Only Large Reductions in Concentrations of Natriuretic Peptides (BNP and NT-proBNP) Are Associated with Improved Outcome in Ambulatory Patients with Chronic Heart Failure

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BACKGROUND: Concentrations of B-type natriuretic peptides (BNPs), including N-terminal probrain natriuretic peptide (NT-proBNP), have been reported to permit the estimation of prognosis in patients with chronic heart failure (HF) (1–6). In many patients with advanced and/or acute decompensated HF, BNP concentrations are markedly increased. BNP concentrations lower than anticipated have also been reported in patients with end-stage HF, however, and these values have been associated with high short-term mortality (7). Large biologic variability (8–11) and molecular heterogeneity (12, 13) may partially explain why, despite robust predictive value, it is difficult to evaluate the impact of increased BNP and NT-proBNP measurements in individual patients. These issues also influence how best to interpret changes in natriuretic peptide concentrations to predict outcomes. For example, studies may show statistically significant changes in BNP and NT-proBNP concentrations, but these changes may not exceed biologic variability, making it difficult to determine the importance of such changes. In some studies, it is argued that these changes are of importance; others say it is less clear (14).

To determine the degree of change necessary to influence prognosis, we assessed BNP and NT-proBNP concentrations in ambulatory HF patients followed up for 2 years. The magnitude and pattern of BNP and NT-proBNP changes were related to the occurrence of (>80%) reduced risk. These data suggest that only robust decreases in natriuretic peptide concentrations should be targeted to reduce mortality and heart failure–related hospitalizations.

METHODS: We studied 172 New York Heart Association class III–IV outpatients. Primary endpoints were death/transplantation or heart failure hospitalization. The magnitude of peptide changes was categorized as no change (<20% increase or decrease from enrollment), ≥20% to ≤80% increase or decrease; and >80% increase or decrease. Changes were also assessed using cutoffs (500 ng/L for BNP and 1000 ng/L for NT-proBNP).

RESULTS: Fifty-two patients died or received transplants during the course of the study. Risk reduction for heart failure hospitalization was demonstrated only for BNP decreases of >80% from enrollment [hazard ratio (HR) 0.318, P = 0.0315]. BNP increases from less than to more than the prespecified cutoff of 500 ng/L were associated with increased mortality risk (HR 2.101, P = 0.0069), whereas decreases from more than to less than the cutoffpoint did not reduce risk. NT-proBNP decreases from more than to less than the cutpoint of 1000 ng/L were associated with reduced risk of death/transplantation (HR 0.119, P = 0.0354).

CONCLUSIONS: BNP increases from less than to more than the cutoffpoint were associated with increased risk of events, whereas further increases did not add to risk. In contrast, only substantial natriuretic peptide decreases
adverse patient outcomes (death, cardiac transplantation, or HF hospitalization) vs event-free follow-up. Our hypothesis was that increases in BNP and NT-proBNP concentrations would presage deleterious events and that reductions would be indicative of a better prognosis. In addition, we aimed to assess what magnitude of changes would be clinically important in predicting these events.

Materials and Methods

Patients and Study Design
We prospectively enrolled a cohort of 200 New York Heart Association class III and IV HF patients during the period of June 2001 to January 2004 to evaluate the use of BNP and NT-proBNP in the follow-up of patients with chronic HF. The overall results of this study have been reported, including the prognostic significance of baseline natriuretic peptide (NP) concentrations (3). We recruited patients from the Mayo outpatient HF Clinic after initial evaluation and establishment of routine follow-up. Some patients were initially identified while in hospital but were not enrolled until they were stabilized as outpatients. We obtained informed consent from each patient after a primary medical evaluation determined their clinical status. Patients were followed with visits scheduled at 3-month intervals (± 3 weeks) for a total of 24 months. Patients were excluded if cardiac revascularization was anticipated within 6 months, if they were already listed for and awaiting cardiac transplantation, or if they had experienced an episode of acute decompensated HF requiring hospitalization within 30 days of anticipated enrollment. The study was approved by the Mayo Foundation Institutional Review Board and included only those patients who provided written informed consent for clinical research analysis as required by Minnesota Statute 144.335/CFR 21 (part 50).

