Prognostic Value of Plasma N-Terminal Pro-Brain Natriuretic Peptide in Patients With Severe Sepsis

Martina Brueckmann, MD*; Guenter Huhle, MD*; Siegfried Lang, PhD; Karl K. Haase, MD; Thomas Bertsch, MD; Christel Weiß, PhD; Jens J. Kaden, MD; Christian Putensen, MD; Martin Borggrefe, MD; Ursula Hoffmann, MD

Background—Increased plasma levels of atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) have been identified as predictors of cardiac dysfunction and prognosis in congestive heart failure and ischemic heart disease. In severe sepsis patients, however, no information is available yet about the prognostic value of natriuretic peptides. Therefore, the aim of the present study was to determine the role of the N-terminal prohormone forms of ANP (NT-proANP) and BNP (NT-proBNP) in the context of outcome of septic patients. Furthermore, the effect of treatment with recombinant human activated protein C [drotrecogin alfa (activated)] on plasma levels of natriuretic peptides in severe sepsis was evaluated.

Methods and Results—Fifty-seven patients with severe sepsis were included. Levels of NT-proANP and NT-proBNP were measured on the second day of sepsis by ELISA. Septic patients with NT-proBNP levels >1400 pmol/L were 3.9 times more likely (relative risk [RR], 3.9; 95% CI, 1.6 to 9.7) to die from sepsis than patients with lower NT-proBNP values ($P < 0.01$). NT-proANP levels, however, were not predictive of survival in our patient population. A highly significant correlation was found between troponin I levels and plasma concentrations of NT-proBNP in septic patients ($r = 0.68$, $P < 0.0001$). In addition, troponin I significantly accounted for the variation in NT-proBNP levels ($P < 0.0001$), suggesting an important role for NT-proBNP in the context of cardiac injury and dysfunction in septic patients. Twenty-three septic patients who received treatment with drotrecogin alfa (activated) presented with significantly lower concentrations of NT-proANP, NT-proBNP, and troponin I compared with patients not receiving drotrecogin alfa (activated).

Conclusions—NT-proBNP may serve as useful laboratory marker to predict survival in patients presenting with severe sepsis. (*Circulation*. 2005;112:527-534.)

Key Words: coagulation ■ infection ■ natriuretic peptides ■ sepsis

Natriuretic peptides play an important role in the regulation of cardiovascular homeostasis and fluid volume. They promote natriuresis and diuresis, act as vasodilators, and exert antimitogenic effects on cardiovascular tissues.1,2 Increased plasma levels of natriuretic peptide hormones have been identified as predictors of cardiac dysfunction and death in many critical care settings, including congestive heart failure, myocardial infarction, and septic shock.3–7 Two members of the natriuretic family, atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP), are secreted by the heart mainly in response to myocardial stretch induced by volume load; ANP is stored as a 126–amino acid prohormone (proANP$^{1-126}$) within granules of atrial cardiomyocytes.8 In response to atrial wall stretch, pro-ANP$^{1-126}$ is cleaved on secretion to yield 2 fragments in equimolar amounts, the biologically active C-terminal peptide (ANP$^{99-126}$) and the residual N-terminal peptide (NT-proANP$^{1-98}$).9 Left ventricular stretch and elevations of end-diastolic pressure and volume regulate the release of BNP from the cardiac ventricle. On secretion, the precursor molecule proBNP$^{1-108}$ is split into NT-proBNP$^{1-76}$ and the physiologically active C-terminal peptide comprising 32 amino acids (BNP$^{77-108}$).

Patients with septic shock show reversible left ventricular systolic dysfunction commonly masked by a concomitant elevation in the cardiac index.10 Drotrecogin alfa (activated) (recombinant human activated protein C; Xigris), which is indicated for the treatment of sepsis-induced multorgan failure, has demonstrated an improvement in cardiovascular...
organ function and a reduction in mortality within the phase III Protein C Worldwide Evaluation in Severe Sepsis (PROWESS; n = 1690) trial.11–13 The mechanisms contributing to improved cardiovascular function and increased survival may result from the antithrombotic, profibrinolytic, and antiinflammatory properties of the compound.

The cardiac-specific contractile protein troponin I (TNI) has been suggested as a sensitive marker of minor myocardial cell injury in systemic inflammation, sepsis, or septic shock.14,15 Whereas TNI has been examined well in the context of severe sepsis, little is known at present about the utility of natriuretic peptides for reflecting left ventricular dysfunction and predicting survival in septic patients.

