Journal of the American College of Cardiology Vol. 57, No. 12, 2011

Published by Elsevier Inc.
ISSN 0735-1097/$36.00
doi:10.1016/j.jacc.2011.01.005

Biomarkers

Pro–B-Type Natriuretic Peptide$_{1–108}$ Circulates in the General Community

Plasma Determinants and Detection of Left Ventricular Dysfunction

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Objectives The purpose of this study was to investigate circulating pro–B-type natriuretic peptide (proBNP$_{1–108}$) in the general community and evaluate its ability to detect left ventricular (LV) dysfunction.

Background The current concept for cardiac endocrine function is that, in response to cardiac stress, the heart secretes B-type natriuretic peptide (BNP$_{1–32}$) and amino-terminal pro–B-type natriuretic peptide (NT-proBNP$_{1–76}$) after intracardiac cleavage of their molecular precursor, proBNP$_{1–108}$. We hypothesized that proBNP$_{1–108}$ circulates in normal human subjects and that it is a useful biomarker for LV dysfunction.

Methods Our population-based study included a cohort of 1,939 adults (age ≥45 years) from Olmsted County, Minnesota, with 672 participants defined as healthy. Subjects underwent in-depth clinical characterization, detailed echocardiography, and measurement of proBNP$_{1–108}$. Independent factors associated with proBNP$_{1–108}$ and test characteristics for the detection of LV dysfunction were determined.

Results ProBNP$_{1–108}$ in normal humans was strongly influenced by sex, age, heart rate, and body mass index. The median concentration was 20 ng/l with a mean proBNP$_{1–108}$ to NT-proBNP$_{1–76}$ ratio of 0.366, which decreased with heart failure stage. ProBNP$_{1–108}$ was a sensitive (78.8%) and specific (86.1%) biomarker for detecting LV systolic dysfunction, which was comparable to BNP$_{1–32}$, but less than NT-proBNP$_{1–76}$, in several subsets of the population.

Conclusions ProBNP$_{1–108}$ circulates in the majority of healthy humans in the general population and is a sensitive and specific biomarker for the detection of systolic dysfunction. The proBNP$_{1–108}$ to NT-proBNP$_{1–76}$ ratio may provide insights into altered proBNP$_{1–108}$ processing during heart failure progression. Thus, this highly specific assay for proBNP$_{1–108}$ provides important new insights into the biology of the BNP system. (J Am Coll Cardiol 2011;57: 1386–95) © 2011 by the American College of Cardiology Foundation

Pro–B-type natriuretic peptide$_{1–108}$ (proBNP$_{1–108}$) is the 108-amino acid prohormone that is cleaved, by either corin or furin, to the 32-amino acid, biologically active brain natriuretic peptide (BNP$_{1–32}$), also known as B-type natriuretic peptide, and to the 76-amino acid, biologically inactive N-terminal pro–B-type natriuretic peptide (NT-proBNP$_{1–76}$) (1). Brain natriuretic peptide augments sodium excretion, lowers blood pressure, suppresses the renin-angiotensin-aldosterone system, inhibits cardiomyocyte hypertrophy, induces angiogenesis, and retards activation of cardiac fibroblasts (2), whereas its prohormone has significantly reduced function (3). The elevation of BNP$_{1–32}$ and NT-proBNP$_{1–76}$ immunoreactivity in heart failure (HF) secondary to myocardial stretch, despite the lack of BNP functionality (the "BNP paradox"), has resulted in their widespread use as diagnostic and prognostic biomarkers (4–6).

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Manuscript received March 1, 2010; revised manuscript received December 27, 2010, accepted January 3, 2011.

Most recently, studies with mass spectroscopy and Western blot analysis have identified that the major immunoreactive form of BNP in plasma of patients with HF is $proBNP_{1–108}$, rather than biologically active $BNP_{1–32}$, which can in part explain the BNP paradox (3,7–10). Although several studies have documented elevated $proBNP_{1–108}$ levels in patients with HF (11–13), to date there are no studies that have investigated circulating $proBNP_{1–108}$ levels in a large sample of normal, disease-free humans to define key independent determinants of plasma levels of this important prohormone. Furthermore, no studies have analyzed the potential of unprocessed BNP forms, such as $proBNP_{1–108}$, to improve detection of left ventricular (LV) dysfunction.

Our central hypothesis was that $proBNP_{1–108}$ circulates in normal human subjects without cardiac or renal disease, is increased in the presence of LV dysfunction, is modulated by age and sex, and serves as a biomarker for the detection of LV dysfunction. This hypothesis is based on previous studies by Costello-Boerrigter et al. (14), Redfield et al. (15), and Wang et al. (16) that demonstrated that these factors are independently correlated with $BNP_{1–32}$ and NT-$proBNP_{1–76}$. To test this hypothesis, we utilized a novel assay for plasma $proBNP_{1–108}$ developed by Giuliani et al. (12) that is sensitive and specific for detecting $proBNP_{1–108}$ and does not cross-react with mature $BNP_{1–32}$ or NT-$proBNP_{1–76}$. Our study cohort was a National Institutes of Health-supported study, the PAVD (Prevalence of Asymptomatic Ventricular Dysfunction) study, which consisted of a community-based cohort of 1,939 persons, of whom 672 were considered clinically normal, in Olmsted County, Minnesota (17).

Methods

The Mayo institutional review board approved this study.

Study population. Medical history and detailed 2-dimensional and color Doppler echocardiography were done on participating residents (n = 1,939; age ≥45 years) in Olmsted County, Minnesota, and were previously reported as the PAVD study (15). Subjects were divided by cardiovascular risk factors into a reference group of stage 0 HF subjects (no risk factors) who were normal healthy humans, and a group of subjects with stages A to C HF as defined by the 2009 HF guidelines (18,19). Persons with stage D HF were previously excluded from the study (5,15,17,20).

