Pro-atrial natriuretic peptide (pro-ANP) level in patients with severe sepsis and septic shock: prognostic and diagnostic significance

M. Lipinska-Gediga · M. Mierzchala · G. Durek

Received: 10 December 2010 / Accepted: 8 December 2011 / Published online: 12 January 2012
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Abstract
Background To establish the prognostic and discrimina-
tive value of the pro-atrial natriuretic peptide (pro-ANP) level in patients with severe sepsis or septic shock.
Patients and methods An observational and prospective study was conducted on 50 critically ill patients with severe sepsis or septic shock. Measurements of the level of pro-calci
tonin (PCT) and mid-regional pro-ANP were deter-
minded in the serum of patients with commercially available immunoluminometric tests.
Results The median pro-ANP level was significantly higher in non-survivors than in survivors \((P < 0.05)\) on all consecutive days. No significant differences in the pro-ANP levels were observed in patients with severe sepsis and septic shock. Measurements of the level of pro-calci
tonin (PCT) and mid-regional pro-ANP were determined in the serum of patients with commercially available immunoluminometric tests. There was a strong correlation between the PCT and pro-ANP levels on admission in non-survivors and in septic shock patients \((r = 0.56, P = 0.007 \text{ and } r = 0.43, P = 0.02, \text{ respectively})\).
Conclusions pro-ANP evaluated in severe sepsis and septic shock patients is a valuable prognostic biomarker, but, in contrast to PCT, which is routinely used as a diagnostic marker of severe sepsis and septic shock, it does not possess diagnostic and discriminative value.

Keywords Pro-atrial natriuretic peptide (pro-ANP) · Procalcitonin (PCT) · Severe sepsis · Septic shock

Introduction

Immune inflammatory reactions, which are responses to infection, are essential to the body’s protective mechanisms. The often excessive and uncontrolled course of such reactions is one of the crucial factors influencing multiple organ dysfunction syndrome (MODS), which impacts intensive care unit (ICU) patient mortality. According to the definition from 1992 [1], there are three clinical forms of response to infection: sepsis, severe sepsis, and septic shock. They are differentiated by the presence and degree of the intensity of cardiovascular dysfunction resulting from the extensity of the inflammatory response [2]. Severe sepsis is defined as sepsis with one or more organ dysfunctions or tissue hypoperfusion [3]. Septic shock is characterized by the most advanced form of circulatory system failure, and is defined as severe sepsis with persisting hypotension, despite adequate fluid resuscitation or blood lactate concentration \(\geq 4 \text{ mmol/L} [3]\). The pathophysiology of severe sepsis and septic shock at the cellular level is associated with an advanced form of microcirculatory and mitochondrial distress syndrome (MMDS). It involves endothelium dysfunction with apoptosis and coagulation disturbances, resulting in global tissue hypoxia [4, 5]. Atrial natriuretic peptide (ANP), consisting of 28 amino acids, is a peptide hormone and a member of the natriuretic peptide family. Its conservative part is composed of 17 amino acids that form a ring structure with disulfide bonds and determine the biological features of ANP. ANP
with a half-life of 3–4 min is cleared out rapidly from the circulation. pro-ANP, composed of 126 amino acids, is the precursor of ANP and is the primary form of storing ANP in atrial cardiomyocytes. The N-terminal part of pro-ANP (1–98) has a longer half-life (60–120 min) than ANP. ANP possesses diuretic and natriuretic properties, resulting from the direct inhibition of sodium absorption in the renal collecting duct. ANP decreases blood pressure, modulates endothelium permeability, and has an effect on the volume and pressure homeostasis in the circulatory system [6]. There are two transmembrane receptors, NPR-A and NPR-C (a clearance receptor), which regulate ANP activity [7, 8]. Natriuretic peptides participate in the innate and acquired immunological response [9]. ANP has been shown to have a modulating effect on macrophage function and the priming of polymorphonuclear neutrophils [10].

ANP interferes with both the mitogen-activated protein kinase (MAPK) network and transcription factors, mainly, NF-κB [11]. Procalcitonin is a 13-kDa prohormone of calcitonin. Carboxyl-terminal (CT) peptides (procalcitonin [PCT] included) have a common origin on the CALC-1 gene on chromosome 11 [12]. It has been suggested that PCT is a prototype of hormokine mediators. The definition of hormokine includes the cytokine-like activity of hormones during inflammation and infection [13]. During the response to infectious stimuli, there is only a limited and transient release of PCT from macrophages and monocytes. LPS, IL-1β, and TNF-α promote the tissue-wide induction of CT mRNA, with the result that PCT is secreted into the circulation.