Thus, the aim of the present study was to determine the role of NT-proANP and NT-proBNP as potential markers of outcome in septic patients receiving different treatment modalities.

Methods

Study Patients

A total of 57 patients presenting with severe sepsis, defined according to the criteria of the American College of Chest Physicians/Society of Critical Care Medicine (ACCP/SCCM) consensus conference,16 were selected for this study. Patients with preexisting reduction of left ventricular function, dilatative cardiomyopathy, acute or chronic cor pulmonale, valve disease, chronic renal failure, or acute coronary ischemia were excluded from the study. Patients presented with a proven infection, 3 criteria of the systemic inflammatory response syndrome, and 1 of the following newly developed, sepsis-induced organ failures: cardiovascular organ failure with need for vasopressors, pulmonary organ failure defined as PaO2/FiO2 < 250, renal organ failure with urine output ≤ 0.5 mL · kg⁻¹ · h⁻¹, hematological organ failure with platelet count < 80 000/μL, or an unexplained metabolic acidosis with pH < 7.30 and lactate levels > 1.5 times of the upper limit of normal. Sepsis-induced organ failures in these patients were strongly connected to infection and were present for < 24 hours. The study was performed at the following 3 sites in Germany: (1) Department of Anesthesiology, University Hospital Bonn (n = 23 patients), (2) Department of Cardiovascular Anesthesiology, University Hospital Bonn (n = 24 patients), and (3) Department of Internal Medicine, University Hospital Mannheim (n = 10 patients). Septic patients were included from March 2001 to August 2002, before Xigris (drotrecogin alfa (activated); Eli Lilly) was legally approved in Europe (August 2002). Twenty-three septic patients at the Department of Anesthesiology, University Hospital Bonn received a 96-hour infusion of drotrecogin alfa (activated) (Xigris; 24 μg per 1 kg body weight per hour) as part of the phase III Extended Evaluation of Recombinant Human Activated Protein C (ENHANCE; Eli Lilly) clinical trial. Thirty-four patients with severe sepsis (control subjects) did not receive drotrecogin alfa (activated) either because study site 2 did not participate in the ENHANCE trial or because of contraindications for drotrecogin alfa (activated) such as a platelet count < 30 000/μm³, need for therapeutic anticoagulation with heparin, increased risk of bleeding, recent gastrointestinal bleeding, or stroke within the last 3 months. The study protocol of the universities of Bonn and Mannheim defined the measurements of natriuretic peptides within ENHANCE study patients and was approved by the local ethics committees. Severity of disease was quantified by the Acute Physiology and Chronic Health Evaluation II (APACHE II) score.57 All patients were followed up for 28 days. This prospective, multicenter, nonrandomized study complies with the Declaration of Helsinki. Written informed consent of patients or their legal representatives was obtained before the investigation.

Blood Sample Collection and Determination of NT-proANP, NT-proBNP, and TNI Levels

Blood samples for the measurement of NT-proANP, NT-proBNP, and TNI were obtained by venipuncture on day 2 of severe sepsis. Citrate plasma was used to determine NT-proANP and NT-proBNP. TNI was determined in serum. Within 30 minutes, blood samples were centrifuged at 2700 g at 4°C for 10 minutes. Serum or plasma was separated, shock-frozen, and stored at −80°C. NT-proANP and NT-proBNP were measured by an enzyme immunoassay (Biosol). The upper limit of normal for apparently healthy persons (95th percentile) is < 1945 pmol/L for NT-proANP and < 250 pmol/L for NT-proBNP in these assays. TNI was measured by a heterogeneous colorimetric immunoassay on the Dimension clinical chemistry system (Dade-Behring). The limit for detection of minor myocardial damage in this assay is 0.1 ng/mL.

Measurement of Cardiovascular Functional Parameters

Cardiac catheterization in septic patients was performed with a pulmonary artery catheter inserted via the jugular vein. Simultaneously, right atrial pressure was measured. Cardiac output was determined by thermodilution technique in triplicate. Arterial pressure was measured by catheters inserted into the radial or femoral artery. Cardiac index, mean arterial pressure (MAP), and systemic vascular resistance were calculated with standard formulas.18 Left ventricular stroke work index (LVSWI) was calculated as MAP times stroke volume index, which is defined as follows: cardiac index divided by heart rate times 0.0136 (constant).18 In addition to invasive measurements by cardiac catheterization, 29 patients received echocardiographic evaluation of left ventricular function on day 2. According to Simpson’s method of estimation of ejection fraction, the left ventricular function was divided into the 3 groups: normal or slightly reduced (ejection fraction ≥ 50%), moderately reduced (ejection fraction between < 50% and ≥ 35%), and severely reduced (ejection fraction < 35%).

