hemodilution secondary to volume overload or, possibly, the increased co-morbidity in patients hospitalized for decompensated heart failure compared with those enrolled in chronic heart failure studies.

Our study identified a significant association between anemia on admission and worsened clinical outcomes. Published data from patients with chronic heart failure, patients referred for cardiac transplant, and an unselected community cohort of patients with heart failure all identified an association between anemia and adverse outcomes.[2,4,5] Our study extends these findings to patients at the time of hospitalization for heart failure.

Our study has several potential limitations. Anemia in heart failure may be in part caused by hemodilution,[9] and this may have been particularly present in patients hospitalized for volume overload. Despite this fact, we found anemia to be a predictor of adverse outcomes even after adjustment was made for noninvasive indicators of volume overload such as elevated jugular venous pressure, pulmonary rales, and peripheral edema.

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Biological Variation for N-Terminal Pro- and B-Type Natriuretic Peptides and Implications for Therapeutic Monitoring of Patients With Congestive Heart Failure

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Given the limitations of low enrollments, this study suggests that a change of 130% for B-type natiuretic peptide (BNP) and 90% for N-terminal (NT)-proBNP are necessary before results of serially collected data can be considered statistically different. This study also shows that there are important differences in the performance of BNP versus NT-proBNP in monitoring patients with congestive heart failure that need to be further explored.
(Am J Cardiol 2003;92:628–631)

B-type natriuretic peptide (BNP)[1] and N-terminal proBNP (NT-proBNP)[2] are plasma biomarkers used in patients with congestive heart failure that are approved for diagnostic purposes. Serial measurement of these peptides may also be useful in monitoring the success of drug therapy. To use these tests for monitoring, it is important to determine the intra- and interindividual biologic variation (BV) and what constitutes a statistically significant change in patient results (Δpt) in BNP and NT-proBNP concentrations (e.g., before and after drug treatment). BV was measured on 3 commercial BNP assays and 1 commercial NT-proBNP assay.

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Four groups of subjects were enrolled in this study. (1) Four blood samples each were collected from 12 apparently healthy subjects and used to calculate the BV for BNP and NT-proBNP. None had a history of heart disease or congestive heart failure. The protocol was reviewed and approved by the Hartford Hospital Institutional Review Board, and all signed a written informed consent. To minimize pre-analytic variations in the collection procedure, each sample was collected every other week at the same time and weekday by the same phlebotomist. (2) Blood from 36 other healthy subjects was tested for estimating the analytic coefficient of variance (CV_A). All had normal BNP and NT-proBNP concentrations. Remaining blood samples from routine investigations from these subjects were used, and no patient consent was deemed neces-

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TABLE 1 Biological Variation and Other Important Statistical Variables

Parameter	Biosite* BNP	Shionogi† BNP	Bayer‡ BNP	Roche‡ NT-pro BNP
Analytic variation, CV_A	8.6%	16.7%	1.8%	1.6%
Biologic variations				
Intraindividual variation, $CV_{I,norm}$	43.6%	58.6%	50.3%	33.3%
Interindividual variation, CV_G	39.4%	44.2%	27.9%	36.5%
Imprecision goals, $\leq 0.5\ CV_I$	21.8%	29.3%	25.1%	16.6%
Inaccuracy goals, $\leq 0.25(CV_I^2 + CV_G^2)^{1/2}$	29.4%	36.7%	28.8%	24.7%
Significant serial change $(\Delta pt)_{norm}$	123%	169%	139%	92%
Index of individuality (CV_I/CV_G)	1.1	1.3	1.8	0.9
$CV_{I,CHF}$	24.0%	NA	NA	NA
Significant serial change $(\Delta pt)_{CHF}$	77.0%	NA	NA	NA

*Point-of-care assay; †manual radioimmunoassay; ‡automated immunoassay.
NA = samples not available for testing.

TABLE 2 Differences in B-type Natriuretic Peptide (BNP) and N-terminal-Pro B-type Natriuretic Peptide (NT-Pro BNP) Concentrations Versus New York Heart Association Classification

	New York Heart Association Classification				
Assay (pg/ml)	I	II	III	IV	Reference
Biosite BNP	95	221*†	459†§	1,006§	9
Biosite BNP	83	235*†	459†§	1,119*†	10
Bayer BNP	211	365§	536§	940§	11
Shionoria BNP‡	49	239*	537§	914§	12
NT-proBNP	1,015	1,666§	3,029*	3,465§	13

*Relative to the previous New York Heart Association classification, this indicates a statistically significant difference ($p < 0.05$) using Δpt_{norm} from Table 1 for the respective assay.
†Significantly different ($p < 0.05$) using Δpt_{CHF} (Biosite only).
‡Presumed to be the Shionoria assay or prototype thereof.
§p = NS.