Study Protocol
Blood samples for BNP and NT-proBNP were drawn at study entry (baseline) and every 3 months for 2 years. Samples were collected in EDTA and immediately stored on ice, processed, and subsequently stored at −70°C until batch analysis for BNP was performed during the course of the study (maximum of every 3–4 weeks). At the end of the study, samples were again thawed, and we measured all values for NT-proBNP using the Dade RXL NT-proBNP assay. Clinicians and investigators were blinded to biomarker results. We also obtained blood samples for plasma electrolytes, serum creatinine, and hemoglobin as clinically indicated. Left ventricular ejection fraction (LVEF) was derived from 2-dimensional echocardiography performed within 3 months of study enrollment and subsequently during routine clinical follow-up as determined by the patient’s HF clinic provider. Patients were seen in the Mayo HF Clinic for routine clinical follow-up as well as for study visits, and every effort was made to coordinate these activities. In addition to blood samples obtained for the measurement of biomarkers, we obtained an updated patient history, determined interim clinical status (such as hospitalizations), and performed a physical examination at each follow-up visit. Changes in the patients’ medical regimens (the responsibility of the primary HF cardiologist and not the study physician or study nurse coordinator) were recorded. Survival was evaluated in follow-up for each member of the cohort; none were lost to follow-up. Mean follow-up duration (SD) was 18.5 (7.7) months (range 3–26.4). We reviewed the records from all hospitalizations. The diagnosis of a hospitalization for decompensated heart failure required the administration of intravenous diuretics, positive inotropes, and/or vasodilators.

Biomarker Measurements
We measured BNP (32 amino acid residues, 77–108) using the Shionoria RIA method. Circulating concentrations of BNP in our institution are reported as age and sex dependent (15), with specific reference intervals (5th to 95th percentiles) for the assay being: age 55–64, male 7–146 and female 9–192 ng/L; age 65–74, male 8–177 and female 11–233 ng/L; and age 75–83, male 10–216 and female 13–284 ng/L. Inter- and intraassay CVs for this assay were both 8% and consistent across all concentrations of BNP. We measured NT-proBNP (amino acid residues 1–76) using the Roche sandwich ELISA method on the Dade RXL Analyzer (Dade-Behring), lower limit of detection 10 ng/L. Circulating concentrations of NT-proBNP are also reported at our institution with age- and sex-dependent reference intervals (16) as validated by mean (median, SD) concentrations observed in healthy individuals in the following age groups: age 55–64, male 37.9 ng/L (13.4, 41.6) and female 39.9 ng/L (18.8, 51.6); age 65–74, male 76.0 ng/L (median 76) and female 84.1 ng/L (59.5, 58.9); age ≥75, male 414.3 ng/L (125.6, 889.0) and female 291 ng/L (131.3, 639.7). Inter- and intraassay CVs were between 8% and 10% for all values between 300 and 1500 ng/L. Increased BNP and NT-proBNP were defined as concentrations exceeding the 95th percentile cutpoints defined for each age and sex group. Renal function was determined at study enrollment (baseline) and at subsequent visits every 3 months by calculation of estimated glomerular filtration rate (GFR, ml/min/1.73m²) using the MDRD (Modification of Diet in Renal Disease) equation (17).
Reduction in BNP/NT-proBNP Related to Improved Outcomes

**STATISTICAL ANALYSIS**

Analyses were done using SAS version 9 and S-plus version 7 (18). Data for continuous variables are reported as mean (SD) and median with 25% and 75% percentiles. Categorical variables are reported as frequencies and proportions. We used 1-way ANOVA to test for statistically significant differences between the BNP and NT-pro BNP categories for continuous baseline data and χ² test to test differences in proportions for the categorical data. Patients were assigned to the categories for BNP and NT-pro BNP by calculating the change from baseline to their last available measurement before an event or last clinical follow-up visit. The goal was to compare the relationship over time between these endpoints and the change in NP values before the primary endpoint event. BNP and NT-proBNP concentrations are influenced by age and sex, and for comparative purposes, we categorized peptide concentrations as either increased or not increased. We defined an increased BNP and NT-proBNP as any value greater than the 95th percentile of normal, adjusted for age and sex, referenced for the assay used (15, 16, 19, 20). Patients were considered at risk for cardiac events from the time of enrollment in the study through the last follow-up visit. The magnitudes of BNP and NT-proBNP changes were prospectively categorized as follows: no change (<20% increase or decrease from enrollment value); ≈20 to ≈80% increase or decrease in concentration relative to enrollment; or >80% increase or decrease from enrollment concentration. These categories reflect the range of analyte changes typically reported in patients with heart failure (19–21), and the larger changes (>80%) are suggested from reference change value (RCV) analyses (8–10). Changes from baseline to endpoint events using pre-specified cutpoint values chosen to be above the “gray zone” (borderline) elevation of these analytes [500 ng/L for BNP (22) and 1000 ng/L for NT-proBNP (23)] were also assessed for association with endpoints. The study endpoints were the time until death/cardiac transplantation, first HF-related hospitalization, and the composite of these events. Analyses are based on the time to the first event. We used the Cochran–Armitage trend test to compare the classifications for the endpoints. The unadjusted Cox proportional hazard model used the no change group as the reference group, and results are presented as hazard ratios (HRs) with corresponding 95% CIs and P values. To be included in this analysis, an individual has to have been followed through at least 1 visit after the baseline/enrollment visit. Because our interest was in the magnitude of change necessary to presage clinical events, clinical variables were not used as covariables.