Statistical Analysis

For normally distributed data, the Student t test was used. The Mann-Whitney U test was used as nonparametric test in the case of nonnormally distributed data. Deviations from a Gaussian distribution were tested by the Kolmogorov-Smirnov test. NT-proBNP data were log transformed, thereby promoting normality, and the unpaired t test was applied. Spearman’s rank correlation for nonparametric data was used to test the association of NT-proANP and NT-proBNP plasma concentrations with cardiovascular functional parameters. Noncontinuous variables were analyzed by use of a 2 × 2 table and Fisher’s exact test. Data are presented as mean ± SD or SEM or as median and interquartile ranges (25th to 75th percentiles), depending on the distribution of the data. Values of P < 0.05 (2 tailed) were considered statistically significant. Cutoff values were determined by receiver-operating characteristic (ROC) curve analysis. Cumulative survival rates in sepsis patients according to the cutoff value of NT-proBNP are presented by Kaplan-Meier diagram, and differences among groups were tested by the log-rank test. To evaluate potential confounding factors on NT-proBNP level variation, multiple regression analysis with backward elimination was performed with NT-proBNP as the dependent variable and renal function, TNI, drotrecogin alfa (activated), NT-proANP, age, and gender as independent variables. The independent contribution to mortality by parameters possibly influencing the outcome was tested by Cox proportional-hazards regression using time to death as the dependent variable and natriuretic peptides, age, gender, TNI, creatinine, and drotrecogin alfa (activated) as independent variables. The calculations were performed with InStat (GraphPad Software), SPSS software (SPSS Software GmbH), and SAS version 8.02 (SAS Institute Inc).

Results

Patient Characteristics

Demographic and laboratory data of septic patients (n = 57) at the onset of severe sepsis (day 0) are given in Table 1. There
were no significant differences in baseline characteristics between patients receiving drotrecogin alfa (activated) and patients not receiving this specific sepsis treatment. Variables presented in Table 1 were comparable across the 3 sites as tested by ANOVA (data not shown). Seventy-nine percent of the patients (n = 45) needed vasopressors at the onset of severe sepsis. Sinus rhythm at baseline was present in 91% (n = 52). Five patients presented with atrial flutter at baseline. Central venous pressure, creatinine, C-reactive protein (CRP), and white blood cell count were elevated in septic patients compared with values usually found in healthy control subjects.18,20 Multiple regression analysis revealed that comorbidities such as arterial hypertension with myocardial hypertrophy (n = 2 patients), coronary artery disease (n = 9 patients), and chronic obstructive pulmonary disease (n = 6 patients), as well as age and gender, did not account for NT-proBNP variation in our patient population (P > 0.05).

Cardiovascular Functional Parameters
Cardiovascular functional parameters are given in Table 2. Septic patients (n = 57) presented with tachycardia, hypotensive blood pressure values, and lowered systemic vascular resistance on the day of diagnosis of severe sepsis (day 0). Cardiac index showed a tendency toward higher values in septic shock compared with normal values of healthy persons, whereas LVSWI in septic patients was considerably lower than normal (data of healthy subjects not shown; for review, see elsewhere18,20). Echocardiographic data performed on 29 patients on day 2 revealed that systolic left ventricular function was normal or slightly reduced (defined as an ejection fraction ≥50%) in 19 patients (65%), moderately reduced (ejection fraction between <50% and ≥35%) in 6 (21%), and severely reduced (ejection fraction <35%) in 4 patients (14%) at the onset of severe sepsis. Left ventricular and atrial diameters were normal in 26 patients (90%). Patients who did not need any vasopressors on day 0 (n = 12) were 1.6 times more likely to survive than patients who were on vasopressors (n = 45) at the onset of sepsis (relative risk [RR], 1.6; 95% CI, 1.2 to 1.9; P = 0.01). Among patients with vasopressors at baseline (n = 45), 8 patients showed resolution of cardiovascular organ failure within the first 2 treatment days of severe sepsis. MAP showed a slight but not significant increase between days 0 and 2 in survivors. Survivors of sepsis presented with slightly higher MAP values on day 2 compared with nonsurvivors (83 ± 16 versus 75 ± 13 mm Hg). However, this difference was not quite significant (P = 0.08). Cardiac index and LVSWI were comparable on days 0 and 2 of severe sepsis. Of the 57 patients, 16 died within 28 days, resulting in a mortality rate of 28%.