Assays and sample processing. Blood samples were drawn into ethylenediaminetetraacetic acid-treated tubes and chilled to 4°C, then centrifuged at 2,500 rpm for 10 min and stored at −80°C. Plasma $proBNP_{1–108}$ was measured with the Bio-Rad assay (Bio-Rad, Hercules, California) on an automated analyzer not yet commercially available. This immunoassay was developed by Giuliani et al. (12) to be specific for $proBNP_{1–108}$ as it uses antibodies directed against the hinge region that is present only in the intact $proBNP_{1–108}$ molecule. The lower limit of detection for $proBNP_{1–108}$ is 2 ng/l and the interassay and intra-assay variabilities for $proBNP_{1–108}$ are 10.3% and 11.6%, respectively. Plasma NT-$proBNP_{1–76}$ was previously measured using the Elecsys electrochemiluminescence immunoassay on the Elecsys 2010 platform (Roche Diagnostics, Indianapolis, Indiana), which is a sandwich assay that at that time utilized 2 different polyclonal antibodies, 1 to amino acids 1–21, and 1 to amino acids 39–50. Samples were previously frozen and sent to Biosite Diagnostics (San Diego, California) for measurement of plasma $BNP_{1–32}$ (14,15). The nonextracted 1-ml aliquots of frozen plasma were batch analyzed with the Biosite fluorescence immunoassay. The total within-day coefficient of variation (9.4% to 15.2%) and total coefficient of variation (10.1% to 16.2%) increased from low to high BNP.

Abbreviations and Acronyms

AUC = area under the curve
BMI = body mass index
BNP = B-type natriuretic peptide
EF = ejection fraction
HF = heart failure
LA = left atrial
LV = left ventricular
NT-proBNP = N-terminal pro–B-type natriuretic peptide

Influence of clinical characteristics. Influences on $proBNP_{1–108}$ were assessed with univariate and multivariable analysis for age, sex, body mass index (BMI), heart rate, left atrial (LA) volume index, LV mass index, LV end-diastolic volume dimension index, systolic and diastolic blood pressure, serum creatinine, and calculated glomerular filtration rate (by Cockcroft-Gault formula). All index values were calculated by dividing by each subject's body surface area.

Statistical methods. Descriptive statistics were used to describe the cohort of subjects: median and interquartile range for continuous data, and count and percentage for categorical data. Patient and clinical characteristic were compared between subjects in the various stages of HF using rank-order tests such as Spearman correlation and the Kruskal-Wallis method. To derive normal values for $proBNP_{1–108}$, the 5th, 50th, and 95th percentile values from the normal subgroup of subjects were used. Multivariable linear least-squares regression was then used to detect independent associations between $proBNP_{1–108}$ levels and clinical cardiovascular risk factors with 2-way interactions considered. Because the variability of $proBNP_{1–108}$ increased with its mean level, a natural log transformation of the $proBNP_{1–108}$ value +1 (since 0 is in its domain, but natural log of 0 is undefined) was applied to satisfy regression modeling assumptions. Similar transformations were applied to the $BNP_{1–32}$ and NT-$proBNP_{1–76}$ biomarkers.

To optimize the number of subjects retained in the multivariable regression, all continuous parameters <90% observed were each categorized, including a category for unknown values. Using stepwise selection with entry and retention criteria of $p < 0.10$ and $p < 0.05$, respectively, a final model was derived for the total population and separately for the stage 0 HF (normal subjects) and stages A to C HF subgroups. Bootstrap resampling was used to evaluate the robustness of the clinical and echocardiographic parameters that were statis-

tically significantly associated with natriuretic peptide levels. The frequency of each variable retained in the model using the pre-specified model selection criteria was computed as a percentage of all 1,000 bootstrap samples. Our modeling strategy required that the factors included in the final model each be retained in at least 60% of the bootstrap samples, thus reducing the chance of type 1 error (21,22).

The diagnostic utility for detecting LV dysfunction as either ejection fraction (EF) ≤40%, or EF ≤50%, and/or moderate to severe diastolic dysfunction was assessed for $BNP_{1–32}$, NT-$proBNP_{1–76}$, and $proBNP_{1–108}$. Diagnostic test characteristics of $proBNP_{1–108}$ were evaluated using receiver-operating characteristic curves to determine areas under the curve (AUCs), which were then compared by the deLong method (23) to those for $BNP_{1–32}$ and NT-$proBNP_{1–76}$ in the total and age- and sex-stratified populations (14). The optimal discriminatory value for each assay was estimated by the point along the receiver-operating characteristic curve that provided the minimum Euclidean distance between the curve and a perfect assay with 100% sensitivity and specificity. The positive likelihood ratio (+LR = sensitivity/[1 − specificity]) and negative likelihood ratio (−LR = [1 − sensitivity]/specificity) were calculated for the optimal discriminatory values. Additional test characteristics estimated include the percentage of patients screened who would need echocardiography secondary to an abnormal $proBNP_{1–108}$ test result (% need echo), the percent of echocardiograms that then would be negative (% −echo), and the percent of those with a reduced EF who would be missed by the $proBNP_{1–108}$ cutoff (% missed), and the odds ratio of having EF ≤40% or 50% and/or diastolic dysfunction for a high $proBNP_{1–108}$ result. Statistical significance was accepted at $p < 0.05$ for all analyses.

Results

$ProBNP_{1–108}$ immunoreactivity in the general community. Plasma $proBNP_{1–108}$ values were determined in all 1,939 subjects of the community-based cohort, of whom 672 were defined as the normal reference group (i.e., stage 0 HF/healthy normals) because they had no cardiovascular or renal disease by clinical examination and normal LV structure and function by echocardiography. The rest of the cohort was considered clinically abnormal, and further classified as having stage A, B, or C HF. Table 1 provides characteristics for the total study population and for subgroups according to stage of HF. Figure 1 displays $proBNP_{1–108}$, NT-$proBNP_{1–76}$, and $BNP_{1–32}$ distributions by HF stage. As illustrated, $proBNP_{1–108}$ was detected at the lowest levels in the reference group. By univariate assessment, $proBNP_{1–108}$ levels increased significantly with advancing stage of HF, as did $BNP_{1–32}$ and NT-$proBNP_{1–76}$ levels. As shown in Figure 2, although higher in female subjects at younger ages, plasma $proBNP_{1–108}$ levels increased with age more markedly in male subjects in the total cohort and in subjects with HF. In normal subjects, however, higher levels of plasma $proBNP_{1–108}$ were associated with increasing age as well as with female sex across the entire age range.

