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Plasma adrenomedullin in critically ill patients with sepsis after major surgery: A pilot study

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ABSTRACT

Purpose: Adrenomedullin is released by different tissues in hypoxia, oxidative stress, and inflammation and is found in general and medical patients and, recently, in sepsis patients in emergency departments. The aim of this study was to evaluate biologically active adrenomedullin that mirrors directly the active peptide levels in plasma of surgical intensive care unit (ICU) patients with sepsis.
Materials and methods: In this single-center observational pilot trial, 42 ICU patients with sepsis and 14 patients after major surgery were included after sepsis diagnosis or ICU admission.
Results: Patients (66% male) were 70 (median) (interquartile range [IQR], 61-77]) years old and had a body mass index of 26.2 (24.2-29.4) kg/m^2. The ICU and hospital length of stay was 8 (1-22) and 17 (8-21) days, respectively. Eight patients had sepsis, 19 developed severe sepsis, and 15 suffered from septic shock. Adrenomedullin increased with severity (sepsis: 25.8 pg/mL [IQR 20.3-40.2], severe sepsis: 84.2 pg/mL [IQR 42.7-118.5], septic shock: 119.7 pg/mL [IQR 83.8-172.6]; $P < .0001$). Higher adrenomedullin was associated with poor 90-day outcomes ($P = .019$) and more frequent vasopressor use ($P = .001$).
Conclusions: This is the first study investigating adrenomedullin in patients with sepsis following major surgery. Higher adrenomedullin on admission is associated with increased vasopressor need and mortality after 90 days. Thus, adrenomedullin may be a useful additional parameter in surgical patients with sepsis.

1. Introduction

More than 1 000 000 cases of sepsis are diagnosed in hospitalized patients each year in the United States. The incidence of sepsis is still increasing primarily because of the growing and aging population and the associated increases in comorbidities [1]. Sepsis is not only a problem in the United States; it is one of the leading causes of mortality and morbidity worldwide with a number of reports from several European countries also [2].

Despite the increased attention focused on sepsis and numerous experimental and clinical trials, the morbidity and mortality of sepsis remain high. Early detection and diagnosis are key points in the treatment of sepsis.

Many biomarkers have been investigated, and different experimental treatment strategies have failed to reduce the mortality associated with sepsis [3].

Adrenomedullin (ADM) is a 52–amino acid peptide that was first isolated from a human pheochromocytoma [4] and participates in a variety of physiological and pathophysiological processes including sepsis. In bacterial sepsis, ADM expression has been described in different vascular systems including the lungs, blood vessels, and kidney and is released by different tissues in hypoxia, oxidative stress, and inflammation [5]. In most cells, ADM stimulation leads to the accumulation of cAMP and thus contributes to vasodilation and the loss of resistance. In infections induced by cytokines, lipopolysaccharide, and hypoxia, increased ADM levels have been demonstrated in animals and humans [5]. From animal experiments, it appears that the most relevant action of ADM is decreasing capillary leakage and hyperpermeability during septic shock [6,7].

In clinical studies, associations between increased ADM blood concentrations in sepsis and increased morbidity and mortality have been found [8-11].

The prognostic value of ADM has been described in general patient populations, in medical patients, in patients following thoracic surgery, and particularly recently in patients with suspected sepsis in the emergency department [12-14], but the prognostic value has not yet been described in patients with sepsis following major surgery. Especially in patients undergoing major surgery, it is important to discriminate between postsurgical

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inflammation and infection as early as possible. Furthermore, in critically ill surgical patients, important factors in therapy are the abilities to predict the severity of disease and to assess the patients' prognoses.

Thus, we conducted a prospective, observational study to evaluate the ADM levels in the plasma of surgical intensive care unit (ICU) patients using a novel assay that specifically measures bioactive adrenomedullin (bio-ADM). We compared bio-ADM levels with conventionally used parameters like C-reactive protein, procalcitonin, and white blood count.