Levels of Natriuretic Peptides and TNI in Septic Patients
For the assay used in this study (enzyme immunoassay from Biozol), the upper limits of normal for apparently healthy men were 109/L, and the limits of normal for apparently healthy women were 109/L. Absolute values were not significantly different between survivors and nonsurvivors (579 ± 152 versus 531 ± 176). NT-proBNP in survivors (25 ± 10) was significantly lower than in nonsurvivors (179 ± 52) (P < 0.001). A positive association between NT-proBNP and TNI was found in survivors of severe sepsis (P < 0.001). A negative association was found in nonsurviving patients (P = 0.003). NT-proBNP showed no significant differences between survivors and nonsurvivors at day 2 (111 ± 47 versus 104 ± 52). The median of NT-proBNP was 25 (19–31) in survivors and reached 213 (81–284) in nonsurvivors. NT-proBNP in survivors at day 2 correlated with TNI at day 2 (P < 0.001).

TABLE 1. Demographic and Laboratory Data of Septic Patients at the Onset of Severe Sepsis (Day 0)

<table>
<thead>
<tr>
<th>Variable</th>
<th>All Septic Patients (n=57)</th>
<th>Drotrecogin Alfa (Activated) (n=23)</th>
<th>No Drotrecogin Alfa (Activated) (n=34)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y**</td>
<td>55.0±16.3</td>
<td>53.2±11.9</td>
<td>55.8±16.8</td>
</tr>
<tr>
<td>Gender, M/F, n (%)</td>
<td>42/15 (74/26)</td>
<td>17/6 (74/26)</td>
<td>25/9 (74/26)</td>
</tr>
<tr>
<td>APACHE II Score†</td>
<td>26 (19–30)</td>
<td>27 (18–30)</td>
<td>24 (19–31)</td>
</tr>
<tr>
<td>Primary site of infection, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lung</td>
<td>28 (49)</td>
<td>11 (48)</td>
<td>17 (50)</td>
</tr>
<tr>
<td>Intra-abdominal</td>
<td>19 (33)</td>
<td>8 (35)</td>
<td>11 (32)</td>
</tr>
<tr>
<td>Other sites or unknown</td>
<td>10 (18)</td>
<td>4 (17)</td>
<td>6 (18)</td>
</tr>
<tr>
<td>On vasopressors, n (%)</td>
<td>45 (79)</td>
<td>17 (74)</td>
<td>28 (82)</td>
</tr>
<tr>
<td>Maximum dose of norepinephrine, mg/h*</td>
<td>2.15±1.92</td>
<td>2.23±2.34</td>
<td>2.09±1.63</td>
</tr>
<tr>
<td>Central venous pressure, mm Hg*</td>
<td>17.7±4.9</td>
<td>16.8±4.7</td>
<td>18.3±5.1</td>
</tr>
<tr>
<td>Myocardial hypertrophy, n</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Chronic obstructive pulmonary disease, n</td>
<td>6</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Coronary artery disease, n</td>
<td>9</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Paroxysmal atrial fibrillation, n</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Preexisting reduction of left ventricular function, dilatative cardiomyopathy, acute or chronic cor pulmonale, valve disease, acute coronary ischemia, n</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Creatinine (normal range, 0.7–1.3 mg/dL), mg/dL†</td>
<td>1.10 (0.92–1.85)</td>
<td>1.40 (0.93–2.03)</td>
<td>1.10 (0.93–1.78)</td>
</tr>
<tr>
<td>C-reactive protein (normal range, 0–5 mg/L), mg/L†</td>
<td>167 (115–198)</td>
<td>194 (132–212)</td>
<td>164 (47–192)</td>
</tr>
<tr>
<td>White blood cells (normal range, 3.80–10.6×10^9/L), 10^9/L†</td>
<td>13.8 (9.0–18)</td>
<td>16.1 (12–19)</td>
<td>14.8 (9.0–16)</td>
</tr>
<tr>
<td>Platelets (normal range, 150–400×10^9/L), 10^9/L†</td>
<td>182 (128–289)</td>
<td>205 (152–311)</td>
<td>139 (98–213)</td>
</tr>
</tbody>
</table>

No significant differences were found between the 2 groups of septic patients.

