Simultaneous Assessment of Unprocessed ProBNP$_{1\text{-}108}$ in Addition to Processed BNP32 Improves Identification of High-Risk Ambulatory Patients With Heart Failure

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Background—B-type natriuretic peptide (BNP) is produced as a biologically inactive prohormone (proBNP$_{1\text{-}108}$), processed, and released as an inactive amino acid N-terminal fragment (proBNP$_{1\text{-}76}$) and a biologically active carboxyl-terminal fragment (proBNP$_{77\text{-}108}$ or BNP32). We hypothesized that simultaneous assessment of proBNP$_{1\text{-}108}$ and active BNP32, as an index of natriuretic peptide processing efficiency, would improve risk stratification in patients with chronic systolic heart failure.

Methods and Results—We quantified plasma proBNP$_{1\text{-}108}$ and BNP32 in 756 participants in the Penn Heart Failure Study, a prospective cohort of outpatients with predominantly systolic heart failure. Cox models were used to determine the association between biomarker level at the time of study entry and incident risk of adverse cardiovascular outcomes. A significant amount of unprocessed proBNP$_{1\text{-}108}$ circulates in patients with systolic heart failure (median, 271 pg/mL; interquartile range, 65 to 825). Higher levels of proBNP$_{1\text{-}108}$ were associated with an increased risk of all-cause death or cardiac transplantation (adjusted hazard ratio, 4.9; 95% CI, 2.5 to 9.7; $P<0.001$, comparing third versus first proBNP$_{1\text{-}108}$ tertile). ProBNP$_{1\text{-}108}$ provided additive information to BNP32 risk assessment, particularly in patients with BNP32 less than the median of 125 pg/mL (adjusted hazard ratio, 1.4; 95% CI, 1.2 to 1.8; $P<0.001$ per doubling of proBNP$_{1\text{-}108}$).

Conclusions—Circulating proBNP$_{1\text{-}108}$ is independently associated with an increased risk of adverse cardiovascular outcomes in ambulatory patients with chronic systolic heart failure. The combined assessment of BNP32 and proBNP$_{1\text{-}108}$ provides additional information in determining risk of adverse clinical outcomes, particularly in patients with low BNP32 values that might otherwise be reassuring to the clinician. **(*Circ Heart Fail*. 2010;3:220-227.)**

Key Words: epidemiology ■ heart failure ■ natriuretic peptides ■ risk factors

The human gene for B-type natriuretic peptide (BNP) encodes a 134-amino acid precursor preproBNP. After removal of a 26-amino acid signal peptide, a 108-amino acid proBNP polypeptide (proBNP$_{1\text{-}108}$) is formed. With further processing of proBNP$_{1\text{-}108}$ by the type 2 transmembrane serine protease corin, the following 2 peptides are generated: (1) the physiologically active 32-amino acid carboxyl-terminal BNP molecule (BNP32, also commonly referred to as BNP$_{1\text{-}32}$ or BNP$_{77\text{-}108}$)[1] and (2) an inactive 76-amino acid N-terminal fragment (NT-proBNP$_{1\text{-}76}$) (Figure 1).[2] Although the precise molecular mechanisms of BNP processing remain incompletely understood, the prevailing hypothesis suggests that proBNP undergoes exocytosis from cardiomyocytes, allowing it to interact with the extracellular catalytic domain of corin resulting in BNP32 and NT-proBNP$_{1\text{-}76}$ (Figure 1).[3,4]

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The natriuretic peptide system exerts compensatory actions in heart failure, including attenuation of adverse neurohormonal activation, balanced venous and arterial vasodilation, reduced endothelin release, enhanced cardiac lusitropy, and intravascular volume regulation by virtue of the ability of BNP to promote natriuresis.[5–7] However, it has been recently shown in 2 independent in vitro systems that BNP must be processed effectively to gain biological activity. Compared with processed BNP32, proBNP$_{1\text{-}108}$ has absent[8] or 8-fold lower biological activity.[9] Consequently, impaired proBNP processing would be expected to reduce the compensatory actions of the endogenous natriuretic peptide system and might contribute to heart failure progression and risk for decompensation leading to heart failure hospitalization, death, or cardiac transplantation.

The overall objective of this study was to define the relationship between circulating levels of unprocessed proBNP$_{1\text{-}108}$ and risk of adverse cardiovascular outcomes, including all-cause death, cardiac transplantation, and heart failure hospitalization, in a chronic heart failure population.

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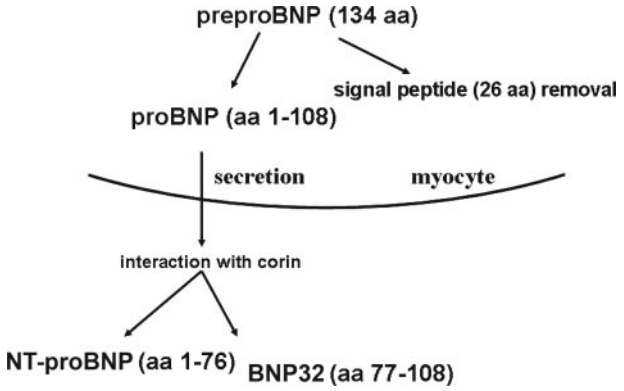

Figure 1. Schemata of BNP processing.

To measure $proBNP_{1\text{-}108}$, we used a highly specific, novel assay that shows no cross-reactivity to either BNP32 (aa77 to 108) or NT-$proBNP_{1\text{-}76}$ (aa1 to 76).[10] In addition, we also sought to determine the prognostic importance of simultaneous assessment of both unprocessed $proBNP_{1\text{-}108}$ and active, circulating BNP32. We hypothesized that discordance in $proBNP_{1\text{-}108}$ and BNP32 levels represents an abnormality in natriuretic peptide processing and is associated with an increased risk of adverse cardiovascular outcomes.

Methods

Study Population

The Penn Heart Failure Study is an ongoing prospective cohort study of outpatients with chronic heart failure recruited from the Penn Heart Failure and Transplantation program.[11,12] The primary inclusion criterion is a clinical diagnosis of heart failure. Patients are excluded if they have a noncardiac condition resulting in an expected mortality of <6 months, as judged by the treating physician.

