PRO-ATRIAL NATRIURETIC PEPTIDE (proANP)
A NEW PROGNOSTIC MARKER IN CLINICAL OUTCOME OF CRITICALLY ILL PATIENTS

HANS B. REITH1, SONYA K. RAUCHSCHWALBE2

Department of General Surgery, Klinikum Konstanz Teaching Hospital of the University in Freiburg1
Kierownik: prof. dr H. B. Reith
Surgical University Clinic of Würzburg, Germany2
Kierownik: prof. dr A. Thiele

The diagnosis of sepsis and the prediction of its outcome are important aims to tackle for this heterogeneous disease. There is a lack of biomarkers to aid in identification of risk groups, inclusion of patients in ongoing studies, and better differentiation of therapeutic strategies. Since the introduction of the prohormone procalcitonin (PCT), many studies have begun to focus on new prohormones.

The aim of the study was to evaluate the prognostic value of pro-atrial natriuretic peptide (proANP) as a new marker of sepsis outcome.

Material and methods. A cohort of 80 patients developing post-surgical sepsis was consecutively included into this study. Blood samples were obtained for analysis of proANP and determination the serum levels of sTNF-R1, TNF-α, IL-6, IL-8, IL-10, procalcitonin and neopterin. Cytokine levels were measured repeatedly until the patients’ discharge from the ICU.

Results. 54/80 (67.5%) fulfilled the criteria for severe sepsis and 36/80 (45.0%) developed septic shock. Multiple organ failure occurred within 60/80 patients (75.0%); the overall mortality was 26/80 (32.5%). Concerning the diagnosis of sepsis, severe sepsis, or septic shock, there was no statistical difference in the proANP values. However, there was a statistical difference in the prediction of outcome in that 26 non-survivors had 803.5 (441.5/1095) pmol/L levels while the survivors level was 315.5 (187/594.5) pmol/L. In addition, there were no statistical significant differences between proANP and other cytokines.

Conclusions. There was a significant correlation between proANP values and the outcome of critically ill patients. ProANP does not differentiate between clinical features like sepsis, severe sepsis or septic shock.

Key words: clinical outcome, pro-atrial-natriuretic peptide (proANP), cytokines, PCT, sepsis

The pathophysiology of septic shock and multiple organ failure is a complex and multifactor process involving an imbalance between proinflammatory and anti-inflammatory cytokine release. Tumor necrosis factor alpha (TNF-α) is recognized as a central mediator of the inflammation cascade and of the pathophysiological changes associated with bacteraemia and sepsis (1). The release of TNF-α, mainly from monocytes, is initiated by microbial components such as lipopolysaccharides and teichnoic acids whereas the amount of production may be modulated by factors like trauma, surgery, nutritional status and underlying disease (1, 2, 3). Biomarkers are on demand to tackle the challenges of sepsis monitoring and treatment. Prohormones, like the well introduced procalcitonin (PCT), give us the opportunity to classify and follow-up with septic patients (4). Other prohormones are called to close the gap of information deficit.
It seems that the release of prohormones due to trauma, stress and sepsis is archaic in the evolution of the response mechanism. The release of prohormones as measured in studies (i.e. PCT) functions as a key player, more or less in a similar time frame as cytokine release. Atrial natriuretic peptide (ANP) is predominantly produced in the atrium of the heart and compromises 98% of the natriuretic peptides in the circulation (5). Beside ANP, proANP has gained interest in the field of sepsis (6). ProANP is the 126 amino acid-long prohormone of ANP which is derived from the carboxyl terminal end of amino acids 99-126 (5). The developed sandwich immune essay of the mid-regional area of proANP may be an advantage for use in further investigations (7).