*Data are presented as mean±SD.
†Data are presented as median (25th to 75th percentiles).
TABLE 2. Cardiovascular Functional Parameters and Day 28 Mortality in Septic Patients

<table>
<thead>
<tr>
<th>Parameter</th>
<th>All Septic Patients (n=57)</th>
<th>Survivors (n=41)</th>
<th>Nonsurvivors (n=16)</th>
<th>P, Survivors vs Nonsurvivors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate at day 0, bpm (n=57)</td>
<td>125±24</td>
<td>124±25</td>
<td>130±21</td>
<td>0.40</td>
</tr>
<tr>
<td>Mean arterial pressure at day 0, mm Hg (n=57)</td>
<td>77±12</td>
<td>79±12</td>
<td>75±13</td>
<td>0.27</td>
</tr>
<tr>
<td>Cardiac index at day 0, L·m⁻¹·min⁻¹ (n=57)</td>
<td>4.5±1.2</td>
<td>4.6±1.3</td>
<td>4.3±0.9</td>
<td>0.40</td>
</tr>
<tr>
<td>Left ventricular stroke work index at day 0, g·m⁻¹·min⁻¹ (n=57)</td>
<td>36.6±11.1</td>
<td>37.1±11.6</td>
<td>35.5±10.4</td>
<td>0.63</td>
</tr>
<tr>
<td>Left ventricular function by echocardiography at day 0, n (%)</td>
<td>29</td>
<td>15</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>Normal or minor reduction (EF ≥50%)</td>
<td>19 (65)</td>
<td>12 (80)</td>
<td>7 (50)</td>
<td>0.13</td>
</tr>
<tr>
<td>Moderate reduction (EF &lt;50% to ≥35%)</td>
<td>6 (21)</td>
<td>3 (20)</td>
<td>3 (21)</td>
<td>1.0</td>
</tr>
<tr>
<td>Severe reduction (EF &lt;35%)</td>
<td>4 (14)</td>
<td>0 (0)</td>
<td>4 (29)</td>
<td>0.042</td>
</tr>
<tr>
<td>On vasopressors at day 0, n (%)</td>
<td>45 (79)</td>
<td>29 (71)</td>
<td>16 (100)</td>
<td>0.013</td>
</tr>
<tr>
<td>Resolution of cardiovascular organ failure (from day 0 to 2), n (%)</td>
<td>8 (18)</td>
<td>6 (21)</td>
<td>2 (12.5)</td>
<td>1.0</td>
</tr>
<tr>
<td>Day 28 mortality, n (%)</td>
<td>16 (28)</td>
<td>...</td>
<td>...</td>
<td></td>
</tr>
</tbody>
</table>

EF indicates ejection fraction. Data are presented as mean±SD.

TABLE 3. Relationship Between Natriuretic Peptides, TNI, Cardiovascular Functional Parameters, and APACHE II Scores

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Spearman’s Rank Correlation Coefficient, r</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>APACHE II vs NT-proANP (n=57)</td>
<td>0.59</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>APACHE II vs NT-proBNP (n=57)</td>
<td>0.42</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>TNI vs NT-proANP (n=57)</td>
<td>0.40</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>TNI vs NT-proBNP (n=57)</td>
<td>0.68</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>NT-proANP vs NT-proBNP (n=57)</td>
<td>0.56</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Left ventricular function vs NT-proBNP as determined by echocardiography (n=29)</td>
<td>0.41</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Relationship Between Plasma Levels of Natriuretic Peptides, TNI, Cardiovascular Functional Parameters, and APACHE II Scores

Severity of disease scores (APACHE II) was positively correlated to NT-proANP and NT-proBNP levels (r=0.59, P<0.01 for NT-proANP; r=0.42, P<0.05 for NT-proBNP; Table 3). NT-proBNP levels were correlated to the severity of reduction in left ventricular function (classified as slightly, moderately, or severely reduced) as determined by echocardiography (r=0.41, P<0.05; n=29 patients). A highly significant correlation was found between NT-proBNP and APACHE II scores (r=0.68, P<0.0001). A less pronounced but statistically significant correlation was also observed between NT-proANP and TNI (r=0.40, P<0.01; Table 3). NT-proANP correlated well with NT-proBNP (r=0.56, P<0.0001; Table 3).