At the time of study entry, detailed clinical data were obtained through questionnaires provided to the patient and treating physician, with verification by medical records. Variables such as New York Heart Association (NYHA) class and cardiomyopathy etiology (ischemic versus nonischemic) were determined by the physician on the basis of all available clinical data and according to standard heart failure clinical practice guidelines.[13] Venous blood samples were obtained at enrollment, processed, and stored at −80°C. Two-dimensional conventional transthoracic echocardiography was performed in all patients typically within 30 days of blood sampling at a laboratory accredited by the Intersocietal Commission for the Accreditation of Echocardiography Laboratories . Ejection fraction was estimated by a level III-certified echocardiographer, according to the standard clinical protocol.

Follow-up events, including all-cause mortality, cardiac transplantation, and hospitalization, were prospectively ascertained every 6 months by direct patient contact and verified through death certificates, medical records, and contact with patients' family members. For hospitalizations, reason for hospitalization was adjudicated based on medical record review by study personnel and classified as a hospitalization primarily for heart failure, other cardiac conditions, or noncardiac conditions. The analyses presented in this article were limited to hospitalizations for heart failure only. All participants provided a written, informed consent, and the Penn Heart Failure Study protocol was approved by our institutional review board.

$ProBNP_{1\text{-}108}$ and BNP32 Assay

$ProBNP_{1\text{-}108}$ was measured with a highly specific, novel Bio-Rad assay.[10] This assay is based on the monoclonal antibody mAb Hinge76, that recognizes with high affinity the cleavage site of $proBNP_{1\text{-}108}$ (Arg^{76}-Ser^{77}), an epitope present only in the precursor form (Figure 1). By combining the mAb Hinge76 with a polyclonal

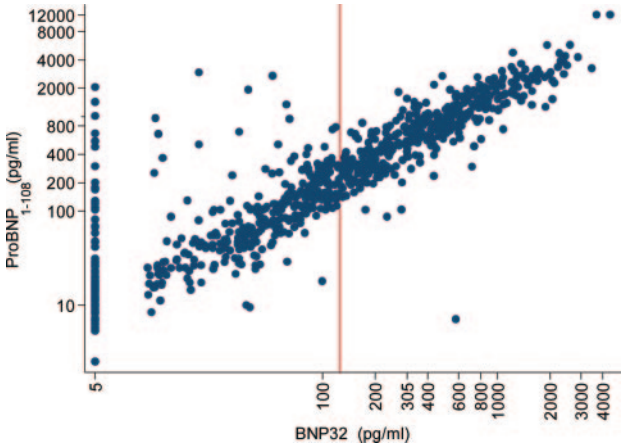

Figure 2. Correlation between $proBNP_{1\text{-}108}$ and BNP32. Spearman ρ=0.89, P<0.001, and n=756; red line denotes median BNP32 of 125 pg/mL.

antibody directed against BNP32, the Bio-Rad-intact $proBNP_{1\text{-}108}$ sandwich immunoassay is specific for the presence of $proBNP_{1\text{-}108}$ in plasma samples. There is no significant cross-reactivity with either recombinant NT-$proBNP_{1\text{-}76}$ or synthetic BNP32. The lower limit of detection of $proBNP_{1\text{-}108}$ was 5 pg/mL, and the upper limit was 12 000 pg/mL. The intra- and interassay coefficients of variations for the assay were 3.0% to 5.0% and 3.5% to 6.1%, respectively.

BNP32 was measured using the Architect BNP immunoassay.[11] Of note, current commercial assays for BNP32 show some cross-reactivity with $proBNP_{1\text{-}108}$. A recent consensus panel estimated 6% to 38% cross-reactivity of the Architect BNP32 assay with $proBNP_{1\text{-}108}$.[14] Hence, we expect some degree of correlation between these 2 measurements based on assay characteristics alone. The intra- and interassay coefficients of variations for the Architect BNP32 assay were 0.9% to 5.6% and 1.7% to 6.7%, respectively. The lower limit of detection of BNP32 was 10 pg/mL, and the upper limit was 4417 pg/mL. Samples below the detectable limit were assigned a value halfway between 0 and the lowest detectable limit of each marker.[15]

Statistical Analysis

The distribution of both $proBNP_{1\text{-}108}$ and BNP32 were skewed, and the log transformation of each marker approximated a normal distribution. We examined the data graphically and fit a simple linear regression model in which we were able to quantify the residuals to determine the relationship between the 2 markers.

Differences in the distribution of clinical variables across tertiles of $proBNP_{1\text{-}108}$ and BNP32 were studied using ANOVA for continuous variables and Pearson χ^2 test for categorical variables. To determine the association between baseline BNP32 or $proBNP_{1\text{-}108}$ level and risk of adverse clinical outcomes, Kaplan–Meier analysis, log-rank tests, and Cox models were used. Two series of univariate and multivariable models were constructed, with $proBNP_{1\text{-}108}$ or BNP32 as the predictor variable and time to the combined end point of all-cause death or cardiac transplantation or the composite end point of all-cause death, cardiac transplantation, or heart failure hospitalization as the outcome variable. We determined univariate hazard ratios (HRs) for each biomarker across categories of biomarker according to tertiles as well as the continuous form of the variable according to a log base 2 transformation in which the HR represents the effect for a doubling of the original variable. Multiple forms of categorical data, including the use of medians, tertiles, quartiles, and quintiles, were examined. For multivariable models, confounders were selected using clinical judgment, cross-sectional associations with $proBNP_{1\text{-}108}$, and statistical evidence of potential confounding. Statistical evidence included a univariate association with death or transplant at a P<0.20.[16]

Given the marked colinearity between $proBNP_{1\text{-}108}$ and BNP32 and consequent limitations in determining the independent effects of either marker by simple confounder adjustment, we used alternative

Table 1. Clinical Characteristics of 756 Patients in the Penn Heart Failure Study According to $ProBNP_{1-108}$ Tertile