The half-life of PCT is about 22–35 h [14]. Under physiological conditions, the level of PCT is lower than <0.5 ng/ml. It has been stated that PCT should be used as a diagnostic marker to monitor the course of severe sepsis and septic shock and antimicrobial therapy [15, 16].

The diagnosis of severe sepsis or septic shock was performed according to the 2001 Consensus Conference Criteria [3].

Infection was confirmed with microbiological tests, radiological analysis, and surgical procedures. In questionable cases, the PCT level was used as confirmation of the presence of infection.

This study was observational and prospective. Table 1 shows the detailed microbiological results and information about the sources of infection. The status of clinical patients was assessed with the Acute Physiology and Chronic Health Evaluation II (APACHE II) score [17] on admission to the ICU, and the extent of multiple organ failure was evaluated using the Sequential Organ Failure Assessment (SOFA) score [18] on admission, and on the 2nd, 3rd, and 5th days. All patients were treated according to accepted standards for severe sepsis and septic shock (antimicrobial therapy, mechanical ventilation, fluid resuscitation, vasopressor therapy). All patients had been receiving empirical antibiotic therapy on admission, which was modified according to ongoing microbiological and laboratory results. The ICU mortality was 48%.

The prognostic and discriminative value of pro-ANP levels was evaluated in patients diagnosed with severe sepsis and septic shock.

The control group included 20 healthy volunteers, comprising 15 females and 5 males (mean age 36.4 years, range 22–56 years), who were recruited from the ICU staff.

Ethical considerations

The study was approved by the Medical Ethics Committee of Wroclaw Medical University and informed consent was obtained from the patients or their legal representatives.

Laboratory measurements

Blood was drawn on admission, and on the 2nd, 3rd, and 5th days. In nine cases, samples from the 5th day were not available (the patients had died or had been discharged earlier). Serum was obtained after 15 min of clotting at room temperature and the blood was centrifuged (10 min, 720g). Serum samples were aliquoted and stored at −80°C to analyze the pro-ANP and PCT concentrations. All measurements were performed with commercially available immunoluminometric tests from BRAHMS Diagnostica GmbH (Hennigsdorf, Germany), according to the manufacturer’s instructions.

Statistical methods

The normality of the distribution was estimated by the Kolmogorov–Smirnov test. The data were analyzed with a
non-parametric test (Mann–Whitney U-test) to compare the two groups. The APACHE II and SOFA score values are presented as the mean ± standard deviation (SD). A P value ≤0.05 was considered to be statistically significant. Correlation analysis with continuous data was conducted using Pearson’s correlation test (r).

All analyses were performed using the STATISTICA data analysis software system, version 9.1 (StatSoft Inc., 2010, http://www.statsoft.com).

Results

Survivors and non-survivors

As an estimation of the patients’ clinical status, the mean value of the APACHE II score on admission in survivors and non-survivors was 18.3 and 27, respectively (Table 2). The value of the SOFA score was significantly lower in survivors than in non-survivors on all the analyzed days (Table 2).

The pro-ANP level was significantly lower in survivors than non-survivors on all the analyzed days (Table 3).

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**Table 1** Source of infection and microbiological results