Natriuretic Peptides and Survival

NT-proBNP levels of survivors (median, 493 pmol/L; 25th and 75th percentiles, 314 and 1126 pmol/L) and nonsurvivors (median, 1431 pmol/L; 25th and 75th percentiles, 712 and 1920 pmol/L) were statistically significant different (P<0.01; Figure 1). Septic patients with NT-proBNP levels >1400 pmol/L were 3.9 times more likely to die of sepsis than patients with lower NT-proBNP values (RR, 3.9; 95% CI, 1.6 to 9.7; P<0.01). With this cutoff, sensitivity (patients who will die with NT-proBNP-test results >1400 pmol/L) was 50.0%, specificity (patients who will survive with NT-proBNP test results ≤1400 pmol/L) was 90.2%, and the positive and negative predictive values were 66.7% and 82.2%, respectively. To correct for potential confounding factors such as use of drotrecogin alfa (activated), renal function, age, gender, NT-proANP, and TNI, we performed Cox regression, which confirmed that NT-proBNP was a highly significant parameter predicting mortality in our patient population (P=0.014).

NT-proANP values, however, were not statistically significant different (P=0.29) among survivors (median, 4484 pmol/L; 25th and 75th percentiles, 2710 and 6373 pmol/L) and nonsurvivors (median, 5613 pmol/L; 25th and 75th percentiles, 3423 and 7019 pmol/L). ROC curves for NT-
proBNP and NT-proANP measurements confirmed that NT-proBNP is a better predictor of survival (area under the curve [AUC], 0.68; 95% CI, 0.52 to 0.86; \(P<0.05\)) than NT-proANP (AUC, 0.60; 95% CI, 0.39 to 0.81; \(P=NS\); Figure 2A and 2B). Kaplan-Meier analysis (Figure 3) estimates the rate of death within 28 days among sepsis patients according to NT-proBNP values to be above or below 1400 pmol/L (cutoff value as determined by ROC curve analysis). Differences between the 2 groups were significant (\(P=0.016\) by the log-rank test).

Effect of Treatment With Drotrecogin Alfa (Activated)

There were no statistically significant differences in cardiovascular functional parameters (heart rate, MAP, cardiac index, LVSWI, resolution of cardiovascular organ failure) between the group of septic patients receiving drotrecogin alfa (activated) and those not receiving this medication (data not shown). Mortality was 22% in patients receiving drotrecogin alfa (activated) compared with 32% in sepsis patients without drotrecogin alfa (activated) (\(P=0.11\)). On treatment day 2 of severe sepsis, TNI serum levels and plasma levels of natriuretic peptides in patients receiving drotrecogin alfa (activated) were significantly lower than in patients not treated with drotrecogin alfa (activated): for TNI: median, 0.00 versus 0.46 ng/mL; 25th and 75th percentiles, 0.00 and 0.15 versus 0.00 to 1.68 ng/mL, \(P<0.01\); for NT-proANP: median, 4474 versus 5000 pmol/L; 25th and 75th percentiles, 2568 and 5170 versus 3533 and 7560 pmol/L, \(P<0.05\); for NT-proBNP: median, 698 versus 876 pmol/L; 25th and 75th percentiles, 425 and 1219 versus 454 and 2163 pmol/L, \(P<0.01\).

Discussion

Natriuretic Peptides and Cardiac Dysfunction in Severe Sepsis

The cardiovascular response to septic shock is peripheral vasodilatation resulting in systemic hypotension, hyporespon-
siveness to vaspressors, and reduced systemic vascular resistance. Cytokines and endotoxins from Gram-negative microorganisms may lead to myocardial depression and ventricular dilatation. A circulating myocardial depressant substance was first proposed >50 years ago. Later, it was shown that tumor necrosis factor-α and interleukin-1 are cardiodepressant. Myocardial depression has been defined by different measures of cardiac performance such as cardiac output, depressed ejection fraction, or ventricular dilatation. Several studies have shown that laboratory markers such as cyclic guanosine monophosphate, ANP, endothelin, or the cardiac-specific protein TNI may reflect cardiac dysfunction or myocardial cell damage in severe sepsis.

