, hazard ratio; KIM-1, kidney injury molecule-1; NGAL, neutrophil gelatinase–associated lipocalin; NT-proBNP, N-terminal pro-B-type natriuretic peptide; PENK, proenkephalin; ST-2, suppression of tumorigenicity-2; and UACR, urine albumin-to-creatinine ratio.

†Adjusted for BIOSTAT risk score, log NT-proBNP, eGFR, and log UACR. Variables in BIOSTAT risk score: all-cause mortality: age, log blood urea nitrogen, log NT-proBNP, hemoglobin, and β-blocker use at baseline HF hospitalization: age, HF hospitalization in the previous year, peripheral edema, systolic blood pressure, and eGFR. Combined end point: age, HF hospitalization in the previous year, systolic blood pressure, log NT-proBNP, hemoglobin, high-density lipoprotein, sodium, and β-blocker use at baseline.

‡Adjusted for BIOSTAT risk score, log NT-proBNP, eGFR, log UACR, log plasma NGAL, log hs-troponin T, log CRP, log GDF-15, log ST-2, log urinary NGAL, and log urinary KIM-1.

§P value <0.05.
associated with higher albuminuria and higher levels of tubular damage markers, suggesting not only an association with glomerular dysfunction but with tubular damage as well. Finally, PENK was a predictor of deterioration of kidney function independent of UACR and tubular markers.

The relationship between PENK and renal function emerges in existing literature. In renal transplant recipients, PENK associated with poorer renal function and an increased risk of graft failure. Both after cardiac surgery and in septic patients PENK predicted acute kidney injury and strongly associated with creatinine and eGFR. In a prospective cohort of patients without chronic kidney disease, PENK predicted deterioration of kidney function and chronic kidney disease onset. PENK has also previously demonstrated a strong relation with renal function in HF. Our study adds to these existing data by providing a more detailed position of PENK in a large number of HF patients including an extensive range of relevant renal function parameters, including several glomerular and tubular renal markers.

PENK appears to be freely filtrated in the glomerulus, and it is indeed the case that, with declining eGFR, PENK levels rise. The association between PENK and renal function could thus be explained by PENK being a filtration signal, for which interest has already previously been shown to use PENK as an easy and reliable determinant of current GFR and possibly a better early indicator of kidney function decline and kidney injury, but PENK might also serve a functional purpose.

Similar to the relationship between PENK and more advanced HF, the relationship between PENK and renal dysfunction could be either of a beneficial nature or of a disadvantageous, damaging nature. PENK could be secreted as a counterregulatory response to renal dysfunction through the effects of enkephalins on renal blood flow and urinary output; activation of δ receptors of enkephalins produces significant diuretic and natriuretic responses without changes in heart rate or blood pressure. Alternatively, through the cardiodepressive response

![Figure 3. Kaplan-Meier curve for the combined end point for quartiles of PENK (proenkephalin).](image)
to PENK,\(^1\)\(^2\) this could, in turn, result in reduced kidney perfusion.

With regards to the association between PENK and tubular damage specifically, this could be explained by increased chronic renal hypoxia and a higher degree of congestion (because of worse HF) with increased tubular damage as a consequence. Furthermore, both renal and HF are associated with a prooxidant status\(^18\) including in tubular cells,\(^36\) and PENK expression is upregulated in response to oxidative stress conditions.\(^37\) In a previous study including both chronic and acute HF,\(^6\) PENK was not associated with tubular damage markers. The chronic HF cohort that focused on tubular damage was conducted in stable, ambulatory patients, whereas our study included patients with either new onset or worsening HF with suboptimal treatment, hence possibly representing more severely ill patients, including presence of tubular damage. Furthermore, the previous data on tubular damage was limited to only 95 chronic HF patients, compared with 2180 in the present study.

**Figure 4.** Higher PENK (proenkephalin) levels exert cardiodepressive effects and improve renal function predominantly by increasing renal blood flow and urinary output.

The relationship of higher PENK levels with (worsening) glomerular dysfunction might be through chronic reduced kidney perfusion, but the response of higher levels of PENK to renal dysfunction might also be of a counterregulatory nature. Higher PENK levels could be connected to tubular dysfunction/damage through chronic renal hypoxia, a higher degree of congestion, and oxidative stress. A wide variety of (renal) biomarkers illustrates this relationship. This association of PENK with renal dysfunction could, in the end, largely contribute to its relationship with increased risk of mortality. eGFR indicates estimated glomerular filtration rate; FGF-23, fibroblast growth factor-23; GAL-3, galectin-3; Hb, hemoglobin; and UACR, urine albumin-to-creatinine ratio. Illustrations were adapted from Servier Medical Art (https://smart.servier.com). Copyright © 2019, Servier. These are Open Access images distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

**Future Perspectives**

Our data confirm and extend the relationship between the heart and kidneys in HF. Dysfunction of both organs often coexists, and cardiac factors can lead to renal damage or vice versa, or there are common pathways that influence both cardiac and renal function.\(^18\)\(^19\) The opioid system, represented by PENK, has mechanistic grounds to be such a common pathway affecting both the heart and kidney (Figure 4). A causal relationship can however only be established by prospective intervention studies. It therefore needs to be established whether the opioid system plays a causal role in the onset and progression of cardiorenal failure. Apart from being used as an analgesic agent in experimental animal models, therapeutic administration of PENK with respect to cardiovascular effects in HF has, to our knowledge, not been investigated.

Furthermore, it remains to be elucidated what the effects of the use of HF therapy, and specifically sacubitril/valsartan will be on circulating PENK levels, because this study was conducted before the incorporation of this
drug in HF management. Because enkephalins are a substrate of nephrilysin, this will be a relevant issue to address.

**Strengths and Limitations of the Study**

The in-depth analysis of PENK across a wide variety of clinical variables and biomarkers is a strength of our study, since it allowed for meticulous positioning of PENK. Furthermore, results were based on a large number of patients that were included in this multicenter, multinational cohort. The likelihood that results are applicable to the general chronic HF population are increased due to this cohort being a very heterogeneous HF population and validation of results in a separate validation cohort that yielded very comparable results.

Shortcomings of this study are the inability to extend results beyond the European population and to show causality instead of associations. The precise stability of the centrally determined biomarkers after frozen storage time is unknown, but we feel it is unlikely that this significantly impacted their concentration, especially for PENK due to extensive testing in this regard. Some analyses included less patients because of missing values. PENK was measured in a subset of patients, and even though baseline characteristics did not differ between in- and excluded patients, informed censoring cannot be fully ruled out. The study populations mainly included HF patients with reduced EF, with different proportions between the study populations, which can be traced back to their study designs. We were also not able to show changes of PENK levels over time which could have contributed to a better understanding of its dynamics.

**Conclusions**

This study shows that higher levels of PENK are associated with worse HF, deterioration of kidney function beyond creatinine, and notably both glomerular dysfunction and tubular damage in a large chronic HF study population. PENK was independently related to poor outcome in HF. This study therefore provides clues for potential pathophysiological mechanisms of PENK and the opioid system and positioned PENK as a potential novel comprehensive renal marker in patients with HF.

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