2. Material and methods

2.1. Population and study protocol

We conducted a prospective, observational clinical pilot trial in the surgical intensive care department of the University Hospital RWTH Aachen, Germany. We enrolled 42 ICU patients with clinical signs of sepsis according to the Society of Critical Care Medicine definitions [15]. All 42 patients underwent major surgery before enrollment and developed sepsis in the postoperative course. Of the 42 ICU patients, 8 patients had sepsis, 19 developed severe sepsis, and 15 suffered from septic shock. We enrolled 14 patients admitted routinely to the ICU directly after major surgery as a control group to evaluate the effect of surgery on bio-ADM levels (Table 1). Four (7%) of 56 patients underwent major trauma surgery, 9 (16%) of 56 patients underwent major brain surgery, 15 (27%) of 56 patients underwent major cardiothoracic surgery, and 28 (50%) of 56 patients underwent major abdominal surgery.

The study was approved by the local ethical committee of the University Hospital RWTH Aachen (EK 021/14). All patients or their legal representatives provided written informed consent. Ethylenediaminetetraacetic acid plasma samples to determine single bio-ADM levels were drawn within 16 hours after patients were classified as having sepsis. In the control group, samples were drawn up to 5 hours after ICU admission. CRP and PCT were measured at the same point of time that the bio-ADM levels were determined and were also measured afterward as clinical routine but were not taken into analysis. The laboratory and clinical parameters were recorded for all patients and presented in the "Results" section. The 28- and 90-day mortalities were recorded. Acute Physiology and Chronic Health Evaluation Score (APACHE) II and Sequential Organ Failure Assessment (SOFA) scores were calculated for all patients. Two patients were excluded because of unknown 90-day survival statuses.

2.2. Bio-ADM measurement

All bio-ADM measurements were performed in a blinded manner in the laboratories of Sphingotec GmbH, Hennigsdorf, Germany, with a previously described, commercially available immunoassay [12]. Briefly, a 1-step sandwich-coated tube chemiluminescence immunoassay based on acridinium NHS-ester labeling was used for the detection of human ADM in the plasma.

Mouse monoclonal antibodies (ABs) directed against the middle region of ADM in the solid phase and labeled mouse monoclonal ABs directed against the amidated C-terminal moiety of ADM were used in the assay. To label the anti–C-terminal antibodies (1 g/L), the samples were incubated with MACN-acridinium-NHS (*N*-hydroxysuccinimide)-ester (1 g/L; InVent GmbH, Hennigsdorf, Germany) at a 1:5 molar ratio for 20 minutes at 22°C, and the process was stopped by the addition of a 1/5 volume of 50 mmol/L glycine for 10 minutes at 22°C. As described previously, the labeled antibody was diluted in the assay buffer (300 mmol/L K-phosphate, 100 mmol/L NaCl, 10 mmol/L sodium ethylenediaminetetraacetic acid, 5 g/L bovine serum albumin [protease-free],1 g/L each of nonspecific bovine and mouse IgG, 0.9 g/L Na-azide, 20 tablets per liter Protease Inhibitor Cocktail [Roche Diagnostics GmbH, Penzberg, Germany], 10 μmol/L amastatin, 20 μmol/L leupeptin; pH 7.0). Dilutions of the full-length human ADM peptide (American Peptide Company, Sunnyvale, CA) in Calibrator Dilution Buffer (10 mmol/L Tris, 250 mmol/L NaCl, 2 g/L Triton X-100, 50 g/L bovine serum albumin [protease-free], and 20 tablets per liter Protease Inhibitor Cocktail [Roche AG]; pH 7.0) served as calibrators [12].

The immunoassays were performed with 50-μL plasma samples or calibrators and 200 μL of the labeled detection antibody (800 000 relative light units per 200 μL). Both were added to the coated tubes, incubated for 18 hours at 4°C, and washed 5 times with wash solution (1 mL each). Finally, the chemiluminescence was measured for 1 second using an LB953 Multi-Tube Luminometer (Berthold Technologies GmbH & Co KG, Bad Wildbad, Germany). The analytical assay sensitivity was 2 pg/mL.