	Entire Cohort (n=756)	Tertile 1 (5–107 pg/mL; n=252)	Tertile 2 (108–572 pg/mL; n=252)	Tertile 3 (>572 pg/mL; n=252)	P*
Age, y	57 (14)	51 (13)	58 (14)	60 (14)	<0.001
Male gender, n (%)	522 (69)	161 (64)	176 (70)	185 (73)	0.07
Race, n (%)					0.97
White	617 (82)	206 (82)	204 (82)	207 (83)	
Black	105 (14)	35 (14)	34 (14)	36 (14)	
Other	28 (4)	9 (4)	11 (4)	8 (3)	
Tobacco use, n (%)					0.008
Never	279 (37)	108 (43)	75 (30)	96 (38)	
Former	428 (57)	124 (49)	158 (63)	146 (58)	
Current	49 (6)	20 (8)	19 (7)	10 (4)	
Hypertension history, n (%)	360 (48)	103 (41)	121 (48)	136 (54)	0.01
Diabetes history, n (%)	210 (28)	48 (19)	86 (34)	76 (30)	<0.001
Creatinine, mg/dL	1.3 (0.7)	1.1 (0.3)	1.2 (0.5)	1.5 (1.0)	<0.001
Body mass index, kg/m^2	29 (6)	30 (7)	30 (7)	28 (7)	0.34
SBP, mm Hg	113 (19)	116 (16)	115 (17)	109 (21)	<0.001
Cardiomyopathy etiology, n (%)					
Ischemic	250 (33)	31 (12)	102 (40)	117 (46)	<0.001
Heart failure type, n (%)					
Diastolic	94 (12)	47 (50)	25 (27)	22 (23)	0.01
NYHA, n (%)					<0.001
I	115 (15)	77 (31)	31 (12)	7 (3)	
II	330 (44)	129 (51)	115 (46)	86 (34)	
III	222 (29)	41 (16)	82 (33)	99 (39)	
IV	89 (12)	5 (2)	24 (9)	60 (24)	
Ejection fraction	32 (16)	41 (14)	30 (15)	24 (16)	<0.001
Cardiac resynchronization, n (%)	208 (28)	38 (15)	74 (29)	96 (38)	<0.001
ACE inhibitor or ARB, n (%)	648 (86)	227 (90)	225 (89)	196 (78)	0.001
β-blocker, n (%)	626 (83)	210 (83)	214 (85)	202 (80)	0.35
BNP32, pg/mL	327 (509)	33 (47)	154 (106)	794 (657)	<0.001

Data are presented as mean (SD) unless otherwise indicated. ACE indicates angiotensin-converting enzyme; ARB, angiotensin receptor blocker; SBP, systolic blood pressure.

*ANOVA for continuous variables; χ^2 test for categorical variables.

measures to understand the value of $proBNP_{1-108}$ assessment in the setting of a known BNP32 level. First, in stratified analyses, we determined the association between $proBNP_{1-108}$ and risk of adverse outcomes according to median BNP32 level. We also formally tested for interaction between the log base 2 transformed $proBNP_{1-108}$ level and median BNP32 level. Second, we divided patients into subgroups according to their median $proBNP_{1-108}$ and BNP32 levels to explore the joint effects of $proBNP_{1-108}$ and BNP32 in determining

Table 2. Unadjusted and Multivariable-Adjusted Risk of All-Cause Death or Cardiac Transplantation According to Tertiles of $ProBNP_{1-108}$ or BNP32

	$ProBNP_{1-108}$			BNP32		
	Tertile 2 vs 1*	Tertile 3 vs 1*	Per 2-Fold Increase in $ProBNP_{1-108}$	Tertile 2 vs 1*	Tertile 3 vs 1*	Per 2-Fold Increase in BNP32
Unadjusted	5.6 (3.0–10.4)	10.1 (5.5–18.4)	1.5 (1.3–1.6)	3.2 (1.9–5.6)	7.6 (4.6–12.7)	1.5 (1.4–1.6)
Model 1†	5.7 (2.9–10.8)	9.5 (5.0–18.0)	1.4 (1.3–1.6)	3.2 (1.8–5.7)	6.9 (4.0–11.8)	1.4 (1.3–1.6)
Model 2‡	5.0 (2.6–9.7)	7.8 (4.0–15.4)	1.4 (1.3–1.5)	2.9 (1.6–5.2)	5.5 (3.1–9.8)	1.4 (1.3–1.5)
Model 3§	3.9 (2.0–7.7)	4.9 (2.5–9.7)	1.3 (1.2–1.4)	2.5 (1.4–4.4)	3.6 (2.0–6.4)	1.3 (1.2–1.4)

Data are presented as HR (95% CI).

All $P<0.001$, including test for trend.

†Model 1 adjusted for age, sex, race, tobacco use, creatinine, and body mass index.

‡Model 2 adjusted for model 1+ejection fraction+cardiomyopathy etiology (ischemic versus nonischemic).

§Model 3 adjusted for model 2+NYHA class (I/II versus III/IV).

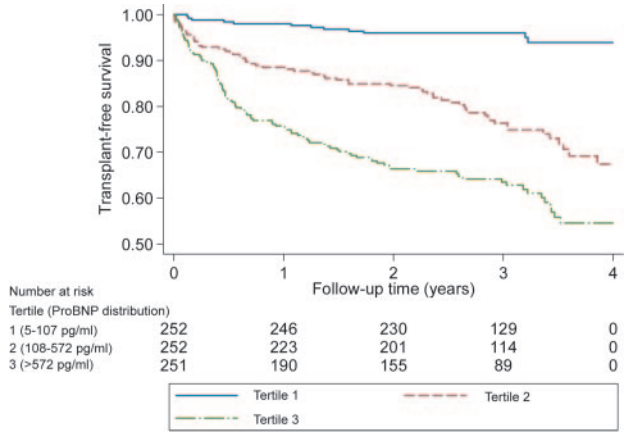

Figure 3. Kaplan–Meier survival estimates for all-cause death or cardiac transplantation according to proBNP$_{1\text{-}108}$ tertiles. $P<0.001$ by log rank test.

risk of adverse outcomes. Cox models were used to compare risk, with the reference group being patients with both proBNP$_{1\text{-}108}$ and BNP32 levels less than median. Third, we determined the association between the residuals generated from the linear regression between proBNP$_{1\text{-}108}$ and BNP32 and risk of adverse outcomes in Cox models.