We sought to investigate whether these age and sex trends for $proBNP_{1–108}$ held after adjustment for potential confounders. Table 2 reports the clinical characteristics that significantly influence $proBNP_{1–108}$ levels within the total population, the reference group (stage 0 HF/healthy normals), and the abnormal group (stages A to C HF). In the multivariable analysis of the reference group, sex, age, heart rate, and BMI were independently associated with $proBNP_{1–108}$ ($p < 0.001$ for age and sex, $p = 0.006$ for heart rate, $p = 0.004$ for BMI 25 to 30 kg/m^2, and $p = 0.021$ for BMI >30 kg/m^2). Echocardiographic measurements were not associated with $proBNP_{1–108}$ in normal subjects, but LV mass index and LV dimension index were significantly associated with $proBNP_{1–108}$ in the total population and in subjects with stages A, B, or C HF. Table 3 displays age- and sex-specific ranges for normal values of $proBNP_{1–108}$, noting that there are few subjects older than 75 years in the normal subgroup. Table 4 illustrates the $proBNP_{1–108}$/NT-$proBNP_{1–76}$ ratio by stage of HF. The $proBNP_{1–108}$/NT-$proBNP_{1–76}$ ratio decreased significantly from stage 0 to stages A to C.

Detection of LV dysfunction. In this community-based sample, 35 (2%) subjects had an EF ≤40%, 114 (6%) had an EF ≤50%, and 135 (8%) had moderate or severe diastolic dysfunction. For detection of LV systolic dysfunction, the AUCs for $BNP_{1–32}$, NT-$proBNP_{1–76}$, and $proBNP_{1–108}$ are each summarized in Online Table 1 for the total population and for subsets stratified by age and sex. In general, the performance for detecting LV systolic dysfunction was comparable between $proBNP_{1–108}$ and $BNP_{1–32}$, although $proBNP_{1–108}$ was more diagnostic of an EF ≤50% in the younger stratum of the total and male populations. Despite this, NT-$proBNP_{1–76}$ was superior to $proBNP_{1–108}$ for detecting EF ≤40% and ≤50% except in the younger subjects. Across the various population groups, each of these natriuretic peptides was consistently more diagnostic of LV dysfunction based on an EF cutoff of 40% as opposed to 50%.

Using a single group-specific cutpoint to optimize sensitivity and specificity in detecting reduced EF, the test characteristics of the $proBNP_{1–108}$ assay were summarized for the total population and for age- and sex-stratified subgroups of the population (Online Table 2). For optimal discrimination of EF >40% or <40% in the total population, the cutpoint $proBNP_{1–108}$ value was 65 ng/l, reflecting a sensitivity of 78.8% and a specificity of 86.1%. The best performance of this assay was in female and younger subgroups.

Similar cutpoint analyses were performed for detecting diastolic dysfunction and overall LV dysfunction, a composite of either systolic (EF ≤40%) or diastolic (moderate or severe based on echocardiography) dysfunction. For discriminating subjects with and without diastolic dysfunction, the AUCs were on average comparable between NT-

Table 1 Characteristics of the Study Population by Stage of HF

Variable*	Total Population (n = 1,939)	Stage 0 HF (n = 672)	Stage A HF (n = 788)	Stage B HF (n = 415)	Stage C HF (n = 64)
Females	1,003 (52%)	347 (52%)	391 (50%)	245 (59%)	20 (31%)
Age, yrs	61.8 (53.6–70.5)	54.5 (50.6–61.3)	64.9 (57.2–72.2)	67.9 (59.3–76.4)	73.8 (64.4–82.0)
Age categories, yrs					
45–54	560 (29%)	346 (51%)	146 (19%)	62 (15%)	6 (9%)
55–64	595 (31%)	222 (33%)	252 (32%)	110 (27%)	11 (17%)
65–74	500 (26%)	89 (13%)	264 (34%)	128 (31%)	19 (30%)
75+	284 (15%)	15 (2%)	126 (16%)	115 (28%)	28 (44%)
BMI, kg/m^2	27.7 (25.0–31.2)	27.1 (24.5–29.8)	28.1 (25.0–31.7)	28.1 (25.3–31.9)	28.0 (25.7–31.9)
Obesity, BMI >30 kg/m^2	623 (32%)	162 (24%)	287 (36%)	152 (37%)	22 (34%)
ProBNP$_{1–108}$, ng/l	20.0 (9.0–42.0)	14.0 (7.0–26.0)	20.0 (9.0–41.0)	37.0 (16.0–79.0)	150.0 (61.5–391.0)
NT-proBNP$_{1–76}$, ng/l	69.9 (28.3–147.5)	39.4 (18.0–82.0)	72.1 (31.3–140.7)	138.5 (61.0–332.2)	777.8 (234.2–1271.0)
BNP$_{1–32}$, ng/l	24.0 (9.5–56.3)	15.3 (6.4–29.8)	24.4 (9.7–53.9)	50.5 (20.3–106.2)	138.5 (80.4–313.6)
Calculated GFR	75.0 (60.4–92.6)	81.2 (66.8–97.0)	72.9 (58.9–91.3)	69.3 (54.6–90.5)	62.4 (44.8–78.5)
Estrogen†	460/961 (48%)	160/325 (49%)	194/381 (51%)	101/235 (43%)	5/20 (25%)
Diabetes mellitus	145 (7%)	0 (0%)	85 (11%)	47 (11%)	13 (20%)
Past or current atrial fibrillation	94 (5%)	0 (0%)	34 (4%)	31 (7%)	29 (45%)
Coronary artery disease	233 (12%)	0 (0%)	113 (14%)	78 (19%)	42 (66%)
Past heart failure diagnosis	49 (3%)	0 (0%)	0 (0%)	0 (0%)	49 (77%)
Hypertension	543 (28%)	0 (0%)	340 (43%)	168 (40%)	35 (55%)
Systolic blood pressure, mm Hg	131.0 (116.0–146.0)	122.0 (111.0–134.0)	135.0 (121.0–149.0)	138.0 (123.0–155.0)	134.0 (119.0–154.0)
Diastolic blood pressure, mm Hg	73.0 (67.0–80.0)	72.0 (66.0–78.0)	75.0 (68.0–82.0)	74.0 (67.0–80.0)	70.0 (66.0–82.5)
LV dimension index	2.61 (2.40–2.82)	2.59 (2.39–2.77)	2.55 (2.36–2.74)	2.77 (2.54–2.97)	3.04 (2.73–3.29)
LV mass index	93.7 (81.9–108.8)	88.2 (77.2–97.0)	92.2 (81.8–104.2)	113.1 (97.7–128.0)	137.2 (116.5–163.3)
LA volume index	23.3 (19.4–28.1)	20.9 (18.0–24.6)	22.7 (19.4–26.2)	33.0 (25.2–36.9)	35.8 (29.0–51.0)
Beta-blocker	284 (16%)	0 (0%)	162 (22%)	99 (25%)	23 (36%)
ACEI/ARB	204 (11%)	0 (0%)	115 (15%)	57 (14%)	32 (50%)
Diuretic	330 (18%)	0 (0%)	193 (26%)	98 (25%)	39 (61%)
EF <40%	35 (2%)	0 (0%)	0 (0%)	0 (0%)	35 (55%)
EF <50%	114 (6%)	0 (0%)	0 (0%)	71 (17%)	43 (67%)
Moderate or severe DD	135/1,709 (8%)	0/672 (0%)	52/668 (8%)	63/330 (19%)	20/39 (51%)