**Results**

The clinical and demographic characteristics of the patients enrolled in this study as a function of category of BNP and NT-proBNP changes, respectively, are presented in Supplemental Tables 1 and 2 (available with the online version of this article at www.clinchem.org/content/vol55/issue1). Of the 200 patients enrolled, 10 were missing baseline peptide values; 18 had enrollment BNP values but no follow-up measurements; and 20 had enrollment NT-proBNP values but no follow-up measurements. Therefore, results are reported on 172 participants with complete BNP data and 170 patients with complete NT-proBNP data. Overall, the BNP concentration was 397 (396) (median 302) ng/L and the NT-proBNP concentration was 5733 (9128) (median 3042) ng/L at study enrollment. Average duration of HF at study enrollment was 42.2 (45.2) months, median 31 months. An unexpected finding was that patients with the largest increase in BNP and NT-proBNP concentrations (>80% increase) on average had the highest LVEF and lowest peptide concentrations at study enrollment among the categories of biomarker change. Paradoxically, this relationship was also associated with highest risk. There was a gradient of risk for mortality and hospitalization across all categories of change. Both of the clinical endpoints were statistically significant for BNP, but only the HF hospitalization endpoint was statistically significant for NT-proBNP.

Fifty-two patients (30%) died or underwent cardiac transplantation during the course of study. Seventy-two patients (42%) were hospitalized 1 or more times. Mortality was 44% in the hospitalized patient group and 20% in the nonhospitalized patients. Based on age and sex, proBNP concentrations were increased (>95th percentile for normal population adjusted for age and sex) at enrollment in 110 (64%) and 152 (89.4%) patients, respectively. Another 32 patients developed new elevations of BNP and NT-proBNP concentrations at study enrollment among the categories of biomarker change. Thus, overall, 83% (142 of 172) had an elevation in BNP and 98% (166 of 170) had an elevation of NT-proBNP at enrollment or during the study period.

The HRs for the risks of death/cardiac transplantation, first HF hospitalization, and the combined risk for death, transplant, and HF hospitalization for the categories of change in BNP and NT-proBNP are shown in Fig. 1. There was a trend of increasing HR for both mortality and hospitalizations from a BNP decrease >80% to a BNP increase >80% for the 3 risk categories (Fig. 1A). However, only BNP decreases >80% were statistically significant for risk reduction of HF-related first hospitalization (HR 0.318, P = 0.0315); a BNP decrease of >80% was of borderline
significance for risk reduction of mortality/transplant (HR 0.393, \(P = 0.0880\)) and remained significant for the combined endpoint (HR 0.370, \(P = 0.0221\)). When the models were analyzed by linear regression comparing >80% decrease in BNP to the other categories of BNP changes, a significant benefit was demonstrated for reduced hospitalization (\(P = 0.0019\)) and the combined endpoint of hospitalization/mortality/transplant (\(P = 0.0020\)), whereas the benefit for the mortality/transplant combined endpoint was of borderline significance (\(P = 0.0760\)). NT-proBNP changes (Fig. 1B) showed a similar trend, with decreases in NT-proBNP being more predictive of a favorable outcome than any other category of change in NT-proBNP level. There was more variability than with BNP, however, and only the HR for HF-related hospitalization associated with...
≥20% to ≤80% decreases in NT-proBNP achieved statistical significance (P = 0.0435).