All analyses were performed using Stata 10.0. All model assumptions, including proportional hazards and linearity on a log scale, were verified. All authors had full access to the data and take full responsibility for their integrity. All authors have read and agreed to the article as written.

Results

Study Population

Between December 2003 and October 2007, 756 patients were enrolled, with the assessments of both unprocessed proBNP$_{1\text{-}108}$ and processed BNP32. Mean±SD age was 57±14 years, 69% were men, and 82% were white. The majority had systolic heart failure (87.6%), with a mean ejection fraction of 32±16%. The full spectrum of NYHA class was represented, with the majority (73%) being NYHA class II or III. One third had ischemic cardiomyopathy, and the remaining two thirds had a nonischemic etiology.

The mean±SD proBNP$_{1\text{-}108}$ was 639±1020 pg/mL, and the median was 271 pg/mL (interquartile range, 65 to 825 pg/mL), with 1% having a proBNP$_{1\text{-}108}$ value less than the detectable limit. The mean BNP32 was 327±509 pg/mL, and the median was 125 pg/mL (interquartile range, 35 to 395 pg/mL), with ≈12% having a BNP32 value less than the detectable limit, similar to findings in other ambulatory patients with chronic heart failure.[17] As shown in Figure 2 and in our linear regression analyses, BNP32 and proBNP$_{1\text{-}108}$ were strongly correlated (R=0.87, $P<0.001$). At the same time, there was evidence of discordance between the 2 markers in a subgroup of patients with BNP32 values less than the median. In particular, at undetectable values of BNP32, there was substantial variability in proBNP$_{1\text{-}108}$. In these patients with a less-than-detectable BNP32, the mean proBNP$_{1\text{-}108}$ was 124±331 pg/mL.

Patients in the highest proBNP$_{1\text{-}108}$ tertile were more likely to be older and to have a history of hypertension and worse renal function (Table 1). In addition, these patients were most likely to have an ischemic cause of their heart failure, worse NYHA class, and lower mean ejection fraction.

Association Between Circulating ProBNP$_{1\text{-}108}$ and BNP32 Levels and Risk of Adverse Cardiovascular Outcomes

Patients were followed for a median of 2.5 years (interquartile range, 1.1 to 3.3 years). During this period, there were 92 deaths, 81 transplants, and 179 first heart failure hospitalizations. For each 2-fold increase in proBNP$_{1\text{-}108}$, there was a significantly increased risk of death or cardiac transplantation (unadjusted HR, 1.5; 95% CI, 1.3 to 1.6; $P<0.001$) that remained independent after multivariable adjustment (Table 2). Similarly, compared with patients in the lowest tertile of proBNP$_{1\text{-}108}$ (<108 pg/mL), patients in the highest tertile (>572 pg/mL) had a markedly increased risk of all-cause mortality or cardiac transplantation (Figure 3), with an unadjusted HR of 10.1 (95% CI, 5.5 to 18.4; $P<0.001$) (Table 2). After adjusting for covariates, such as demographics, creatinine, ejection fraction, heart failure etiology, and disease severity, the risk of death or transplantation was still significant (HR, 4.9; 95% CI, 2.5 to 9.7; $P<0.001$) (Table 2). Patients with a circulating proBNP$_{1\text{-}108}$ <107 pg/mL represented a low-risk subset with a small number of adverse events (Table 3).

As expected for highly correlated predictors, there was a similarly increased risk associated with increased BNP32 levels. For each doubling of BNP32, there was also a 50% increased risk of death or transplant that remained independent after confounder adjustment (Table 2). The risk associated with the highest BNP32 tertile was increased in both unadjusted (HR, 7.6; 95% CI, 4.6 to 12.7; $P<0.001$) and adjusted (HR, 3.6; 95% CI, 2.0 to 6.4; $P<0.001$) models.

We also examined the association between biomarker level and risk of death, transplantation, or heart failure hospitalization. A striking relationship also was observed, although the absolute risk estimates were not as markedly increased (Table 4). This finding in part may be secondary to a relatively greater number of events in the lowest tertile groups (Table 3).

Table 3. Number of Adverse Cardiovascular Outcomes According to Tertile of ProBNP$_{1\text{-}108}$ or BNP32

		ProBNP$_{1\text{-}108}$ Tertile			BNP32 Tertile		
		1	2	3	1	2	3
	Total	5–107 pg/mL (n=252)	108–572 pg/mL (n=252)	>572 pg/mL (n=251)	4–54 pg/mL (n=252)	55–264 pg/mL (n=252)	>264 pg/mL (n=251)
All-cause death or cardiac transplantation	177 (23.4)	13 (5.1)	63 (25.0)	101 (40.1)	18 (7.1)	53 (21.0)	106 (42.1)
All-cause death, cardiac transplantation, or heart failure hospitalization	355 (46.9)	54 (21.4)	137 (54.4)	164 (65.1)	64 (25.4)	123 (48.9)	168 (66.7)

Data are presented as n (% of tertile).

Table 4. Unadjusted and Multivariable-Adjusted Risk of All-Cause Death, Cardiac Transplantation, or First Heart Failure Hospitalization According to Tertiles of $ProBNP_{1-108}$ or BNP32

	$ProBNP_{1-108}$			BNP32		
	Tertile 2 vs 1*	Tertile 3 vs 1*	Per 2-Fold Increase in $ProBNP_{1-108}$	Tertile 2 vs 1*	Tertile 3 vs 1*	Per 2-Fold Increase in BNP32
Unadjusted	3.1 (2.3–4.3)	4.4 (2.3–4.3)	1.3 (1.2–1.3)	2.2 (1.6–3.0)	3.8 (2.9–5.1)	1.3 (1.2–1.3)
Model 1†	3.1 (2.2–4.2)	4.3 (3.1–6.0)	1.3 (1.2–1.3)	2.2 (1.6–3.0)	3.7 (2.7–5.0)	1.2 (1.2–1.3)
Model 2‡	2.7 (1.9–3.8)	3.4 (2.3–4.8)	1.2 (1.2–1.3)	1.9 (1.4–2.7)	2.8 (2.0–3.9)	1.2 (1.1–1.3)
Model 3§	2.4 (1.7–3.3)	2.5 (1.7–3.6)	1.2 (1.1–1.2)	1.8 (1.3–2.5)	2.1 (1.5–3.0)	1.1 (1.1–1.2)

Data are presented as HR (95% CI).
All $P<0.001$, including test for trend.
†Model 1 adjusted for age, sex, race, tobacco use, creatinine, and body mass index.
‡Model 2 adjusted for model 1+ejection fraction+cardiomyopathy etiology (ischemic versus nonischemic).
§Model 3 adjusted for model 2+NYHA class (I/II versus III/IV).