Values are n (%) or median (interquartile range). *The top block of variables were each tested for an association with advancing stage of heart failure (HF) using a Spearman correlation rank-order test or a Cochran-Armitage trend test as appropriate for continuous and categorical variables (each had a highly significant association [$p < 0.001$], except for sex [$p = 0.796$] and female estrogen use [$p = 0.047$]; the lower block of variables was used in defining the subgroups of HF, so no statistical tests were conducted. †Percent of estrogen use based only on female subgroup.

ACEI = angiotensin-converting enzyme inhibitor; ARB = angiotensin-receptor blocker; BMI = body mass index; BNP = B-type natriuretic peptide; DD = diastolic dysfunction; EF = ejection fraction; GFR = glomerular filtration rate; LA = left atrial; LV = left ventricular; NT-proBNP = N terminal pro–B-type natriuretic peptide; proBNP = pro–B-type natriuretic peptide.

proBNP$_{1–76}$ and BNP$_{1–32}$, and significantly greater than those of proBNP$_{1–108}$ (Table 5). The optimal cutpoint for detection of diastolic dysfunction by proBNP$_{1–108}$ was 25.1 ng/l, with a resulting sensitivity of 66.1% and a specificity of 63.1% in the total population. Finally, the optimal proBNP$_{1–108}$ cutpoint for detection of overall LV dysfunction as a composite of either systolic or diastolic dysfunction was 39 ng/l, with a sensitivity of 56.8% and a specificity of 74.5% in the total population (AUCs not displayed for composite LV dysfunction).

Given their strong influence on proBNP$_{1–108}$ levels, age- and sex-specific cutpoints were obtained for detecting a reduced ejection, with their corresponding test characteristics compared to those based on 1 unadjusted proBNP$_{1–108}$ cutpoint (Online Tables 3 and 4). In the total population, the use of age- and sex-adjusted (vs. unadjusted) proBNP$_{1–108}$ cutpoints increased the relative risk estimate of having an EF ≤40% by a factor of 2 (95% confidence interval of odds ratio: 23 to 46).

Discussion

ProBNP$_{1–108}$ is a circulating prohormone. The major finding in our study is that proBNP$_{1–108}$ circulates in the majority of normal, disease-free humans, which changes our understanding of the secretion of proBNP$_{1–108}$ and its processing to mature, biologically active BNP$_{1–32}$. Although prior studies have shown that proBNP$_{1–108}$ exists in circulation, our translational study is the first to our knowledge to confirm at the population level the relative predominance of this prohormone compared to multiple immunoreactive BNP forms that circulate and which are nonspecifically detected by conventional BNP$_{1–32}$ and NT-proBNP$_{1–76}$ assays (7,9,10,24).

To date, studies have focused on circulating proBNP$_{1–108}$ in humans with advanced HF (8,10–13). With BNP$_{1–32}$ reported to be at low levels or undetectable in human HF despite high immunoreactivity using conventional assays, the concept has been proposed that the presence of proBNP$_{1–108}$ in plasma reflects a defect in proBNP$_{1–108}$