The present study demonstrated that NT-proBNP may be a valuable laboratory marker to allow risk stratification of sepsis patients. Whether NT-proBNP levels may also be useful to reflect early cardiovascular dysfunction in severe sepsis is also indicated by our study, but larger trials are still needed to verify this possible relationship of NT-proBNP levels and cardiac dysfunction in septic patients.

Whereas plasma concentrations of the C-terminal peptides BNP and ANP are low and fluctuate rapidly, half-life times of the N-terminal peptides NT-proANP and NT-proBNP are considerably longer, resulting in at least 5- to 15-times-greater plasma levels. The N-terminal peptides may therefore be even more sensitive for the detection of left ventricular dysfunction in critical care settings because they are less influenced by the conditions under which the blood sample is taken. In contrast to numerous studies examining the role of natriuretic peptides among patients with congestive heart failure and ischemic heart disease, less information is available about the relationship of natriuretic peptides with cardiac dysfunction in severe sepsis patients. Withhaut et al have described significant increases of ANP and BNP plasma levels on the day of diagnosis of septic shock and an inverse correlation of BNP with cardiac index. Moreover, ANP and NT-proANP have been shown to be negatively correlated to cardiac index and LVSWI, suggesting that natriuretic peptides may serve as markers of cardiac depression in septic patients.

The results of our study agree with previous examinations demonstrating that increased plasma levels of NT-proBNP are very likely related to cardiovascular (dys)function as determined from hemodynamic and echocardiographic assessment. We calculated LVSWI to further elucidate left ventricular systolic function because reduced cardiac function is commonly masked by a concomitant elevated cardiac index at the onset of sepsis. As a measure of lower external left ventricular work, this is a frequently used parameter for quantifying cardiac function in sepsis patients. Corresponding to echocardiographic assessment, LVSWI was lowered in our patients, indicating reduced systolic left ventricular function. Taken together, our data indicate that NT-proBNP may very likely serve as a useful biochemical index of cardiac dysfunction and myocardial depression early in severe sepsis.

Moreover, elevated NT-proBNP levels were highly significant correlated to increased levels of the myocardial cell damage marker TNI. In addition, TNI significantly accounted for the variation in NT-proBNP levels (P<0.0001), emphasizing the important role for NT-proBNP in the context of cardiac injury and dysfunction in septic patients. In agreement with previous observations, 40% of septic patients (n=23) in this study presented with cardiac troponin levels greater than the threshold of significant myocardial damage (>0.1 ng/mL) on day 2 of severe sepsis. Previous studies found TNI elevations in an even greater percentage of septic patients (63% and 85%, respectively). Several studies have offered evidence of ongoing structural myocardial cell injury during the course of septic shock reflected by an increase of TNI levels. However, the mechanism of troponin elevation in sepsis is not completely understood. It has been speculated that alteration of permeability occurs at the levels of myocyte cell membranes, thus leading to leakage of TNI. It is possible that additional factors such as prolonged hypotension, shock state, cardiomyocyte apoptosis, or use of inotropic agents may contribute to the myocardial release of TNI. Increased blood cardiac troponin levels may thus be manifestations of both reversible and irreversible injuries.

**Natriuretic Peptides and Survival**

Data compiled from large clinical trials indicate that an increase in circulating levels of natriuretic peptides during early phases of myocardial infarction is an independent predictor of left ventricular dysfunction and death. Here, we were able to demonstrate that NT-proBNP plasma levels may also be predictive of survival of patients with severe sepsis or septic shock. In this study, septic patients with NT-proBNP plasma levels >1400 pmol/L were 3.9 times more likely to die within 28 days than patients with lower values. Moreover, there were significant correlations detectable between levels of natriuretic peptides and severity of disease scores (APACHE II scores; Table 3). To the best of our knowledge, a relationship between elevated levels of NT-proBNP and both APACHE II scores and the likelihood of death has not been reported before for severe sepsis patients. We would like to suggest that, in the early course of sepsis, NT-proBNP may serve as a reliable laboratory marker for risk stratification of septic patients. For NT-proANP, however, there were no significant differences detectable among survivors and nonsurvivors of sepsis, possibly because of the limited number of patients in our study. Withhaut et al have demonstrated a strong correlation between the proinflammatory cytokine interleukin-6 and ANP, suggesting an interleukin-6–promoted transcription and secretion of ANP in atrial cardiomyocytes during septic shock. This mechanism of inflammation-triggered ANP release might contribute to the fact that, unlike the finding in patients with congestive heart failure, NT-proANP might be a less discerning marker than NT-proBNP in septic patients.

