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Elevated asymmetric dimethylarginine levels predict short- and long-term mortality risk in critically ill patients☆

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ABSTRACT

Objective: Serum concentrations of asymmetric dimethylarginine (ADMA), an endogenous inhibitor of nitric oxide synthase, may contribute to endothelial dysfunction and organ failure in sepsis. We aimed at investigating ADMA levels as a potential diagnostic or prognostic biomarker in critically ill patients.
Methods: Two hundred fifty-five patients (164 with sepsis, 91 without sepsis) were studied prospectively upon admission to the medical intensive care unit (ICU) and on day 7, in comparison to 78 healthy controls. ADMA serum concentrations were correlated with clinical data and extensive laboratory parameters. Patients' survival was followed up for up to 3 years.
Results: ADMA serum levels were significantly elevated in critically ill patients at admission compared to controls. ADMA levels did not differ between patients with or without sepsis, but were closely related to hepatic and renal dysfunction, metabolism and clinical scores of disease severity. ADMA levels further increased during the first week of ICU treatment. ADMA serum levels at admission were an independent prognostic biomarker in critically ill patients not only for short-term mortality at the ICU, but also for unfavorable long-term survival.
Conclusion: Serum ADMA concentrations are significantly elevated in critically ill patients, associated with organ failure and related to short- and long-term mortality risk.

1. Introduction

Systemic inflammation and sepsis, associated with multiple organ dysfunctions, are key characteristics of patients with critical illness requiring treatment at the intensive care unit (ICU). Nonspecific clinical and physiologic criteria of systemic inflammatory response syndrome and sepsis are used to identify patients at immediate need for ICU therapy. A complex network of biological mediators is dysregulated in critically ill patients, and several circulating parameters have been evaluated as potential biomarkers with diagnostic or even prognostic value [1].

There is increasing experimental evidence that the arginine-nitric oxide (NO) pathway is crucially involved in inflammation, infection and organ injury [2]. NO is a potent vasodilatator, which is produced from L-arginine by the enzyme nitric oxide synthase [3]. Asymmetric dimethylarginine (ADMA), on the other hand, is a naturally occurring non-selective inhibitor of NO synthase [4]. Circulating ADMA is mainly derived from protein catabolism, because dimethylarginines are released as proteins are hydrolyzed, thus representing an obligatory product of protein turnover [5]. Functionally, ADMA impairs (beneficial) NO-dependent endothelial functions such as vascular dilatation or anti-inflammatory processes [4]. In line, circulating ADMA levels have been linked to endothelial dysfunction in cardiovascular diseases and risk factors for atherosclerosis [6].

It had been speculated that ADMA is also involved in the pathogenesis of microvascular dysfunction in sepsis, as increased ADMA could possibly decrease NO bioavailability at the endothelium [7]. Few small studies have investigated circulating ADMA as a novel biomarker in critical care medicine and found elevated ADMA serum concentrations in patients with acute infections and sepsis [7–11]. Although the exact mechanisms of ADMA regulation in these patients remained obscure, ADMA was suggested as a predictor of ICU mortality [7,8,12]. However, these initial studies were limited by their focus on patients with sepsis, by their relatively small cohorts and by the short observation period with respect to mortality as an endpoint. The aim of this study was to investigate ADMA serum concentrations in a large cohort of 255 consecutively enrolled critically ill patients, with or without sepsis, identify associations between ADMA and organ dysfunction, metabolism, and disease severity as well as to assess the prognostic

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value of ADMA to predict ICU and long-term mortality in critically ill patients.

2. Materials and methods

2.1. Study design and patient characteristics

Written informed consent was obtained from the patient, his or her spouse or the appointed legal guardian. Patients who were expected to have a short-term (<72 h) intensive care treatment due to post-interventional observation or acute intoxication were not included in this study [13]. All patient data, clinical information and blood samples were collected prospectively. The clinical course of patients was observed in a follow-up period by directly contacting the patients, the patients' relatives or their primary care physician. Patients who met the criteria proposed by the American College of Chest Physicians & the Society of Critical Care Medicine Consensus Conference Committee for severe sepsis and septic shock were categorized as sepsis patients, the others as nonsepsis patients [14].

The study protocol was approved by the local ethics committee and conducted in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki (ethics committee of the University Hospital Aachen, RWTH-University, Aachen, Germany, reference number EK 150/06).

2.2. ADMA measurements

Blood samples were collected upon admission to the ICU as well as in the morning of day 7 after admission. Importantly, ADMA levels at admission were obtained prior to therapeutic inventions at the ICU, which could potentially influence glucose metabolism, such as parenteral nutrition or insulin administration. After centrifugation at 4°C for 10 minutes, serum and plasma aliquots of 1 mL were frozen immediately at −80°C. ADMA serum concentrations were analyzed using a commercial enzyme immunoassay (Immundiagnostik, Bensheim, Germany). The scientist performing experimental measurements was fully blinded to any clinical or other laboratory data of the patients or controls.