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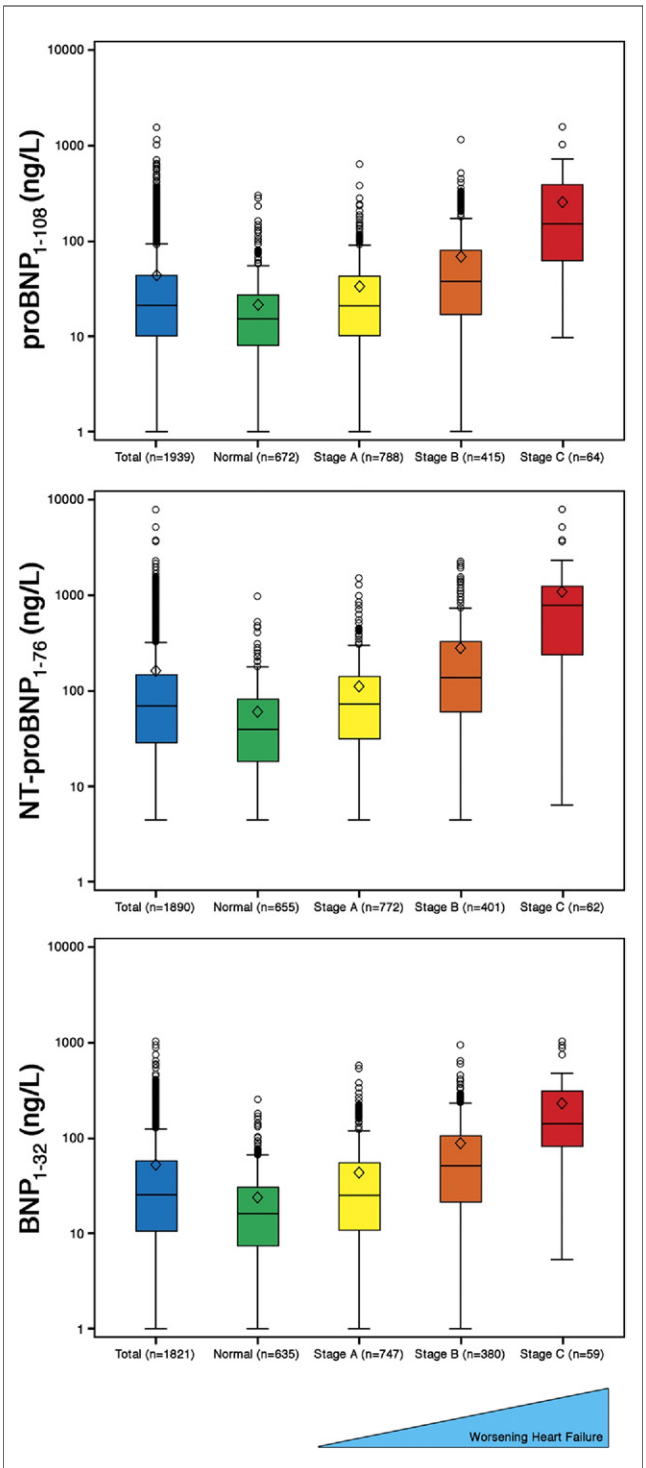

Figure 1 BNP Levels in the General Population by Stage of HF

Plasma B-type natriuretic peptide$_{1–32}$ (BNP$_{1–32}$), N-terminal pro–B-type natriuretic peptide$_{1–76}$ (NT-proBNP$_{1–76}$), and pro–B-type natriuretic peptide$_{1–108}$ (proBNP$_{1–108}$) levels in the total population, the healthy subjects (i.e., cardiovascular and renal disease-free subjects, stage 0 heart failure [HF]), and subjects with stages A, B, and C heart failure.

processing in the heart with spillover of unprocessed and nonbiologically active proBNP$_{1–108}$ into the plasma (3,6,9). Our study changes that paradigm with the use of this newly developed and specific assay for proBNP$_{1–108}$ that does not cross-react with NT-proBNP$_{1–76}$ or BNP$_{1–32}$. Specifically, our data are consistent with the concept of the physiological release of proBNP$_{1–108}$ from the normal heart, implying peripheral processing to mature BNP$_{1–32}$ either in plasma and/or at target organs. Indeed, we have recently documented the presence of the proBNP$_{1–108}$ processing convertase corin in normal human plasma and the in vitro processing of proBNP$_{1–108}$ to BNP$_{1–32}$ in normal human plasma (25) together with a step-up in the proBNP$_{1–108}$ gradient across the normal human heart (26). With

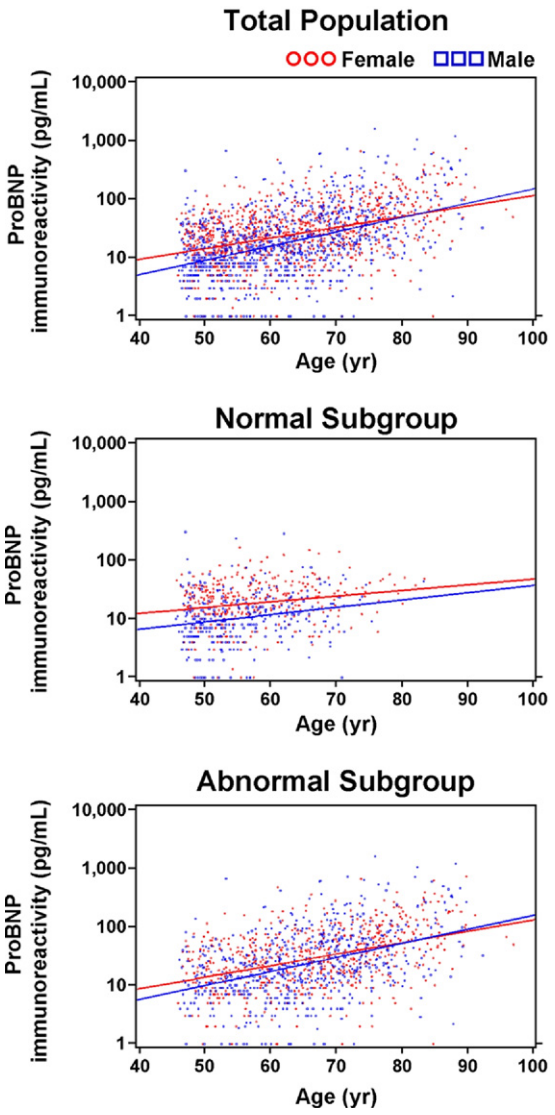

Figure 2 Plasma ProBNP$_{1–108}$ Levels by Age, Separated by Sex

Plasma pro–B-type natriuretic peptide$_{1–108}$ (proBNP$_{1–108}$) distributions shown for the total population, stage 0 heart failure subjects, and stages A to C heart failure subjects.