| Number of patients | Site of infection | Details | Microbiological results |
|--------------------|-------------------|---------|-------------------------|
| 21                 | Respiratory       | Lungs   | Neisseria sp. (1)       |
|                    |                   |         | Ralstonia mannitolitlyca (1) |
|                    |                   |         | Enterococcus faecium (2) |
|                    |                   |         | Pseudomonas aeruginosa (5) |
|                    |                   |         | Staphylococcus aureus (3) |
|                    |                   |         | Acinetobacter baumannii (3) |
|                    |                   |         | Candida albicans (3) |
|                    |                   |         | Unknown (3) |
| 18                 | Abdominal         | Peritonitis, pancreatitis, cholecystitis, UTI | Pseudomonas aeruginosa (1) |
|                    |                   |         | Citrobacter freundii (1) |
|                    |                   |         | Enterobacter cloacae (1) |
|                    |                   |         | Acinetobacter baumannii (1) |
|                    |                   |         | Enterococcus faecalis (3) |
|                    |                   |         | Staphylococcus aureus (1) |
|                    |                   |         | Escherichia coli (1) |
|                    |                   |         | Unknown (9) |
| 5                  | Blood             |         | Acinetobacter baumannii (1) |
|                    |                   |         | Pseudomonas aeruginosa (1) |
|                    |                   |         | Candida albicans (1) |
|                    |                   |         | Neisseria meningitis (1) |
|                    |                   |         | Enterobacter cloacae (1) |
|                    |                   |         | Pseudomonas aeruginosa (2) |
|                    |                   |         | Escherichia coli (1) |
|                    |                   |         | Unknown (1) |
| 6                  | Other             | Eye, meningitis, skin, wound | Neisseria sp. (1) |
|                    |                   |         | Ralstonia mannitolitlyca (1) |
|                    |                   |         | Enterococcus faecium (2) |
|                    |                   |         | Pseudomonas aeruginosa (5) |
|                    |                   |         | Staphylococcus aureus (3) |
|                    |                   |         | Acinetobacter baumannii (3) |
|                    |                   |         | Candida albicans (3) |
|                    |                   |         | Unknown (3) |

**Table 2** Acute Physiology and Chronic Health Evaluation II (APACHE II) and Sequential Organ Failure Assessment (SOFA) scores in survivors and non-survivors

|                   | Survivors (n = 26) | Non-survivors (n = 24) | P value* |
|-------------------|--------------------|------------------------|----------|
| APACHE II1st day  | 18.3 (8–32)        | 27 (11–44)             | 0.000007 |
| SOFA1st day       | 6.6 (0–14)         | 11.2 (4–18)            | 0.00005  |
| SOFA2nd day       | 5.8 (0–14)         | 10.6 (3–20)            | 0.00004  |
| SOFA3rd day       | 4.8 (0–12)         | 10.5 (2–20)            | 0.0001   |
| SOFA5th day       | 4.2 (0–14)         | 11.5 (3–19)            | 0.000007 |

* P value for differences between survivors and non-survivors

**Table 3** Pro-atrial natriuretic peptide (pro-ANP) level (pmol/L) (median and 95% confidence interval [CI]) in survivors and non-survivors

|                   | Survivors (n = 26) | Non-survivors (n = 24) | P value* |
|-------------------|--------------------|------------------------|----------|
| pro-ANP1st day    | 218 (98–385)       | 372 (277–740)          | 0.008    |
| pro-ANP2nd day    | 227 (131–402)      | 472 (263–878)          | 0.016    |
| pro-ANP3rd day    | 249 (155–376)      | 456 (197–1,128)        | 0.043    |
| pro-ANP5th day    | 158 (113–275)      | 531 (272–1,706)        | 0.001    |

* P value for differences between survivors and non-survivors
In the receiver operating characteristic (ROC) curve analysis of survival, on the day of admission, the cut-off value was 220.58 pmol/L and area under the curve (AUC) = 0.72, sensitivity 0.917, and specificity 0.538 (a); 2nd day with a cut-off value of 262.5 pmol/L (AUC = 0.699, sensitivity 0.75, and specificity 0.654) (b); 3rd day with a cut-off value of 409.94 pmol/L (AUC = 0.673, sensitivity 0.591, and specificity 0.84) (c); 5th day with a cut-off value of 270.34 pmol/L (AUC = 0.789, sensitivity 0.789, and specificity 0.76) (d).

Severe sepsis and septic shock

On admission, there was a statistically significant difference in the APACHE II score value of both groups (P = 0.05). The differences in the SOFA score value were statistically significant in the course of the study, except for the 5th day (Table 4).

There was no statistically significant difference in the pro-ANP level between the groups (Table 5).