We evaluated the association of survival and hospitalizations with overall BNP and NT-proBNP changes. Survivors showed more change (increases and decreases ≥20%) in BNP than nonsurvivors [103 of 120 (86%) in survivors vs 32 of 52 (62%) in nonsurvivors] but not in NT-proBNP [92 of 119 (77%) vs 41 of 51 (80%)]. Increases in BNP >80% were more frequent than decreases >80% [42 of 69 (61%) vs 28 of 76 (37%)]; again, most of these changes occurred in survivors (60%–80%). Very large increases (>150%) occurred in 31 of 69 (45%) patients; 11 of 31 were nonsurvivors. During the course of this study, 100 patients avoided hospital admission, whereas 72 patients experienced 1 or more HF hospitalizations. Decreases in BNP >80% were associated with better hospital-free survival (23 of 28, 82%) than lesser (20%–80%) BNP decreases (30 of 48, 63%) or any BNP increases ≥20% (32 of 69, 46%). Similar trends were not noted for NT-proBNP change categories. Decreases in NT-proBNP >80% were not associated with a better hospital-free survival (10 of 17, 59%) compared with lesser decreases (36 of 46, 78%) in NT-proBNP, and any increase in NT-proBNP (33 of 70, 47%) showed a rate similar to that of BNP increases (46%). In the cohort of patients hospitalized during the course of this study, 51% had BNP increases ≥20%, whereas the nonhospitalized group had only 27% of patients with BNP increases ≥20%. Similar findings were seen for NT-proBNP, with 53% and 33%, respectively, having NT-proBNP increases ≥20% in the hospitalized and nonhospitalized groups.

We also analyzed risk of death/cardiac transplantation, risk of HF hospitalization, and combined risk using prespecified cutpoints for BNP (500 ng/L) and NT-proBNP (1000 ng/L) for changes from baseline to last measurement. Fig. 1 also shows these results. For BNP, an increase from less than to more than the cutpoint was associated with an increased risk for combined endpoints (HR 2.101, P = 0.0069), whereas a reduction from more than to less than the cutpoint was not associated with any significant change (reduction) in risk. Changes to less than or more than the cutpoint for NT-proBNP were generally associated with no significant changes in risk, with the exception of a reduction in risk of death or cardiac transplantation associated with a decrease in NT-proBNP from more than to less than the cutpoint value (HR 0.119, P = 0.0354).

The overall relationship of NT-proBNP changes to BNP changes is shown in Table 1. This table demonstrates considerable overlap of peptide change within each category and high degree of variability, such that changes in BNP and NT-proBNP concentrations do not track closely enough to be mutually predictive of the same magnitude or direction of change over time.

**Discussion**

BNP and NT-proBNP concentrations are used extensively in the diagnosis and assessment of patients with acute and chronic heart failure. Few studies, however, report how large the changes in the values of these biomarkers need to be of clinical significance. Some would argue that changes must be above the biologic variability of the peptide values (8–11). Several studies suggest that changes in BNP concentrations defined by RCV need to be 80% or greater to exceed biologic and analytic variability, particularly in long-term (weeks to months) follow-up (8–10). This magnitude is uncommon, however, and thus rarely used for the purposes of analysis (19–21). Our data from a carefully followed outpatient cohort documents a continuum of risk associated with changes in NP concentrations that appear to have detectable impact on clinical events only when values go down substantially (80% or more). We cannot exclude the possibility that a much larger cohort would have shown significant differences with lower values of change, but our data do conform reasonably well with the reported magnitude.

| Table 1. Relation of BNP changes to NT-proBNP changes (n = 170). |
|-------------------|-------------------|-------------------|-------------------|
| BNP               | NT-proBNP         |                  |
|                   | >80% decrease    | ≥20% to ≤80%     | No change         | ≥20% to ≤80%     | >80% increase    |
| >80% decrease     | 13               | 9                | 3                | 1                | 2                |
| ≥20% to ≤80%      | 3                | 23               | 10               | 3                | 8                |
| No change         | 1                | 7                | 10               | 4                | 4                |
| ≥20% to ≤80%      | 0                | 2                | 11               | 8                | 6                |
| >80% increase     | 0                | 5                | 3                | 6                | 28               |
| All               | 17               | 46               | 37               | 22               | 48               | 170              |
of biologic variability. It is possible that changes in BNP and NT-proBNP may occur in the absence of therapeutic maneuvers due to biologic variability. If so, many of the reported changes that are within a range of 30%–50% (4, 6, 19–21) may be statistically significant but may also be within the biologic variability of these NPs. This issue has important implications for the care of patients and for clinical trials using NP concentrations to monitor and define adequacy of therapy.