2.3. Statistical analysis

The values are expressed as the median and interquartile range (IQR) or as the count and percentage as appropriate. Group comparisons of the continuous variables were performed using the Kruskal-Wallis test. The

Table 1
Patient characteristics on admission

Variable	Total population (N = 56)	Control group (n = 14)	Sepsis (n = 8)	Severe sepsis (n = 19)	Septic shock (n = 15)	*P* value
Demographics						
Sex (male), n (%)	37 (66.1)	9 (64.3)	7 (87.5)	13 (68.4)	8 (53.3)	.440
Sex (female), n (%)	19 (33.9)	5 (35.7)	1 (12.5)	6 (31.6)	7 (46.7)	.440
Age (y), median (IQR)	70 (61-77)	69 (63-74)	58 (35-77)	74 (57-79)	73 (65-78)	.380
BMI (kg/m^2), median (IQR)	26.2 (24.2-29.4)	27.1 (24.5-29.1)	26.1 (23.4-29.6)	25.8 (23.9-28.4)	27.0 (23.7-32.0)	.936
Medical history						
Diabetes (yes), n (%)	12 (21.4)	4 (28.6)	0 (0.0)	3 (15.8)	5 (33.3)	.256
Laboratory variables						
Bio-ADM, (pg/mL), median (IQR)	54.8 (20.3-107.6)	16.2 (11.8-20.0)	25.8 (20.3-40.2)	84.2 (42.7-118.5)	119.7 (83.8-172.6)	<.001
PCT (ng/mL), median (IQR)	0.37 (0.14-1.50)	0.07 (0.05-0.16)	0.31 (0.11-0.57)	0.44 (0.25-3.41)	1.62 (0.39-5.72)	<.001
Crea clearance, (mL/min), median (IQR)	87.2 (59.1-99.7)	87.3 (67.2-96.3)	96.9 (92.7-115.7)	85.4 (32.6-100.2)	80.6 (44.6-91.5)	.113
Creatinine, (mg/dL), median (IQR)	0.87 (0.72-1.20)	0.86 (0.71-1.04)	0.79 (0.69-0.86)	0.88 (0.76-2.58)	0.9 (0.73-1.39)	.449
CRP (mg/dL), median (IQR)	92.3 (15.6-201.4)	3.5 (2.1-5.2)	87.3 (58.5-118)	234.3 (92.0-267)	99.7 (88.1-194)	<.001
WBC (10^9 cells/L), median (IQR)	12.2 (9.0-16.0)	11.8 (10.3-14.3)	12.4 (10.4-13.3)	12.5 (10.8-20.0)	11.4 (7.6-13.7)	.354
Platelets (10^9 cells/L), median (IQR)	217 (144-338)	199 (184-219)	169 (104-299)	246 (180-359)	284 (117-375)	.418
Other						
APACHE II score (points), median (IQR)	16 (11-18.3)	10 (7.5-11)	13.5 (9.8-16)	18 (15-19)	18 (15.5-24.5)	<.001
SOFA score (points), median (IQR)	7 (4-9)	5 (3-6)	4 (3.5-5)	7 (6-9)	8 (8-11)	<.001
Length of ICU stay (d), median (IQR)	8 (1-22)	1 (1-1)	14 (8-21)	11 (6-33)	8 (5-24)	<0.001
Length of hospital stay (d), median (IQR)	17 (8-21)	11 (7-18)	19 (15-23)	17 (11-35)	19 (10-21)	.252
28-d mortality (%)	6 (10.7)	0 (0)	1 (12.5)	2 (10.5)	3 (20.0)	.121
90-d mortality (%)	14 (25.0)	1 (7.1)	2 (25.0)	6 (31.6)	5 (33.3)	.388

BMI indicates body mass index; Crea, creatinine.