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**Table 4** APACHE II and SOFA score in severe sepsis and septic shock

|                      | Severe sepsis (n = 19) | Septic shock (n = 31) | P value* |
|----------------------|------------------------|-----------------------|----------|
| APACHE II 1st day    | 19.5 (8–35)            | 24.3 (13–44)          | 0.05     |
| SOFA 1st day         | 6.3 (0–16)             | 10.4 (5–18)           | 0.002    |
| SOFA 2nd day         | 5.6 (0–12)             | 9.6 (2–20)            | 0.006    |
| SOFA 3rd day         | 5.2 (0–15)             | 8.7 (1–20)            | 0.04     |
| SOFA 5th day         | 6.8 (1–19)             | 8.3 (0–19)            | 0.6      |

*P value for differences between severe sepsis and septic shock

**Table 5** pro-ANP level (pmol/L) (median and 95% CI) in severe sepsis and septic shock

|                      | Severe sepsis (n = 19) | Septic shock (n = 31) | P value* |
|----------------------|------------------------|-----------------------|----------|
| pro-ANP 1st day      | 295 (103–750)          | 356 (229–713)         | 0.27     |
| pro-ANP 2nd day      | 249 (132–584)          | 369 (204–509)         | 0.57     |
| pro-ANP 3rd day      | 215 (142–532)          | 344 (201–592)         | 0.289    |
| pro-ANP 5th day      | 143 (87–1,416)         | 295 (179–809)         | 0.18     |

*P value for differences between severe sepsis and septic shock
In the ROC curve analysis of the diagnosis, on the day of admission, the cut-off value was 215 pmol/L and AUC = 0.59, the sensitivity was 0.81, and the specificity was 0.47 (Fig. 2a). On the 2nd therapy day, the cut-off value was 258 pmol/L and AUC = 0.548, the sensitivity was 0.61, and the specificity was 0.58 (Fig. 2b). On the 3rd therapy day, the cut-off value was 162 pmol/L and AUC = 0.594, the sensitivity was 0.80, and the specificity was 0.47 (Fig. 2c). On the 5th therapy day, the cut-off value was 143 pmol/L and AUC = 0.632, the sensitivity was 0.81, and the specificity was 0.53 (Fig. 2d).

There was a strong correlation between the PCT and pro-ANP levels on admission in non-survivors and in septic shock patients ($r = 0.56, P = 0.007$) (Fig. 3a) and $r = 0.43, P = 0.02$ (Fig. 3b), respectively.

**Discussion**

This study was performed in order to determine the prognostic and discriminative properties of pro-ANP in critically ill patients. In the study, the level of pro-ANP was lower in survivors than non-survivors, with statistical significance reached on all the study days; this was similar to the results obtained by Vazquez et al. [19]. A decline in the pro-ANP level was observed starting from the 2nd day in survivors. Similar results were presented by Morgenthaler et al. [20] and Boeck et al. [21], but were in contrary to those of Prucha et al. [22]. In Prucha et al.’s study, the pro-ANP level did not show any tendencies to increase or decrease. There was a correlation between the levels of PCT and pro-ANP on the day of admission in the non-survivors and septic shock groups. In Morgenthaler et al. [20] study, the circulating pro-ANP levels showed a similar increase when categorized on the increasing PCT in all the study groups.

The highest values of pro-ANP were observed in non-survivors and was directly associated with the highest APACHE II and SOFA score results, reflecting the clinical status and progress of multiple organ failure in this group. There was a correlation between the SOFA value and pro-ANP level in the non-survivors and septic shock group on the 1st day. APACHE II and SOFA score values were lower in the survivors group on admission.
The pro-ANP level, which was not significant for severe sepsis and septic shock subgroups, did not reflect statistical significance in the APACHE II and SOFA score results. These results were similar to Morgenthaler et al. study [20], where there were no statistically significant differences in the pro-ANP levels between the severe sepsis and septic shock groups. All those results indicate that pro-ANP measured in severe sepsis or septic shock patients is a valuable prognostic biomarker without diagnostic and discriminative value, which is in contrast to PCT [23, 24].

Boeck et al. [21] found that patients with the highest pro-ANP quartile at ventilator-associated pneumonia (VAP) onset were at increased risk for death, and pro-ANP was identified as the best predictor of survival, followed by the SOFA and Simplified Acute Physiology Score (SAPS) II values.

Morgenthaler et al. [20] did not observe any significant differences in the pro-ANP levels between survivors and non-survivors with no infection. This suggests that pro-inflammatory factors like TNF-α and IL-6 have a greater influence on the release of pro-ANP than the hemodynamic changes linked to circulatory dysfunction that is essential for severe sepsis or septic shock [25].

The question as to why the level of pro-ANP is similar in such clinically differentiated conditions like severe sepsis and septic shock is still open for discussion and further study.

Acknowledgments The present study was financially supported by Wroclaw Medical University grant number 1430.

Conflict of interest None.

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