Table 2 Parameters That Significantly Contribute to $ProBNP_{1–108}$* in Multivariable Analysis

Population	Parameters Included in Model	Regression Coefficient	SE	p Value
Total population†				
	Age‡			
	Males	0.431	0.035	<0.001
	Females	0.308	0.032	<0.001
	BMI ≤25 kg/m^2 (reference)	—	—	—
	25–30 kg/m^2	−0.193	0.060	0.001
	>30 kg/m^2	−0.184	0.067	0.006
	LVMI high (>134 males, >110 females)	0.203	0.085	0.017
	LVDI high (>2.6)	0.151	0.059	0.010
	Creatinine	0.297	0.129	0.021
Normal subgroup (stage 0 HF)	Age‡	0.250	0.046	<0.001
	Sex, female	0.485	0.073	<0.001
	Heart rate	−0.010	0.004	0.006
	BMI ≤25 kg/m^2 (reference)	—	—	—
	25–30 kg/m^2	−0.244	0.085	0.004
	>30 kg/m^2	−0.227	0.099	0.021
Abnormal subgroup (stages A–C HF)	Age‡	0.406	0.031	<0.001
	Sex, female	0.162	0.063	0.010
	BMI ≤25 kg/m^2 (reference)	—	—	—
	25–30 kg/m^2	−0.153	0.079	0.054
	>30 kg/m^2	−0.179	0.085	0.036
	LVMI high (>134 males, >110 females)	0.225	0.092	0.014
	LVDI high (>2.6)	0.146	0.077	0.059

*Dependent variable in all linear regression models above was log-transformed proBNP value+1. †For left ventricular mass index (LVMI) and left ventricular dimension index (LVDI) covariates, each was fit as categorical with categories abnormal/high (effect shown), normal (reference level), and unknown (effect not shown). ‡Age, expressed per 10-year change.
Abbreviations as in Table 1.

$proBNP_{1–108}$ now a circulating hormone, it is logical that there now is a need for such an assay to better assess direct secretion of this cardiac hormone from the heart and its peripheral processing from a biological perspective as well as to assess its potential diagnostic significance across a wide spectrum of human cardiovascular disease.

Importantly, we have documented in a large population both free of HF and in stages A to C what Dries et al. (11) has labeled the "natriuretic peptide processing efficiency," and which has previously been investigated in the PENN HF study, which was mostly in patients with advanced systolic HF. Our results show that progressing from stage 0 to stages A to C is associated with increasing processing efficiency due to either decreased secretion of $proBNP_{1–108}$ or an increase in peripheral processing to NT-$proBNP_{1–76}$ as demonstrated by the decrease in the $proBNP_{1–108}$/NT-$proBNP_{1–76}$ ratio. It appears that NT-$proBNP_{1–76}$ is increasing faster than $proBNP_{1–108}$ in stages A to C. We speculate as one moves into stage D, secretion outpaces processing and efficiency decreases. Because of our population, we are unable to prove whether HF worsens processing efficiency or if decreasing processing efficiency pre-dates advanced HF, as we had no stage D subjects. To show this, an important future use of the $proBNP_{1–108}$ assay, using step-up studies in subjects with varying degrees of HF and at different time points, will be needed to determine exactly what proportion of BNP forms the heart secretes in physiological and pathophysiological states, and where and by what mechanism precisely extracardiac processing occurs. We speculate that local processing of $proBNP_{1–108}$ may be an efficacious way of promoting renal function without causing systemic actions such as hypotension.

Table 3 Age- and Sex-Specific Median Values and Ranges for Plasma $ProBNP_{1–108}$ in Normal Subjects

	Age, yrs			
	45–54	55–64	65–74	≥75
Women				
Median (5th–95th percentiles), ng/l	17 (3–48)	21 (3–81)	23 (4–128)	37 (5–56)
n	168	117	49	13
Men				
Median (5th–95th percentiles), ng/l	7 (1–40)	12 (2–44)	16 (2–53)	25 (8–42)
n	178	105	40	2

ProBNP = pro–B-type natriuretic peptide.

Table 4 $ProBNP_{1–108}$ to $NT\text{-}proBNP_{1–76}$ Ratio by Stage of Heart Failure

Ratio	Stage 0	Stage A	Stage B	Stage C	p Value
$ProBNP_{1–108}/NT\text{-}proBNP_{1–76}$	0.366 (0.209, 0.612)	0.300 (0.178, 0.504)	0.271 (0.147, 0.444)	0.301 (0.140, 0.416)	<0.001

Median (quartile 1, quartile 3); both tested for an association with advancing stage of heart failure using a Spearman correlation rank-order test. Abbreviations as in Table 1.

Association of clinical and echocardiographic parameters on $proBNP_{1–108}$ levels. We present the first analysis of the factors independently associated with circulating $proBNP_{1–108}$ in subjects without cardiovascular and renal disease and then compare those factors to those of $BNP_{1–32}$ and $NT\text{-}proBNP_{1–76}$ in the same population. To our knowledge, this is the first study of natriuretic peptides that utilized the internal validation technique of bootstrap resampling to ensure the robustness of the multivariable modeling. We found that age and sex were the principal determinants of $proBNP_{1–108}$ levels, with higher levels associated with increasing age and female sex, but we also observed that this association increased at a higher rate with age in males in the total population. Despite significant associations, the effects of age and sex on $proBNP_{1–108}$ in our study were not as strong as those on $NT\text{-}proBNP_{1–76}$, suggesting a difference between the effects of age and sex on cardiac $proBNP_{1–108}$ production and downstream processing and renal clearance (13,14,27,28). These important findings underscore the need for considering age and sex when developing ranges for normals; they also emphasize that production and processing are distinct processes with unique determining characteristics. Of relevance is the recent report by Ichiki et al. (25) that corin levels in the plasma are also determined by sex, with males possessing higher levels than females.

Although the mechanisms by which age contributes to an increase in $proBNP_{1–108}$ levels are not clearly understood, we demonstrate that the aging heart produces more $proBNP_{1–108}$, even with normal myocardial function and structure in the general population. Expression of the BNP gene has been shown to be up-regulated with aging (29), but some studies have speculated that circulating $BNP_{1–32}$ immunoreactivity rises because of impaired renal function with aging, including reduced natriuretic peptide receptor function (30,31). Thus, the increase in $proBNP_{1–108}$ with aging may be due to 1 or more mechanisms: increased cardiac production and release or impaired downstream processing or clearance.