sary (no extra blood and all identifiers permanently removed). (3) Blood from 5 patients diagnosed with stable systolic congestive heart failure (New York Heart Association, NYHA class I to III) was collected every 2 hours for 24 hours. Separate approval and written consent was obtained. Congestive heart failure diagnosis was made based on physical examination, history, and echocardiographic data (left ventricular ejection fraction <35%). Subjects were excluded if they had hypothyroidism, diabetes mellitus, kidney, or liver disease. The patient's clinical status did not change during on the day of blood collections. (4) Thirty-seven serial blood samples leftover from routine laboratory studies were tested for 11 hospitalized patients with congestive heart failure, and the percent change from baseline concentration was calculated. Blood was typically collected on consecutive days while patients underwent treatment with diuretics, β blockers, and/or angiotensin-converting enzyme inhibitors. The number of occurrences where serial data points were significantly different from each other (using the Δpt from this study) was computed for each assay. This group was tested to assess the impact of BV on serial measurements for patients with heart failure. No patient consent was necessary for this group.

All blood was collected into plastic tubes containing ethylenediaminetetraacetic acid and centrifuged; the plasma was then aliquotted and stored at −70°C before analysis. Samples were tested under manufacturer's recommendations for BNP using the Triage point-of-care BNP test (Biosite Inc., San Diego, California), Shionoria radioimmunoassay BNP (Shionogi & Co., Ltd, Osaka, Japan), and Centaur analyzer (Bayer Diag., Tarrytown, New York); NT-proBNP was analyzed using the Elecsys 2010 analyzer (Roche Diagnostics, Indianapolis, Indiana). All assays were performed singlularly for samples from groups 1, 3, and 4, except for the Shionoria assay (duplicate), because the manufacturer recommended duplicate analysis. For group 2, all samples were assayed in duplicate, as recommended by Fraser and Harris,[3] for the determination of CV_A. For samples from group 3, only the Triage assay was used as blood was not available for the other assays.

The intra- (CV_I) and interindividual coefficients of variance (CV_G) were determined from group 1. Three subjects were excluded because they had BNP concentrations that were below the respective detection limits for the assays. The Reed and Cochran tests were used to find outliers from the total range of values, resulting in the removal of 1 subject at $p < .05$. The BV study was conducted on the remaining 8 subjects (3 men, 5 women, age range 21 to 45 years). One half of them reported regular aerobic exercise. Analysis of variance was determined from Excel (Microsoft, Seattle, Washington). The goals for analytic precision were calculated as $\leq 0.5\ CV_I$ and for inaccuracy as $\leq 0.25(CV_I^2 + CV_G^2)^{1/2}$. The percent change in serial results was determined as $2.77(CV_A^2 + CV_I)^{1/2}$. These calculations were derived from Fraser and Harris.[3]

The CV_A determined from group 2 was more superior for the 2 automated immunoassays than the point-of-care assay and the manual radioimmunoassay (Table 1). The $CV_{I,norm}$ and CV_G for BNP and NT-

proBNP (range 27.9% to 58.6%, Table 1) were higher than other chemistry analytes (e.g., sodium at 0.7% to 1.0%)[4] and other cardiac markers (e.g., myoglobin at 11% to 14%).[5] As such, the analytic goals for imprecision and inaccuracy were attained by these BNP and NT-proBNP tests. Given the high BV for BNP and NT-proBNP, high assay precision may be unnecessary. The relatively high index of variability (Table 1) indicated that population-based reference intervals were appropriate for BNP and NT-proBNP. Markers with a high index were more useful for diagnostic purposes when approval had been rendered than for monitoring[6] (where these indications are pending).

Of particular importance to clinical cardiology is the calculation of the significant serial change of patient results (Δpt_{norm}) of 123% to 169% for BNP and 92% for NT-proBNP. The lower value for NT-proBNP suggested that day-to-day concentrations were more consistent. These differences are likely to related to the differences in the pathophysiology of the peptide release and clearance rather than test analytic performance. The biologic half-life for BNP is 20 versus 70 minutes for NT-proBNP (observed in sheep),[7] and may be the result of differences in the clearance rate of these peptides from blood. The NT-proBNP has more of an "averaging" effect, whereas BNP is more sensitive to acute changes in the disease processes.

The $CV_{I,norm}$ and Δpt_{norm} for healthy subjects for the Biosite BNP assay over an 8-week period exceeded the corresponding values for the 5 patients with congestive heart failure (group 3) over 24 hours (Table 1). The differences in the frequency of blood collections may be responsible for the lower $CV_{I,CHF}$ and Δpt_{CHF} values for patients with congestive heart failure. If BNP and NT-proBNP are to be used for monitoring the immediate or daily effect of drug therapy (e.g., inpatients), the Δpt_{CHF} value may be appropriate. However, to measure long-term successful treatment of congestive heart failure (e.g., weeks as for outpatients), the Δpt_{norm} may be more appropriate.

The difference in the BV among healthy subjects versus those with congestive heart failure was observed after controlled exercise. McNairy et al[8] showed that the change in BNP concentration before and after a stationary bicycle protocol was higher in healthy controls (55%) than in patients with congestive heart failure (30% in New York Heart Association classes I to II and 18% in classes III to IV). However, in both cases, results were not statistically significant because they were less than Δpt_{norm} and Δpt_{CHF}, as obtained in this study.