Ongoing studies demonstrate proANP as a new marker for diagnosis of sepsis. Morgensthaler et al. (8) found proANP to be a valuable tool for stratification and individual risk assessment in sepsis patients. They were able to compare the proANP results with the clinical status of sepsis, severe sepsis and septic shock. Brueckmann et al. (9) also studied proANP; however, they focused on patients with severe sepsis and the proBNP marker (pro-brain natriuretic peptide), a marker originally introduced for ischemic heart disease. Other cytokines such as IL-1 act synergistically with TNF-α, stimulating the release of mediators such as IL-6, IL-8 and IL-10 which participate in the host inflammatory response. IL-6 is a cytokine which potently stimulates B- and T-lymphocytes and is involved in the induction of fever and the synthesis of proteins by the liver. It is shown that IL-6 is closely correlated with the severity and outcome of sepsis; therefore it is likely to play a pathogenic role (10, 11).

IL-8 has chemo attractant activity and is able to activate and degranulate neutrophils; it is also involved in the pathogenesis of pneumonia (12).

IL-10 is a cytokine produced by TH2 lymphocytes, monocytes and epithelial cells and possesses both anti-inflammatory and immunosuppressive properties. Experimental animal studies have demonstrated that IL-10 can reduce the inflammatory response and improve outcome in sepsis models. Nevertheless, IL-10 can also exacerbate T-cell dysfunction, decrease T-cell apoptosis, reduce antimicrobial function and increase mortality in other models of sepsis (13).

Procalcitonin, a precursor protein of calcitonin and katacalcin, is highly suitable to identify patients with severe infective postoperative complications in the early postoperative period, enabling for early antibiotic treatment (4). It presents itself as a marker to identify whether the applied therapy is adequate and allows the prediction of sepsis outcome in critically ill patients (14).

Finally, neopterin is a low molecular weight pteridine compound secreted by macrophages stimulated with IFN-γ. Although its physiological function is currently unknown, neopterin has been used as an indicator of cellular immune activation, representing the sum of positively and negatively regulating influences on IFN-γ (15).

In the presented study we investigated eighty-five patients in our surgical intensive care unit for the prognostic value of proANP and panels of TNF-α, sTNF-R1, IL-6, IL-8, IL-10, procalcitonin and neopterin for mortality due to sepsis and septic shock. A population of 406 surgical patients who stayed in our ICU postoperatively were observed for comparison of mortality rates.

**MATERIAL AND METHODS**

Between June 1997 and November 1999, 80 patients developing post-surgical sepsis were enrolled into the study. The Ethics Committee of the University of Wuerzburg approved the protocol and informed consent was obtained from all patients or their relatives. Blood samples were drawn after admission to the ICU for analysis of proANP and determination of serum levels of sTNF-R1, TNF-α, IL-6, IL-8, IL-10, procalcitonin and neopterin. Cytokine levels were measured daily until the patients' discharge from the ICU. A Goris-Score was calculated by the addition of less than or equal to two points for each of the seven organ systems included (lung, heart, kidney, liver, blood, gastrointestinal tract, central nervous system). This system was developed and evaluated for simplified scoring of organ failure. A Score of more than or equal to four points was defined as multiple organ dysfunction (16). The Acute Physiology and Chronic Health Evaluation Score II were also evaluated (17). Diagnosis of SIRS, sepsis, severe sepsis and septic shock was made according to Bone et al. (18). Detailed results have been reported earlier (19).
For comparison of mortality, a total of n=406 surgical patients consecutively admitted to our ICU were observed for diagnosis and outcome without regard to proANP or other cytokines.