The results of this study provide additional insight into the role of serial BNP and NT-proBNP measurements in the management of ambulatory patients with stable chronic heart failure. It would appear from these data that whereas increases in BNP and NT-proBNP, particularly increases >80% from baseline, suggest a trend for worsening outcomes, lesser increments are overall fairly neutral with regard to predicting mortality or HF-related hospitalization (HR analyses) unless initial comparative values are below the cutpoint value. Decreases in BNP, particularly large decreases >80% from baseline, however, do seem to be effective in predicting better survival and lack of hospital admission. Changes in NT-proBNP concentrations were more highly variable than BNP changes, and only moderate (and therefore somewhat indeterminate) decreases in NT-proBNP were associated with a reduced risk for HF-related hospitalization. It is unclear why NT-proBNP was not equivalent to BNP in this analysis. The reason may be related to stored samples being batch-analyzed for NT-proBNP, which may allow degradation of the samples despite storage at −70 °C and the known resistance of NT-proBNP to degradation over time (24).

Although overall BNP increases >80% above baseline were associated with a trend for increased risk of events, when cutoffs were used to assess relative risks associated with changes in BNP concentrations, changes from less than (at baseline) to more than the cutpoint of 500 ng/L at follow-up were associated with statistically significant increases in the risk for events (Fig. 1A). Changes from more than to less than the cutpoint, however, were not associated with reduced risk. This suggests that the starting concentration from which BNP changes occur influences the predictive and clinical meaning of the change. If BNP is relatively low at baseline and increases substantially above a cutpoint, in this instance 500 ng/L, then such a change contributes to an increase in risk. If, however, BNP at baseline is already substantially increased above the cutpoint, then further increases do not seem to further impact risk, and any decreases need also to be substantial (>80%) to have an impact on risk reduction. These observations would seem to be supported by the changes in NT-proBNP, where relatively substantial decreases to <1000 ng/L (mean baseline level >5000 ng/L) were associated with a reduced risk of death/transplantation and a trend for reduction in HF-related hospitalizations as well as the combined endpoint. These data are consistent with the concept that once BNP (and perhaps NT-proBNP) concentrations are substantially increased, further changes (moderate increases or decreases) have little additional impact on outcome (3). Only very robust decreases, which are not routinely observed clinically, are associated with improved outcomes.

It is of interest that the BNP and NT-proBNP groups with >80% increases demonstrated the highest LVEFs and lowest peptide concentrations at baseline among the categories of biomarker changes. This cohort also demonstrated the highest mortality and HF-related hospitalization rate. Patient characteristics or other measurements that might explain this observation were assessed, but none were apparent. Age, sex, duration of heart failure, renal function, etiology of heart failure, and medication regimen or change in medication regimen were not different among the BNP categories of change. For NT-proBNP, this was not the case and may explain some of the larger variability observed in the NT-proBNP measurements; statistically, however, there were no differences in NT-proBNP concentrations among the groups. These observations may also, in part, explain the variable results reported using BNP and NT-proBNP in guiding therapy, and also underscore our previously reported data demonstrating a limited of sensitivity of NP measurements for monitoring response to therapy (14). Changes in BNP and NT-proBNP concentrations in either magnitude or direction were also highly independent and did not demonstrate a great deal of overlap across categories of change (Table 1). This observed lack of association most likely reflects differences in half-life and mechanism of metabolism and clearance of these analytes, but may suggest that changes in one peptide cannot be extrapolated to changes in the other for purposes of clinical comparisons or comparisons of different trial findings.

**STUDY LIMITATIONS**

Twenty-eight patients of the initially enrolled 200 patients did not complete follow-up because of lack of clinical follow-up, biomarker values unavailable for technical reasons, or decision at various times during follow-up not to continue participation in the study. Therefore, not all patients had samples at all time points, and some did not complete the 2-year follow-up period. Because our study is hypothesis-generating, we did not correct for multiple comparisons and chose to report uncorrected P values. Confidence intervals should be noted; some subgroups are small in number and hence more variable. The consistency of
these data, however, argues that the principles put forth are not likely due to statistical artifact.

Conclusions

Our study findings suggest that both clinicians and clinical trial investigators should target robust decreases in BNP and NT-proBNP if they wish to reduce mortality and the need for hospitalization. More modest increases or decreases seem to confer little additional predictive value; however, the baseline NP concentration from which peptide changes are derived does seem to impact the predictive value of observed changes and may interact with the percent change. Serial changes in BNP or NT-proBNP concentrations in association with the clinical follow-up of chronic HF patients, therefore, need to be interpreted with great care to avoid conclusions of benefit or detriment when changes may be due to biological variability and/or the relative peptide concentration used for comparison.

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All authors contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data, (b) drafting or revising the article for intellectual content, and (c) final approval of the published article.

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References


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