Table 2 shows the median BNP and NT-proBNP concentrations as a function of New York Heart Association classification from published reports and manufacturer's package inserts.[9–13] Using analysis of variance, the differences in BNP and NT-proBNP concentrations among the groups were significant. However, when applying the values for Δpt_{norm} and Δpt_{CHF}, many of these differences were not significant. This observation was likely caused by the subjective nature of the New York Heart Association classification system, rather than the inability of these objective assays to detect differences between classes.

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The values for Δpt_{norm} and Δpt_{CHF} can be used to interpret data for the use of BNP and NT-proBNP in congestive heart failure clinical trials. In a study of tissue necrosis factor-α (etanercept), initial data suggested that BNP concentrations were associated with improved clinical status.[14] However, a closer examination of the data showed that the change in BNP values were not different, which was consistent with findings of no efficacy of etanercept treatment.[15] In a study of carvedilol, BNP concentrations using a radioimmunoassay decreased from 127 to 69 pg/ml.[16] In another study involving angiotensin-converting enzyme inhibitors, a BNP-guided protocol resulted in a BNP decrease of 42.1%.[17] These differences, however, did not exceed the Δpt_{norm} for either study.

In our pilot study of 11 subjects with congestive heart failure (group 4), the significant changes were observed in 2 (Shionogi) and 3 pairs of serial samples (Bayer, Biosite) for BNP using the Δpt_{norm} value as the discriminatory value. In contrast, there were 8 pairs of samples that were different using NT-proBNP. The lower CV_I for NT-proBNP may enable a smaller difference to be significant. BNP is the biologically active hormone that is tightly regulated by precise endocrine control. NT-proBNP is an inactive metabolite of proBNP, the blood concentrations of which do not influence the rate of myocardial hormonal secretion. Therefore, the higher frequency of significantly different results between serial samples from the same patients for NT-proBNP relative to BNP may be unrelated to therapeutic success or failure. Studies using BNP and NT-proBNP on the same patient populations will be necessary to determine which marker is best for therapeutic monitoring.

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Economic Implications of *Nesiritide* Versus *Dobutamine* in the Treatment of Patients With Acutely Decompensated Congestive Heart Failure

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Pooled data from trials comparing nesiritide with dobutamine for treatment of acute decompensated congestive heart failure were combined with national hospital cost data in an economic model. Results indicate that the acquisition cost of nesiritide is fully offset by decreased hospital costs.

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Although nesiritide improves patient outcomes relative to dobutamine,[1] the drug is more expensive, and its impact on overall cost of care and cost effectiveness is uncertain. Using data from 2 recent clinical trials (Comparative and Prospective Randomized Evaluation of Cardiac Ectopy With Dobutamine or Nesiritide Therapy [PRECEDENT] trials), we modeled clinical and economic outcomes of nesiritide versus dobutamine for patients emergently hospitalized with symptomatic decompensated heart failure (HF)

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Designed primarily to gather safety and clinical experience, the Comparative study enrolled 305 patients at 46 clinical sites during early 1997.[2] Patients were randomly assigned to either standard care (n = 101) or 1 of 2 doses of nesiritide: 0.015 μg/kg/min (n = 102) or 0.030 μg/kg/min (n = 102) with investigators blinded as to nesiritide dosage. Choice of standard care agent and dosage was left to the discretion of the investigators and therefore unblinded; dobutamine was selected in 57% of cases (n = 58).

The PRECEDENT study compared the effects of nesiritide with those of dobutamine on ventricular arrhythmias and heart rate while accumulating safety and clinical experience.[3] In late 1998, 255 subjects were enrolled at 46 clinical sites and randomly assigned to 1 of 3 regimens on an open-label basis: nesiritide 0.015 μg/kg/min (n = 85), nesiritide 0.030 μg/kg/min (n = 84), or dobutamine (n = 86). All patients underwent Holter monitoring during the 24 hours before initiation of the study drug and throughout the first 24 hours of treatment.

Cost of medical care is ideally determined by collecting billing data or by tabulating resource utilization during treatment and valuing each resource according to a standard unit price. Because neither of the clinical trials documented charges or resource utilization, we used the Monte Carlo simulation to estimate treatment cost and survival in hypothetical cohorts of 1,000 patients treated with nesiritide or dobutamine. The model was programmed in Microsoft Excel (Microsoft, Redmond, Washington). Clinical parameters were derived using pooled data from the Comparative and PRECEDENT studies for patients treated with dobutamine and those who received nesiritide 0.015 μg/kg/min. Patients receiving nesiritide 0.030 μg/kg/min were excluded because this dose was not used in subsequent nesiritide trials.

From an exhaustive list of side effects and adverse events among patients enrolled in the trials, we identified events during the initial hospital admission that were both clinically significant and likely to generate consumption of additional medical resources. For example, we included symptomatic hypotension but excluded asymptomatic hypotension because the latter would not be expected to result in additional treatments or medical procedures. These event rates were used as model parameters to predict clinical course

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