Measurement of cytokine levels

Mid-regional proANP was detected in patients EDTA plasma with a new sandwich assay (BRAHMS Seristra® LIA, BRAHMS AG Henningsdorf/ Berlin, Germany). The details of measurements were described in detail elsewhere (7). TNF-α, sTNF-R1, IL-6, IL-8 and IL-10 were determined using the MEDGENIX ELISA-Test (Biosource, Ratingen); neopterin was measured by ELISA-Test (BRAHMS AG, Henningsdorf/ Berlin). For procalcitonin, the LUMItest (BRAHMS AG, Henningsdorf/ Berlin) was used. The normal ranges were as follows: TNF-α <20 pg/ml; sTNF-R1 0.3-2.9 ng/ml; IL-6 3-8.5 pg/ml; IL-8 0-47 pg/ml; IL-10 0-112 pg/ml; neopterin <20 nmol/ml; procalcitonin 0.1-1 ng/ml. ProANP levels were measured by BRAHMS Seristra® LIA and expressed in pmol/L. The lower detection limit of the assay was 4.3 pmol/L. The 97.5th percentile in 325 healthy individuals was 163.9 pmol/L (median 45 pmol/L), with no difference in gender noted.

Statistical analysis

Descriptive results of continuous variables were expressed as a median (25th /75th percentile). Cytokine levels were expressed as a median (25th /75th percentile) of peak plasma values. For comparison of proANP levels between shock and non-shock patients and survivors and non-survivors, the Mann-Whitney-U-test was used; it was also used for comparing peak plasma levels of cytokines vs. survival. Analysis was completed using commercial software (SPSS Sigma Stat); a two-tailed p<0.05 was deemed significant.

RESULTS

80 patients meet all inclusion criteria and the follow-up. Table 1 demonstrates the demographic characteristics of the study population. Underlying diseases relevant preoperative illnesses are in all cases severe infections with sepsis, severe sepsis and septic shock. Gram-negative organisms caused the majority of infections.

Of the 406 control patients consecutively admitted to our ICU, 100 suffered from infection. 35/406 (8.6%) fulfilled criteria for sepsis, 24/406 (5.9%) fulfilled criteria for severe sepsis, and 27/406 (6.6%) developed septic shock. Mortality was 11.4% for sepsis patients, 25.0% for patients with severe sepsis, and 44.4% within the septic-shock-group.

Levels of cytokines and prohormones were statistically significantly different between survivors and non-survivors (p=range between 0.026 and 0.002) for peak plasma levels (tab. 2). The values are statistically significantly different for levels of proANP and also TNF-α, sTNF-R1, IL-8, IL-10, PCT and neopterin (tab. 2, fig. 1).

The peak plasma concentrations of proANP in correlation to the severity of disease are shown in tab. 3. It is obvious that proANP did not correlate with the clinical findings of sepsis, severe sepsis and septic shock. The same

<table>
<thead>
<tr>
<th></th>
<th>Females</th>
<th>Males</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>29</td>
<td>51</td>
<td>80</td>
</tr>
<tr>
<td>Age *</td>
<td>71 (62.5/82.3)</td>
<td>62 (53.3/71.5)</td>
<td>65.5 (56.6/74)</td>
</tr>
<tr>
<td>APACHE II *</td>
<td>17 (12/22.5)</td>
<td>18 (12.3/22.75)</td>
<td>17.5 (12/22.5)</td>
</tr>
<tr>
<td>Days on ICU *</td>
<td>8.5 (4.5/25.5)</td>
<td>16 (8/30)</td>
<td>14 (6/29)</td>
</tr>
<tr>
<td>Ventilation time *</td>
<td>5 (2/17.5)</td>
<td>8.5 (4/19)</td>
<td>8 (3/19)</td>
</tr>
<tr>
<td>Sepsis</td>
<td>29</td>
<td>51</td>
<td>80</td>
</tr>
<tr>
<td>Severe sepsis</td>
<td>17 (58.6%)</td>
<td>37 (72.5%)</td>
<td>54 (67.5%)</td>
</tr>
<tr>
<td>Septic shock</td>
<td>11 (37.9%)</td>
<td>25 (49%)</td>
<td>36 (45%)</td>
</tr>
<tr>
<td>MOF</td>
<td>22 (75.9%)</td>
<td>38 (74.5%)</td>
<td>60 (75%)</td>
</tr>
<tr>
<td>Goris-score *</td>
<td>5 (3.8/7)</td>
<td>6 (4/9)</td>
<td>6 (4/9)</td>
</tr>
<tr>
<td>Survivor</td>
<td>18 (27.6%)</td>
<td>36 (70.6%)</td>
<td>54 (67.5%)</td>
</tr>
<tr>
<td>Non-survivor</td>
<td>11 (7.9%)</td>
<td>15 (29.4%)</td>
<td>26 (32.5%)</td>
</tr>
</tbody>
</table>