Association Between Circulating $ProBNP_{1-108}$ and Risk of Adverse Cardiovascular Outcomes According to BNP32 Level

As noted earlier, ≈12% of the cohort, or 88 patients, had a BNP32 level that was below detection. In this subgroup, there were 3 adverse events (death or transplantation), and among these 3 patients, $proBNP_{1-108}$ values were substantially increased at 548, 1440, and 2043 pg/mL and in the upper quartile of $proBNP_{1-108}$. Furthermore, we determined that the relationship between $proBNP_{1-108}$ and risk of death or transplantation differed significantly according to BNP32 level (interaction $P=0.003$). In patients with a BNP32 less than the median of 125 pg/mL, a 2-fold increase in $proBNP_{1-108}$ was associated with an increased risk of death or transplantation in both unadjusted and adjusted models (adjusted HR, 1.5; 95% CI, 1.3 to 1.8; $P=0.001$) (Table 5). However, in patients with BNP32 levels greater than the median, this association was less pronounced and significant in the unadjusted model but not after multivariable adjustment (adjusted HR, 1.1; 95% CI, 0.9 to 1.3; $P=0.35$) (Table 5). We also examined the interaction between $proBNP_{1-108}$ and BNP32 on a continuous scale and found a highly significant interaction in the unadjusted and adjusted models ($P=0.001$ for both). This model was also consistent in demonstrating a more pronounced effect of $proBNP_{1-108}$ when BNP32 was low.

In examining the association between biomarker level and risk of death, transplantation, or heart failure hospitalization, we observed a similar relationship (Table 6). The risk of an event associated with $proBNP_{1-108}$ was greater in those patients with a BNP32 level less than the median (adjusted HR, 1.3; 95% CI, 1.2 to 1.4; $P<0.001$) than in those with BNP32 levels greater than the median (adjusted HR, 1.0; 95% CI, 0.9 to 1.1; $P=0.52$). Again, the interaction was highly significant, using BNP32 on a categorical and continuous scale (adjusted $P<0.001$ for both).

We also examined the predictive value of the residual values derived from the linear regression of BNP32 on $proBNP_{1-108}$. A positive residual was indicative of more $proBNP_{1-108}$ than predicted by BNP32. Interestingly, we found that an increased residual also was associated with a significant risk of all-cause death and cardiac transplantation (online-only Data Supplement Table I). There were some significant, although much weaker, associations between the residuals and the composite risk of death, transplantation, and first heart failure hospitalization (supplemental Table II).

Joint Effects of $ProBNP_{1-108}$ and BNP32 Assessment on Risk of Adverse Cardiovascular Outcomes

We then tested the combined influence of $proBNP_{1-108}$ and BNP32 assessment to further determine the joint effects of $proBNP_{1-108}$ and BNP32. Patients were divided into 4 subgroups based on median $proBNP_{1-108}$ and BNP32 levels. A number of patients (11.6%) showed discordance between the 2 biomarkers, with 44 having a $proBNP_{1-108}$ level less than the median and BNP32 greater than the median and 44 with a $proBNP_{1-108}$ level greater than the median and BNP32 less than the median. As shown in Table 7, for the composite end point of all-cause death or cardiac transplantation, patients with a $proBNP_{1-108}$ greater than the median but a BNP32 less than the median had an unadjusted risk of death or transplantation of 4.1 (95% CI, 2.1 to 7.9; $P<0.001$) compared with the group with low levels of both biomarkers. This association remained significant in multivariable-adjusted analyses. As shown in Kaplan–Meier analyses, patients with an increase in either BNP32 or $proBNP_{1-108}$ had a significantly increased risk of adverse events compared with patients with

Table 5. Association Between $ProBNP_{1-108}$ and Risk of All-Cause Death or Cardiac Transplantation According to Median BNP32 Level

BNP32 Level	n	Unadjusted HR per 2-Fold Increase in $ProBNP_{1-108}$ (95% CI)	Interaction *P*	Adjusted HR per 2-Fold Increase in $ProBNP_{1-108}$ (95% CI)*	Interaction *P*
BNP32 <125 pg/mL	378	1.7 (1.4–2.0)	0.001	1.5 (1.3–1.8)	0.001
BNP32 >125 pg/mL	377	1.2 (1.1–1.4)		1.1 (0.9–1.2)	

*Adjusted for all covariates in Table 2, Model 3.

Table 6. Association Between ProBNP$_{1\text{-}108}$ and Risk of All-Cause Death, Cardiac Transplantation, or First Heart Failure Hospitalization According to Median BNP32 Level

BNP32 Level	n	Unadjusted HR per 2-Fold Increase in ProBNP$_{1\text{-}108}$ (95% CI)	Interaction *P*	Adjusted HR per 2-Fold Increase in ProBNP$_{1\text{-}108}$ (95% CI)*	Interaction *P*
BNP32 <125 pg/mL	378	1.4 (1.3–1.5)	<0.001	1.3 (1.2–1.4)	<0.001
BNP32 >125 pg/mL	377	1.1 (0.9–1.2)		1.0 (0.9–1.1)	

*Adjusted for all covariates in Table 4, Model 3.

low levels of both markers (Figure 4). These findings were similar for the combined end point of death, transplantation, or incident heart failure hospitalization (Table 8).