**Treatment Effect of Drotrecogin Alfa (Activated)**

Within both the PROWESS and ENHANCE trials, the 28-day mortality rate in drotrecogin alfa (activated) treated patients was reduced (absolute reduction of mortality, 6.1%). Because our sample size was limited (n=57), we were not able to show a significant reduction in mortality in the group of patients receiving drotrecogin alfa (activated) compared with control sepsis patients. However, patients receiving drotrecogin alfa (activated) for 2 days presented with significantly lower levels of NT-proANP, NT-proBNP, and TNI.
compared with control sepsis patients. No statistically significant differences in baseline characteristics between the 2 treatment groups were found, excluding the possibility that age, gender, or renal function might have influenced these results. The presence of cardiac disease has been suggested to be the most important determinant for BNP, NT-proBNP, and TNI variations in critically ill patients presenting with renal impairment, diabetes, myocardial infarction, symptomatic heart failure, or pneumonia. Age and gender, however, might also have a minor impact on plasma BNP and NT-proBNP concentrations.36,37

Cardiovascular functional parameters were not statistically different among the 2 patient groups on days 0 and 2 of severe sepsis. This lack of significance may be attributable to the limited number of patients in each group and the relatively early time points (day 0 to 2) in which measurements were performed. Within the large PROWESS trial, ~1500 patients with cardiovascular organ failure at baseline were necessary to demonstrate a significantly higher likelihood of resolution of organ failure during sepsis days 1 to 7 in patients receiving drotrecogin alfa (activated) (n=736) compared with control sepsis patients (n=746; hazard ratio, 1.19; 95% CI 1.04 to 1.36; P=0.009).12,13 The molecular mechanisms responsible for the beneficial effects of drotrecogin alfa (activated) on cardiac depression and survival have not been fully elucidated and remain an open field of discussion. In addition to its well-defined role in coagulation, there is evidence that drotrecogin alfa (activated) exhibits direct antiinflammatory properties independent of its antithrombotic effects as indicated by decreased interleukin-6 levels in patients with severe sepsis.11 Moreover, activated protein C has been shown to improve capillary perfusion and to protect from lipopolysaccharide-mediated microcirculatory dysfunction in animal models.38

Study Limitations
Because this was a nonrandomized study, the effect of drotrecogin alfa (activated) on levels of TNI, NT-proANP, and NT-proBNP should not be overemphasized. Either drotrecogin alfa (activated) itself or several other reasons [e.g., site-specific treatment modalities or the inclusion and exclusion criteria for the administration of drotrecogin alfa (activated)] may have contributed to the observed differences in plasma levels of natriuretic peptides. Thus, the study may only serve as “proof of principle” for the assumption that natriuretic peptide levels may reflect improvement of cardiac dysfunction observed in patients treated with drotrecogin alfa (activated). However, the fact that patients were not randomized to treatment groups should have only a slight or no effect at all on the most important observation of the study, namely the close association between the severity of sepsis and the increased activation of the endocrine function of the injured heart as assessed by circulating levels of NT-proANP and NT-proBNP. Larger clinical trials are needed to confirm these results. Additional data are necessary to determine whether the predictive value of NT-proBNP is indeed superior to that of NT-proANP in sepsis patients and how both natriuretic peptides may influence each other. Whether NT-proBNP may be a sensitive marker to indicate cardiovascular dysfunction in septic patients also needs confirmation by a larger, prospectively defined trial.

Conclusions
In summary, we observed that NT-proANP and NT-proBNP levels are increased in patients with severe sepsis. NT-proBNP may serve as a useful laboratory marker to predict time to death in patients presenting with severe sepsis.

Acknowledgments
This work was supported by a grant from the Faculty of Clinical Medicine Mannheim, University of Heidelberg, Mannheim, Germany, and an unrestricted research grant from Lilly, Germany. We thank Julia Noll, Genevieve Guillier, and Claudia Liebetrau for their excellent technical assistance.

Disclosure
Dr Huhle was an employee of Lilly, Germany, and has received an unrestricted grant from the University of Heidelberg, Germany. Dr Brueckmann has received a research grant from Eli Lilly for in vitro experiments on recombinant human activated protein C and has served as a consultant to Eli Lilly.

References