* mediana (25/75 percentyl) / expressed as median (25/75 percentile)
Pearson Product Moment Correlation, *no significant differences (p > 0.05). 

of the new prohormone (tab. 4).

rences between first and last value (results can be obtained in measuring the differences between first and last value (Δ proANP) of the new prohormone (tab. 4).

DISCUSSION

Pro atrial-natriuretic peptide (proANP) is a new prohormone associated with the pathophysiology of sepsis. ANP is recognized as a marker for heart failure and is well introduced in different clinical features (5, 6, 7, 20, 21).

In the present study we evaluate the value and prognostic significance of proANP in a clinical setting of 80 patients with sepsis, severe sepsis and septic shock in the surgical ICU. We compare the results with different cytokines and other markers to differentiate between clinical situation and outcome.

TNF-α levels were different between the groups of survivors and non-survivors and between patients developing septic shock and those who did not. Within the group of septic shock patients, TNF-α values were comparable to those reported previously (2, 22). On the other hand, others reported lower TNF-α values in shock patients who survived rather than those who expired (13).

Plasma levels of the soluble TNF-receptor protein 1 showed a significant difference concerning sepsis outcome. Spielmann and co-workers (22) found elevated levels of sTNF-R1 in the early hours after trauma and posttraumatic SIRS, but differences vanished during later measurements. They stated that "single shot analyses" of cytokine levels in plasma at one more or less arbitrary time point after injury or onset of sepsis does not reveal reproducible associations with clinical parameters. This is surely true, but more frequent blood sampling is mostly not possible to apply in clinical routines.

Plasma levels of IL-6 have been described to correlate well with severity and outcome of

<table>
<thead>
<tr>
<th>Pro ANP [pmol/l]</th>
<th>Survivors</th>
<th>Non-survivors</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>315,5 (187/394,5)</td>
<td>803,5 (441/1095)</td>
<td>0,008*</td>
<td></td>
</tr>
</tbody>
</table>

* wartość p, test U Mann i Whitney’a, *różnica istotna statystycznie / p-values of Mann-Whitney-test, *statistically significantly different

Table 2. Peak plasma cytokine levels vs mortality, median (25 /75 percentile)

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Survivors</th>
<th>Non-survivors</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6 [pg/ml]</td>
<td>690,4 (289,3/1873,8)</td>
<td>946,0 (547,0/2765)</td>
<td>0,166</td>
</tr>
<tr>
<td>IL-10 [pg/ml]</td>
<td>6,2 (3,2/14,5)</td>
<td>17,3 (5,8/41)</td>
<td>0,026*</td>
</tr>
<tr>
<td>Neopterin [nmol/l]</td>
<td>64,4 (22,1/110,4)</td>
<td>157,0 (62,1/279)</td>
<td>0,002*</td>
</tr>
</tbody>
</table>

Table 3. Peak plasma levels of proANP in correlation to severity of disease

| Severe Sepsis | 17 | 392,533 | 326,68 | 84,348 | 180,909 | 1300 | 101 | 247 |
| Septic Shock | 35 | 658,471 | 375,522 | 64,401 | 131,026 | 1320 | 1 | 557,5 |