Discussion

This study is the first to determine the use of proBNP$_{1\text{-}108}$ as an individual biomarker and in conjunction with BNP32 measurement in ambulatory patients with primarily chronic systolic heart failure. Our results show that a significant amount of unprocessed proBNP$_{1\text{-}108}$ circulates in patients with systolic heart failure, and this amount is positively associated with risk of adverse outcomes. Furthermore, measurement of unprocessed proBNP$_{1\text{-}108}$ in ambulatory patients with heart failure adds value to BNP32 assessment. In particular, a low BNP32 value of <125 pg/mL in conjunction with an increased proBNP$_{1\text{-}108}$ identifies a subset of patients with a significant risk of adverse outcomes that might otherwise be overlooked. Our findings thus suggest that simultaneous assessment of proBNP$_{1\text{-}108}$ and BNP32 provides important additional information regarding risk for death, cardiac transplantation, or heart failure hospitalization, particularly for those patients with low BNP32 values and may be useful as a strategy to further guide the management of outpatients with chronic systolic heart failure.

We used a highly specific assay for proBNP$_{1\text{-}108}$ that shows no significant cross-reactivity for current commercial assays for BNP32 or NT-proBNP$_{1\text{-}76}$. However, most of the commercially available BNP immunoassays (Architect, AxSYM, Centaur, Access, Triage) use antibodies aimed at epitopes in the ringed structure of the BNP molecule formed by disulfide bonds. This domain is also present in proBNP$_{1\text{-}108}$ and, as expected, these immunoassays cross-react to varying degrees with proBNP$_{1\text{-}108}$. The degree of cross-reactivity seems to depend on the degree of proBNP glycosylation. For example, the Architect assay (used in this analysis) shows 38% cross-reactivity with glycosylated proBNP (expressed in CHO cells) but only 6% with nonglycosylated recombinant proBNP (expressed from *Escherichia coli*).[14] The degree of glycosylation of circulating proBNP in heart failure has not been determined.

Several previous reports have highlighted the fact that unprocessed proBNP$_{1\text{-}108}$ is a major circulating component of circulating BNP in humans with advanced heart failure. Using mass spectrometry, Hawkridge et al[18] did not detect any processed BNP32 in the plasma of patients with advanced heart failure but instead detected a number of higher molecular weight forms likely representing unprocessed proBNP. Their finding has since been verified using proBNP$_{1\text{-}108}$ immunoassays[10] and is consistent with the findings in this study. Although these studies have shown that the absolute amount of proBNP$_{1\text{-}108}$ correlates with the severity of heart failure, there is substantial interindividual variability in the proportion of proBNP$_{1\text{-}108}$ in patients with advanced heart failure.[10] The basis for this variation remains unclear, but work by our own group suggests that in specific populations, inherited genetic variation in corin may confer variation in efficiency of natriuretic processing and thus lead to different proportions of proBNP$_{1\text{-}108}$ and BNP32.[19,20] It may be that patients having low processed BNP32 levels but high proBNP$_{1\text{-}108}$ levels are "impaired BNP processors."

We hypothesize that the clinical significance and prognostic importance of impaired BNP processing relates to an attenuation of the compensatory actions of the endogenous natriuretic peptide system in heart failure. The natriuretic peptide system exerts a variety of beneficial actions in advanced heart failure that include increased lusitropy, reduced endothelin release, venous and arterial vasodilation, reduced activation of the renin-angiotensin-aldosterone and sympathetic neurohormonal

Table 7. Joint Effects of BNP32 and ProBNP$_{1\text{-}108}$ Assessment and Risk of All-Cause Death or Cardiac Transplantation*

BNP32	ProBNP$_{1\text{-}108}$	n	Unadjusted HR (95% CI)†	n	Adjusted HR (95% CI)†
Low	Low	334	1	327	1
High	Low	44	3.4 (1.6–6.9)	43	3.0 (1.4–6.4)
Low	High	44	4.1 (2.1–7.9)	43	2.3 (1.1–4.6)
High	High	333	5.5 (3.6–8.4)	329	2.9 (1.8–4.7)

*Low or high BNP32 defined as below or above median (125 pg/mL); low or high proBNP$_{1\text{-}108}$ defined as below or above median (271 pg/mL).

†Adjusted for all covariates in Table 2, Model 3.

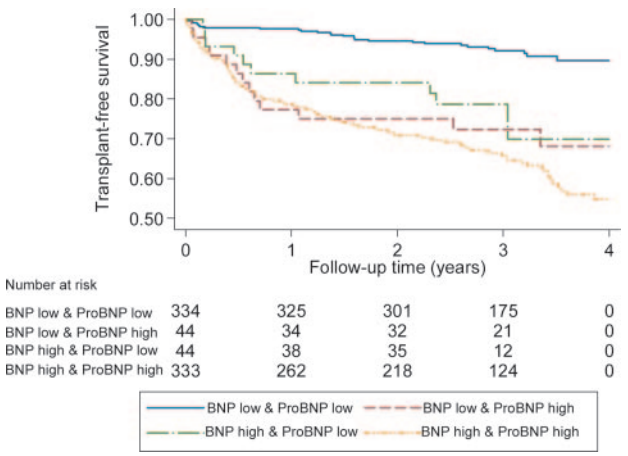

Figure 4. Kaplan–Meier survival estimates for all-cause death or cardiac transplantation in groups stratified by median BNP32 and proBNP$_{1\text{-}108}$ values. $P<0.001$ by log rank test.

Table 8. Joint Effects of BNP32 and ProBNP$_{1-108}$ Assessment and Risk of All-Cause Death, Cardiac Transplantation, or First Heart Failure Hospitalization

BNP32	ProBNP$_{1-108}$	n	Unadjusted HR (95% CI)†	n	Adjusted HR (95% CI)†
Low	Low	334	1	327	1
High	Low	44	2.0 (1.2–3.2)	43	1.8 (1.1–2.9)
Low	High	44	2.5 (1.6–3.9)	43	1.6 (1.0–2.6)
High	High	333	3.2 (2.5–4.1)	329	1.9 (1.4–2.6)

*Low or high BNP32 defined as below or above median (125 pg/mL); low or high proBNP$_{1-108}$ defined as below or above median (271 pg/mL).