2.3. Statistical analysis

Data are given as median and range due to the skewed distribution of most of the parameters. Differences between two groups were assessed by Mann-Whitney *U* test and multiple comparisons between more than two groups have been conducted by Kruskal-Wallis analysis of variance and Mann-Whitney *U* test for post hoc analysis. Box plot graphics illustrate comparisons between subgroups and they display a statistical summary of the median, quartiles, range, and extreme values. The whiskers extend from the minimum to the maximum value excluding outside and far out values which are displayed as separate points. An outside value (indicated by an open circle) was defined as a value that is smaller than the lower quartile minus 1.5 times interquartile range, or larger than the upper quartile plus 1.5 times the interquartile range. A far out value was defined as a value that is smaller than the lower quartile minus three times interquartile range, or larger than the upper quartile plus three times the interquartile range [15]. All values, including "outliers", have been included for statistical analyses. Correlations between variables have been analyzed using the Spearman correlation tests, where values of $P < .05$ were considered statistically significant. All single parameters that correlated significantly with ADMA levels at admission were included in a multivariate linear regression analysis with ADMA as the dependent variable to identify independent (meaningful) predictors of elevated ADMA. The prognostic value of the variables was tested by univariate and multivariate analysis in the Cox regression model. Kaplan Meier curves were plotted to display the impact on survival [16]. Receiver operating characteristic (ROC) curve analysis and the derived area under the curve (AUC) statistic provide a global and standardized appreciation of the accuracy of a marker or a composite score for predicting an event. ROC curves were generated by plotting sensitivity against 1-specificity [17]. All statistical analyses were performed with SPSS (SPSS, Chicago, IL).

3. Results

3.1. ADMA serum levels are significantly elevated in critically ill patients, but not related to sepsis

In order to investigate ADMA in critical illness, we measured ADMA serum concentrations in a large cohort of medical ICU patients at admission (before therapeutic intervention) and on day 7 (Table 1). We enrolled 255 patients (149 male, 106 female with a median age of 63 years; range 18-90 years) who were admitted to the General Internal Medicine ICU at the RWTH-University Hospital Aachen, Germany (Table 1). As a control population we analyzed 78 healthy blood donors (50 male, 28 female; median age 30, range 18-67 years) with normal values for blood counts, C-reactive protein and liver enzymes. ADMA serum levels were significantly higher in ICU patients ($n = 255$, median 0.48 μmol/L, range 0.14-2.0) as compared with healthy controls ($n = 78$, median 0.36 μmol/L, range 0.23-0.57, $P < .001$; Fig.1 A). No association between ADMA levels and sex or age were observed in controls (data not shown).

Among the 255 critically ill patients enrolled in this study, 164 patients conformed to the criteria of bacterial sepsis (Table 1). Pneumonia was identified in the majority of sepsis patients as origin of infection (not shown). Non-sepsis patients were admitted to the ICU mainly due to cardiopulmonary diseases (myocardial infarction, pulmonary embolism, and acute decompensated heart failure), decompensated liver cirrhosis or other critical conditions and did not differ in age or sex from sepsis patients. Sepsis patients were more often in need of mechanical ventilation in longer terms as compared to the non-sepsis patients' cohort (Table 2). In sepsis patients significantly higher levels of routinely used biomarkers of inflammation (ie, C-reactive protein, procalcitonin, white blood cell count) were found (data not shown). Both groups did not differ in Acute Physiology and Chronic Health Evaluation (APACHE) II-, Sequential Organ Failure Assessment (SOFA) and simplified acute physiology score-2 score, vasopressor demand, or laboratory parameters indicating liver or renal dysfunction (Tables 1-2, and data not shown). Among all critical care patients about 25% died at the ICU; during the follow-up period of up to 3 years, a total of 47% of the initial cohort had died (Table 2). By direct comparison between septic and non-septic patients, ADMA serum levels did not differ between patients with or without sepsis (Table 1, Fig. 1B).

In 44 patients, paired blood samples were available for ADMA measurements at ICU admission and at day 7 of ICU treatment. Remarkably, individual ADMA levels increased during the first week

Table 1
Baseline patient characteristics and ADMA serum measurements

Parameter	All patients	Sepsis	Non-sepsis
Number	255	164	91
Sex (male/female)	149/106	96/68	53/38
Age median (range) [years]	63 (18-90)	64 (20-90)	61 (18-85)
APACHE-II score median (range)	17 (2-43)	19 (3-43)	15 (2-33)
SOFA score median (range)	9.5 (0-19)	11 (3-19)	7 (0-16)
pre-existing diabetes n(%)	75 (29.4%)	46 (28%)	29 (31.9%)
ADMA day 1 median (range)[μmol/L]	0.48 (0.14-2.00)	0.47 (0.14-2.00)	0.49 (0.16-1.65)
ADMA day 7 median (range) [μmol/L]	0.71 (0.43-1.94)	0.70 (0.43-1.94)	0.78 (0.51-1.62)

For quantitative variables, median and range (in parenthesis) are given.