Previous studies have shown a relationship between sex and $BNP_{1–32}$ and $NT\text{-}proBNP_{1–76}$, and some have noted an association between estrogen use (hormone replacement therapy) and increased $BNP_{1–32}$ levels (14,15). We did not find an independent association between estrogen use and $proBNP_{1–108}$ in our study, whereas a significant association was found for $NT\text{-}proBNP_{1–76}$ in the Costello-Boerrigter et al. (14) study, suggesting a differential effect by androgens and estrogens on production and processing. Studies of sex differences in natriuretic peptide production and processing may lead to better explanation of the predominance of men with HF at younger ages than women.

Another important finding in our study was that cardiac structure and function parameters, LV mass index and LV dimension index, were independently associated with increased $proBNP_{1–108}$ in stage A, B, and C HF subgroups, suggesting that decreasing myocardial function and worsening fibrosis, dilation, and hypertrophy may contribute to increasing $proBNP_{1–108}$ by an alteration in production or processing capability in persons with cardiovascular and renal disease.

Previously, $NT\text{-}proBNP_{1–76}$ and $BNP_{1–32}$ were found to be independently associated with LA volume index, but in our study, $proBNP_{1–108}$ was not. It was believed that LA volume index and LV dimension index had effects on the variability of $BNP_{1–32}$ because both atrial and ventricular myocardium are responsible for $BNP_{1–32}$ production (5,32–34). Importantly, our results suggest a stronger role for ventric-

Table 5 Detection of Moderate or Severe Diastolic Dysfunction by $ProBNP_{1–108}$, $NT\text{-}proBNP_{1–76}$, and $BNP_{1–32}$

Population/Subgroup	n	AUC (95% CI)			p Value of Overall Difference
		$ProBNP_{1–108}$	$NT\text{-}proBNP_{1–76}$	$BNP_{1–32}$ (Bio-site)	
Total (n = 1,558)	118	0.71 (0.66, 0.76)	0.77 (0.72, 0.82)*	0.78 (0.73, 0.82)*	0.028
Age ≥65 yrs (n = 594)	77	0.65 (0.58, 0.72)	0.73 (0.67, 0.79)*	0.76 (0.70, 0.82)*	0.006
Age <65 yrs (n = 964)	41	0.71 (0.63, 0.79)	0.73 (0.63, 0.83)	0.72 (0.63, 0.81)	0.871
All men (n = 742)	54	0.77 (0.71, 0.83)	0.78 (0.71, 0.86)	0.78 (0.72, 0.85)	0.929
All women (n = 816)	64	0.66 (0.58, 0.74)	0.76 (0.70, 0.83)*	0.79 (0.72, 0.85)*	0.008
Men, age ≥65 yrs (n = 265)	33	0.71 (0.61, 0.80)	0.76 (0.67, 0.86)	0.78 (0.70, 0.87)	0.228
Women, age ≥65 yrs (n = 329)	44	0.61 (0.50, 0.72)	0.70 (0.61, 0.80)	0.75 (0.67, 0.83)*	0.022
Men, age <65 yrs (n = 477)	21	0.77 (0.67, 0.87)	0.72 (0.57, 0.86)	0.71 (0.59, 0.84)	0.644
Women, age <65 yrs (n = 487)	20	0.66 (0.53, 0.80)	0.76 (0.64, 0.89)	0.76 (0.62, 0.89)	0.345

*Area under the curve (AUC) of biomarker is significantly different from that of $proBNP_{1–108}$.
CI = confidence interval; other abbreviations as in Table 1.

ular myocardium than atrial myocardium in secretion of unprocessed $proBNP_{1–108}$ in diseased subjects compared with normal subjects, on the basis of a lack of significant association between LV echocardiographic characteristics and $proBNP_{1–108}$ in subjects with stage 0 HF (healthy normals) and the presence of significant associations between LV dimension index and LV mass index and $proBNP_{1–108}$ in subjects with cardiovascular disease. Atrial myocardium may play a part in secretion of mature ($BNP_{1–32}$) or may be involved in processing.

We did not find a statistically significant correlation between glomerular filtration rate and $proBNP_{1–108}$ levels in our multivariable analysis of the normal population. This finding is similar to previous studies with NT-$proBNP_{1–76}$ in our study population (14). However, several groups have reported that $BNP_{1–32}$ and NT-$proBNP_{1–76}$ levels are inversely correlated with renal function in abnormal patients, such as in those with varying degrees of HF or renal disease (35–37). It was further shown that cardiovascular disease was required for $BNP_{1–32}$ elevation in patients on dialysis for end-stage renal disease (38). Given that our study had so few subjects with the severity of disease that was examined in those studies, it was unlikely that we would have found an association. Another consideration is that $proBNP_{1–108}$, NT-$proBNP_{1–76}$, and $BNP_{1–32}$ may all have different mechanisms for renal clearance (39–41), which could explain the variability in the associations among these 3 forms of BNP and renal function, especially in the stages A to C HF group.

Finally, we note that the decrease in $proBNP_{1–108}$ with obesity may support the overall concept that decreased circulating $BNP_{1–32}$ levels in obesity may indeed be secondary to reduced $BNP_{1–32}$ production by the heart in this clinical setting (42). This observation supports the need for better understanding the impact of obesity on the heart, which may influence BNP production, and, here, use of $proBNP_{1–108}$ measurements may have an important role beyond HF alone.

In our analysis of clinical factors, because of lack of data, we were unable to correlate $proBNP_{1–108}$ secretion with functional status as measured by New York Heart Association functional class of HF.

Detection of LV dysfunction. We are the first to report that the Bio-Rad $proBNP_{1–108}$ assay detected LV systolic dysfunction in the general community with high sensitivity and specificity and was comparable to $BNP_{1–32}$ in discriminative performance (14). We also show the efficacy of an unprocessed natriuretic peptide for detection of diastolic dysfunction in a large population. Importantly, because of a lack of cross-reactivity, we are able to emphasize that this novel assay is a true reflection of physiological and pathological secretion of $proBNP_{1–108}$, and, therefore, of the endocrine function of the heart. A specific $proBNP_{1–108}$ assay will be of tremendous value for future studies evaluating the peripheral processing of $proBNP_{1–108}$. Regarding its use as a novel biomarker for HF, it is also important to state that $proBNP_{1–108}$ is a more robust biomarker for the detection of systolic versus diastolic dysfunction, and that our findings will have to be replicated in other populations and in other scenarios.