†Adjusted for all covariates in Table 4, Model 3.

systems, and the promotion of natriuresis, even in advanced stages of heart failure.[5] For example, the acute administration of HS-121, a competitive antagonist of the natriuretic peptide receptor-A, to dogs with severe pacing-induced systolic heart failure resulted in an increase in adverse neurohormonal activation, increased systemic vascular resistance, an increase of cardiac filling pressures, and reduced fractional excretion of sodium. Conversely, the administration of recombinant, active BNP (nesiritide) to patients with NYHA class II to IV heart failure reduces pulmonary capillary pressures acutely and improves dyspnea scores.[21,22] Therefore, it is not unexpected that impaired natriuretic peptide processing might be causally related to heart failure progression or the risk for decompensation leading to heart failure hospitalization. This paradigm is supported by in vitro models showing a decreased ability of recombinant proBNP$_{1-108}$ compared with recombinant BNP32 to stimulate cGMP production in cardiomyocytes and fibroblasts.[8,9]

Alternatively, the presence of impaired proBNP processing might simply be a marker of an underlying "molecular signature" that is associated with the risk of heart failure progression. Although the molecular mechanisms that underlie BNP processing in human heart failure remain incompletely understood, recent data have provided important insights. Corin has been identified as a contributor to natriuretic peptide processing, although other enzymes such as furin also are postulated to play a role. Corin is expressed abundantly in cardiomyocytes, and it possesses an extracellular serine protease that has shown the ability to process pro-atrial natriuretic peptide and proBNP in vitro and in vivo.[23,24] Abnormalities in corin abundance or corin bioactivity might contribute to impaired BNP processing, but this remains speculative. Elucidating the underlying molecular mechanisms that account for impaired BNP processing in human heart failure may provide novel insights into heart failure progression and new targets for pharmacological intervention.

The clinical implications of our findings suggest that the use of both biomarkers might permit the identification of high-risk ambulatory patients with heart failure that otherwise might be misclassified on the basis of a low BNP32 value. Although one might raise the issue that the number of patients with discordant BNP32 and proBNP$_{1-108}$ values (11.6% of this cohort) is relatively small, we believe that these findings are important from a mechanistic standpoint and clinically because biomarkers become increasingly applied as a multimarker strategy. The limitations of our study do include confirmation in additional cohorts. In addition, there exists a lack of ability to precisely define the cross-reactivity of the BNP32 isoform with proBNP$_{1-108}$. Although we sought to adjust for an extensive list of confounders, there may be unknown covariates that we did not adjust for in our multivariable models. Finally, this study population represents patients recruited from a tertiary referral heart failure clinic with primarily systolic heart failure, with nonischemic heart failure, and of a relatively young age. In accordance with a high-volume heart failure and transplant center, our outcomes assessment included a significant number of cardiac transplantations. As such, our results may not be generalizable to all populations with heart failure, particularly those with diastolic heart failure.

In conclusion, we have shown that in ambulatory patients with heart failure, increased proBNP$_{1-108}$ levels are associated with a significantly increased risk of adverse cardiovascular outcomes. In addition, joint assessment of both proBNP$_{1-108}$ and BNP32 leads to improved risk stratification. In particular, in patients with low measured BNP32 levels, assessment of proBNP$_{1-108}$ levels may permit the identification of a high-risk cohort for death, cardiac transplantation, or heart failure hospitalization that cannot be adequately identified using BNP32 values alone. It also permits the identification of a group with a low risk for these events. If confirmed in additional studies, we propose that the simultaneous assessment of BNP32 and proBNP$_{1-108}$ may be useful for improved risk stratification in patients with systolic heart failure, especially patients with a BNP32 value $<$125 pg/mL that might otherwise reassure the clinician.

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Disclosures

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References

1. Lam CS, Burnett JC Jr, Costello-Boerrigter L, Rodeheffer RJ, Redfield MM. Alternate circulating pro-B-type natriuretic peptide and B-type natriuretic peptide forms in the general population. *J Am Coll Cardiol*. 2007;49:1193–1202.
2. Wu Q, Xu-Cai YO, Chen S, Wang W. Corin: new insights into the natriuretic peptide system. *Kidney Int*. 2009;75:142–146.
3. Wu Q. The serine protease corin in cardiovascular biology and disease. *Front Biosci*. 2007;12:4179–4190.
4. Wu Q, Kuo HC, Deng GG. Serine proteases and cardiac function. *Biochim Biophys Acta*. 2005;1751:82–94.
5. Potter LR, Abbey-Hosch S, Dickey DM. Natriuretic peptides, their receptors, and cyclic guanosine monophosphate-dependent signaling functions. *Endocr Rev*. 2006;27:47–72.
6. Burnett JC Jr. The atrial peptide system in cardiac disease. *Am J Hypertens*. 1988;1:410S–420S.
7. Burnett JC Jr, Kao PC, Hu DC, Heser DW, Heublein D, Granger JP, Opgenorth TJ, Reeder GS. Atrial natriuretic peptide elevation in congestive heart failure in the human. *Science*. 1986;231:1145–1147.
8. Heublein DM, Huntley BK, Boerrigter G, Cataliotti A, Sandberg SM, Redfield MM, Burnett JC Jr. Immunoreactivity and guanosine 3′,5′-cyclic monophosphate activating actions of various molecular forms of human B-type natriuretic peptide. *Hypertension*. 2007;49:1114–1119.
9. Liang F, O'Rear J, Schellenberger U, Tai L, Lasecki M, Schreiner GF, Apple FS, Maisel AS, Pollitt NS, Protter AA. Evidence for functional