* Pearson Product Moment Correlation, **no significant differences (p > 0,050)
sepsis (10). Since TNF-α and IL-1 are known to act synergistically, the conclusion has been drawn that elevated levels of IL-6 in patients with septic shock represent the net effect of biologically active TNF-α and IL-1 (1). However, we could not reproduce that observation. As well, the ratio of IL-10/TNF-α showed a non-significant trend to be larger for non-survivors than for survivors of septic shock (0.29 vs 0.11, respectively). This does not fit the observation that IL-10 suppresses gene expression and synthesis of IL-1 and TNF-α in mice resulting in a reduced lethal endotoxinemia. However, it is consistent with reports from other animal models observing exacerbated T-cell function and increased mortality. Our patients’ IL-10 levels were significantly different between survivors and non-survivors as reported earlier (23). The contribution and importance of IL-10 on the outcome of sepsis patients has to be evaluated very critically because of its challenging simultaneous anti-inflammatory and immunosuppressive properties.

In addition, IL-8 plasma levels were higher for non-survivors of septic shock compared to survivors. In community-acquired pneumonia, IL-8 is elevated at the site of infection (as confirmed by broncho-alveolar lavage) but not in the contralateral lung or blood (24). Plasma IL-8 increases with generalization of the local infection into sepsis and septic shock and is then directly correlated with mortality (25).

Likewise, procalcitonin and neopterin levels were significantly higher in non-survivors compared to survivors, allowing a prediction of sepsis outcome as has been described previously (4, 26).

Trouillet and co-workers (24) stressed that the predominant pathogens associated with hospital-acquired infections vary between hospitals as well as between specialized units within individual hospitals; therefore, clinicians should be aware of the prevailing bacterial pathogens in their hospitals and their susceptibilities. This should help guide the empiric therapy of suspected nosocomial infections. Therefore, empirical treatment with either of the antibiotics studied is adequate and is an equivalent first-shot-therapy for the microbiologic spectrum in our ICU.

Sepsis, severe sepsis and septic shock are well-defined clinical diagnoses with widely accepted guidelines; however, there are a lot of recommendations and comments about it. In routine bedside use these clinical guidelines are not 100% certain in the classification of critical ill patients (27).

The ideal sepsis markers are not yet found, but prohormones like procalcitonin have come close (28). The first results of proANP as a sepsis marker were shown by Morgenthaler et al. (8). They found that proANP better indicates the survival question as found with CRP, PCT and IL-6 and with slightly better or similar results to the APACHE II Score. They predicted that proANP might become a new and useful additional prognostic marker for individual risk assessment (8). In our study we were able to confirm these results; proANP was able to distinguish between survivors and non-survivors. However, the severity of illness could not be discriminated.

Regarding the plasma levels in our study, we found a broad range of values in the beginning of the evaluation. Also, the average age of our patient population was over 60 years. Therefore, the question “does the age of the patients influence the proANP values?” has to be answered in further investigations. Also, the cut-off level for normal patients (160 pmol/L) needs to be determined.

In conclusion, there seems to be a new diagnostic tool as an outcome predictor in patients with sepsis, severe sepsis and septic shock. More investigations are needed to confirm these first results and ascertain additional knowledge about the pathophysiological pathway of proANP.

### Table 4. Plasma level differences (Δ) of proANP in correlation to severity of disease

<table>
<thead>
<tr>
<th></th>
<th>No</th>
<th>Mean</th>
<th>Std Dev</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Δ proANP sepsis</td>
<td>26</td>
<td>31.55</td>
<td>178.833</td>
<td>35.072</td>
</tr>
<tr>
<td>Δ proANP severe sepsis</td>
<td>18</td>
<td>38.956</td>
<td>270.92</td>
<td>63.856</td>
</tr>
<tr>
<td>Δ proANP shock</td>
<td>35</td>
<td>69.649</td>
<td>475.831</td>
<td>80.43</td>
</tr>
</tbody>
</table>

* one Way Repeated Measures Analysis of Variance: no statistically significant differences (p = 0.908)
REFERENCES