Based on statistically significant differences in their AUCs, the NT-$proBNP_{1–76}$ assay performed better than the $proBNP_{1–108}$ and $BNP_{1–32}$ assays in several subgroups for the detection of reduced EF, whereas the $proBNP_{1–108}$ assay was comparable with $BNP_{1–32}$ in most others. These results illustrate that all of these assays hold some diagnostic value for the detection of LV systolic dysfunction in the general population, but individual differences in their biologic meaning must be taken into account in the clinical setting. We caution against over-interpretation of their differences because of the small number of subjects in some of the subgroups, especially females. In fact, the lack of a large number of diseased persons in the general population is an important limitation of the cohort study design.

Our findings illustrate the heterogeneity of factors in cardiac disease that may influence $proBNP_{1–108}$ secretion and processing. We noted that age- and sex-adjusted cutpoints generally improved test characteristics for detecting EF $\leq$40%. Furthermore, we noted that processed forms of BNP, including $BNP_{1–32}$ and NT-$proBNP_{1–76}$ were for the most part better at detection of systolic HF, implying that processes that adversely affect ventricular filling may have a differential effect on processing compared to secretion. The heterogeneity is also evident from the fact that when $proBNP_{1–108}$ is used to detect composite, namely, systolic and/or diastolic dysfunction, the sensitivity and specificity both decrease relative to systolic dysfunction alone.

A limitation of our study was that we had no information regarding right ventricular function. Therefore, we had no way to assess the diagnostic accuracy of $proBNP_{1–108}$ for right ventricular dysfunction.

Physiologic, pathophysiologic, and diagnostic significance of $proBNP_{1–108}$. In addition to its value as a novel diagnostic tool for HF, the Bio-Rad $proBNP_{1–108}$ assay reveals both the physiologic and pathophysiologic importance of $proBNP_{1–108}$ in human plasma. It confirms and extends previous studies by Lam et al. (24) that utilized an assay for $proBNP_{3–108}$, which reported its presence in the general population. With the $proBNP_{1–108}$ assay, which has been documented not to cross-react to $BNP_{1–32}$ or $NTproBNP_{1–76}$ we can now be confident that the heart releases this prohormone of BNP in normal humans (12). Indeed, the presence of $proBNP_{1–108}$ in most normal humans in this large population study and the recent report that $proBNP_{1–108}$ can be processed to mature $BNP_{1–32}$ in human plasma clearly change our understanding of the heart as an endocrine organ and the BNP system (25). These observations strongly suggest that in part $proBNP_{1–108}$ is an important carrier protein, which delivers the mature peptide to the plasma or tissues for local processing and conversion. From a biological perspective, measurement of circulating $proBNP_{1–108}$ may be important

from the perspective of fully understanding the intactness of $proBNP_{1–108}$ secretion from the heart and its processing in the circulation.

In HF, the BNP paradox is the disparity between diagnostically elevated $BNP_{1–32}$ immunoreactivity in patients with HF and a lack of BNP-derived vasodilatory and cardiorenal protective effects. We can now state that the elevation of $proBNP_{1–108}$, which has reduced biologic activity and cross-reacts with conventional BNP assays, in patients with HF can account for at least some of this phenomenon (3,8). Yet, in stages A, B, and C HF, there may be increased processing efficiency based upon the decrease in the $proBNP_{1–108}$/NT-$proBNP_{1–76}$ ratio. Perhaps the increased secretion of $proBNP_{1–108}$ in stages A to C, and its efficient conversion, represents successful compensation to maintain a state of compensated HF. We would predict that this efficiency is impaired in advanced HF, as supported by studies by Dries et al. (11). The exact reason for this possible $proBNP_{1–108}$ processing deficiency is unknown, but there may be an enzymatic deficiency, whether due to saturation or constitutive dysfunction, that underlies the progression of HF. Such a deficiency may also represent a therapeutic target and opportunity. Use of either corin or a corin-like drug could have potential as a therapy to delay disease progression and warrants investigation, especially in experimental models of progressive HF. In addition to aberrant upstream processing, there may be downstream receptor insensitivity or decreased renal clearance, as other authors have speculated. These may be similar pathophysiological mechanisms to those that cause decreasing $BNP_{1–32}$ activation and processing in early stages of hypertension as well (43). It should also be noted, however, as indicated in the preceding text, that decreased overall $proBNP_{1–108}$ processing may serve to achieve high local levels of $BNP_{1–32}$ activity in areas with convertase activity while at the same time avoiding systemic $BNP_{1–32}$ activity, which could result, for example, in hypotension.

Conclusions

$ProBNP_{1–108}$ circulates in the majority of normal persons in the general population, supporting the conclusion that $proBNP_{1–108}$ is normally released from the heart and may serve a role as a carrier protein to deliver the biologically active peptide into the circulation or to target tissues for processing. Importantly, age and sex were the major factors influencing $proBNP_{1–108}$ in the general population. In addition, $proBNP_{1–108}$ was a sensitive and specific biomarker for the detection of systolic dysfunction and less so for diastolic dysfunction equal to that of $BNP_{1–32}$, but in general, less so than NT-$proBNP_{1–76}$. Importantly, the ratio of $proBNP_{1–108}$/NT-$proBNP_{1–76}$ provides insight into possible changes in $proBNP_{1–108}$ processing during the progression of HF. Thus, this highly specific assay for $proBNP_{1–108}$ provides important new insights into the biology of the BNP system as well as potential diagnostic applications for this important new technology.

Acknowledgments

The authors thank Ivan Nenadic, Matthew Mrazek, Wael Salem, Rishi Wadhera, Dr. J. Michael Bostwick, Dr. Eddie Greene, David Hodge, and Dr. Tony Windebank for their most helpful and thoughtful discussions of this work.

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Key Words: biomarker ■ BNP ■ heart failure ■ natriuretic peptide ■ NT-proBNP ■ proBNP.

APPENDIX

For supplementary Tables 1 through 4, please see the online verison of this article.