heterogeneity of circulating B-type natriuretic peptide. *J Am Coll Cardiol*. 2007;49:1071–1078.
10. Giuliani I, Rieunier F, Larue C, Delagneau JF, Granier C, Pau B, Ferriere M, Saussine M, Cristol JP, Dupuy AM, Merigeon E, Merle D, Villard S. Assay for measurement of intact B-type natriuretic peptide prohormone in blood. *Clin Chem*. 2006;52:1054–1061.
11. Vorovich E, Chuai S, Li M, Avena J, Marwin M, Wolfe D, Reilly MP, Cappola TP. Comparison of matrix metalloproteinase 9 and brain natriuretic peptide as clinical biomarkers in chronic heart failure. *Am Heart J*. 2008;155:992–997.
12. Ky B, Kimmel SE, Safa RN, Putt ME, Sweitzer NK, Fang JC, Sawyer DB, Cappola TP. Neuregulin-1beta is associated with disease severity and adverse outcomes in chronic heart failure. *Circulation*. 2009;120:310–317.
13. Jessup M, Abraham WT, Casey DE, Feldman AM, Francis GS, Ganiats TG, Konstam MA, Mancini DM, Rahko PS, Silver MA, Stevenson LW, Yancy CW. 2009 focused update: ACCF/AHA guidelines for the diagnosis and management of heart failure in adults: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines: developed in collaboration with the International Society for Heart and Lung Transplantation. *Circulation*. 2009;119:1977–2016.
14. Luckenbill KN, Christenson RH, Jaffe AS, Mair J, Ordonez-Llanos J, Pagani F, Tate J, Wu AH, Ler R, Apple FS. Cross-reactivity of BNP, NT-proBNP, and proBNP in commercial BNP and NT-proBNP assays: preliminary observations from the IFCC Committee for Standardization of Markers of Cardiac Damage. *Clin Chem*. 2008;54:619–621.
15. Astor BC, Yi S, Hiremath L, Corbin T, Pogue V, Wilkening B, Peterson G, Lewis J, Lash JP, Van Lente F, Gassman J, Wang X, Bakris G, Appel LJ, Contreras G. N-terminal prohormone brain natriuretic peptide as a predictor of cardiovascular disease and mortality in blacks with hypertensive kidney disease: the African American Study of Kidney Disease and Hypertension (AASK). *Circulation*. 2008;117:1685–1692.
16. Mickey RM, Greenland S. The impact of confounder selection criteria on effect estimation. *Am J Epidemiol*. 1989;129:125–137.
17. Tang WH, Girod JP, Lee MJ, Starling RC, Young JB, Van Lente F, Francis GS. Plasma B-type natriuretic peptide levels in ambulatory patients with established chronic symptomatic systolic heart failure. *Circulation*. 2003;108:2964–2966.
18. Hawkridge AM, Heublein DM, Bergen HR III, Cataliotti A, Burnett JC Jr, Muddiman DC. Quantitative mass spectral evidence for the absence of circulating brain natriuretic peptide (BNP-32) in severe human heart failure. *Proc Natl Acad Sci U S A*. 2005;102:17442–17447.
19. Dries DL, Victor RG, Rame JE, Cooper RS, Wu X, Zhu X, Leonard D, Ho SI, Wu Q, Post W, Drazner MH. Corin gene minor allele defined by 2 missense mutations is common in blacks and associated with high blood pressure and hypertension. *Circulation*. 2005;112:2403–2410.
20. Wang W, Liao X, Fukuda K, Knappe S, Wu F, Dries DL, Qin J, Wu Q. Corin variant associated with hypertension and cardiac hypertrophy exhibits impaired zymogen activation and natriuretic peptide processing activity. *Circ Res*. 2008;103:502–508.
21. Publication Committee for the VMAC Investigators (Vasodilatation in the Management of Acute CHF). Intravenous nesiritide vs nitroglycerin for treatment of decompensated congestive heart failure: a randomized controlled trial. *J Am Med Assoc*. 2002;287:1531–1540.
22. de Lemos JA, McGuire DK, Drazner MH. B-type natriuretic peptide in cardiovascular disease. *Lancet*. 2003;362:316–322.
23. Antos LK, Abbey-Hosch SE, Flora DR, Potter LR. ATP-independent activation of natriuretic peptide receptors. *J Biol Chem*. 2005;280:26928–26932.
24. Yan W, Wu F, Morser J, Wu Q. Corin, a transmembrane cardiac serine protease, acts as a pro-atrial natriuretic peptide-converting enzyme. *Proc Natl Acad Sci U S A*. 2000;97:8525–8529.

CLINICAL PERSPECTIVE

Clinicians managing ambulatory patients with heart failure may use measurement of B-type natriuretic peptide (BNP) as an additional tool to identify patients at risk for decompensation. In patients with moderate to severe chronic heart failure, a substantial proportion of circulating BNP consists of the 108-amino acid prohormone proBNP. Usually, $proBNP_{1\text{-}108}$ is proteolytically cleaved by corin into an inactive amino acid N-terminal fragment (NT-BNP) and a biologically active 32-amino acid carboxyl fragment (BNP32). In vitro experiments also have shown that $proBNP_{1\text{-}108}$ has substantially reduced biological activity. Commercially available C-terminal BNP immunoassays incompletely cross-react with $proBNP_{1\text{-}108}$, creating the potential to underestimate the total amount of circulating BNP, particularly in patients with increased ratios of unprocessed or processed BNP. Using a novel immunoassay specific for unprocessed $proBNP_{1\text{-}108}$, we showed that in patients with low BNP32 levels ($<$100 pg/mL), the additional measurement of $proBNP_{1\text{-}108}$ can separate patients into a low- or a high-risk group for heart failure progression based on the amount of unprocessed $proBNP_{1\text{-}108}$; patients with low BNP32 levels but increased $proBNP_{1\text{-}108}$ levels were at increased risk for heart failure progression. We suspect that the improved risk stratification related to the simultaneous measurement of both processed and unprocessed BNP provided relates to several factors, including the fact that measuring both processed and unprocessed BNP more accurately reflects the total physiological demand for BNP gene transcription by cardiomyocytes, and it allows an assessment of the degree attenuation of the compensatory actions of the natriuretic peptide system since unprocessed proBNP1-108 is substantially less biologically active compared with processed BNP. In summary, we propose that the simultaneous measurement of processed and unprocessed BNP1-108 improves risk stratification in ambulatory heart failure patients, particularly in patients with low BNP values obtained using commercially available immunoassays directed at the carboxyl domain of BNP.