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Original Article

Dimethylarginines in critically ill patients with severe sepsis or septic shock

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ABSTRACT: ***Objective:*** *We investigated the trend of asymmetric dimethylarginine (ADMA) and symmetric dimethylarginine (SDMA) in patients with severe sepsis and septic shock, and the relationship between dimethylarginines, organ failure, sepsis status and intensive care unit (ICU) mortality.*
Design: *A prospective observational study carried out in three general adult Italian ICUs. Patients: Consecutive patients with severe sepsis or septic shock admitted to ICUs between December 2004 and March 2007.*
Methods: *Plasma ADMA and SDMA were determined immediately after enrolment and on the third, sixth and twelfth days. Organ failure score, sepsis status, standard blood parameters and ICU outcome were collected.*
Results: *Twenty-nine consecutive patients were enrolled. ADMA and SDMA were higher in patients than in healthy volunteers. Sixteen patients who suffered from septic shock during ICU stay had a higher average value of daily ADMA and SDMA. ADMA was significantly related to arterial pH. SDMA was related to diabetes mellitus, Simplified Acute Physiology Score II, arterial pH level, daily Sequential Organ Failure Assessment score, and creatinine. Independent variables predicting ICU mortality were Simplified Acute Physiology Score II, mean Sequential Organ Failure Assessment score, and SDMA.*
Conclusion: *Dimethylarginines are not only markers of body catabolism but may have a high metabolic activity, tightly related to the risk of adverse outcome of sepsis syndrome. (Nutritional Therapy & Metabolism 2007; 25: 189-94)*

KEY WORDS: Sepsis, Dimethylarginines, Nitric oxide, Organ failure, Intensive care unit, Critically ill

INTRODUCTION

The biosynthesis of symmetric dimethylarginine (SDMA) and asymmetric dimethylarginine (ADMA) occurs during the methylation of protein arginine residues in a process catalyzed by the action of protein arginine methyltransferases I and II (1). Type I protein arginine methyltransferase is responsible for the formation of ADMA, while type II protein arginine methyltransferase produces SDMA. Protein methylation is principally irreversible and arginine-methylated proteins are mainly engaged in RNA processing, transcriptional control and nucleocytoplasmic transport (2, 3). Methylated proteins release ADMA and SDMA upon their proteolytic degradation during physiological protein turnover.

A major metabolic route for ADMA is decomposition to citrulline and dimethylamine in a reaction catalyzed by dimethylarginine dimethylaminohydrolase, highly expressed under normal conditions in liver, kidney, pancreas and endothelial cells. It is besides eliminated by renal excretion (4, 5). SDMA seems to be strictly eliminated by renal excretion (6, 7). Dimethylarginines compete with arginine for cationic amino acid transporters of system y+ (8), whereas ADMA is also an endogenous inhibitor of all isoforms of nitric oxide synthase (NOS) (9, 10).

The free radical nitric oxide (NO), produced from L-arginine by constitutive and inducible isoforms of synthase, is recognized as a potent multifunctional molecule which may play a both beneficial and detrimental role in vivo. Constitutive NO acts as a neurotransmitter and regulates vascular tone as well as deregulating the interac-

tion between vascular endothelium and blood platelets and leukocytes (11). NO synthesized by inducible NOS has an immunoregulatory function: it modulates cytokine production and cytoprotection as a free radical scavenger (12). Increased NO production by inducible NOS and decreased production by constitutive NOS seem to occur in infection, inflammation, trauma, heart attack, renal failure, sepsis resulting in oxidative stress, excessive inflammation, disseminated coagulopathy, alteration of microvascular endothelium, organ perfusion and resulting organ injury. However, administration of NG-monomethylarginine, a pharmacological nonspecific NOS inhibitor analogous to ADMA, led to increased mortality in septic patients (13), documenting an adverse effect on nonselective NO inhibition.

Critically ill patients are hypercatabolic and may present impaired renal and hepatic function. In addition, dimethylarginines may be elevated due to the combination of increased elaboration and reduced elimination. Moreover, there is experimental and clinical evidence indicating a causal relationship between development of oxidative stress and elevation of ADMA. For example, oxidative stress enhances the activity of protein arginine methyltransferase and compromises the activity of dimethylarginine dimethylaminohydrolase. This subsequently provokes profound accumulation of ADMA. In the presence of high ADMA levels, active dimeric isoforms of NOS may become uncoupled and a new catalytic function of NOS is established: generation of superoxide anion. In this way, ADMA is not just a marker but also a mediator of oxidative stress (14).

Variable levels of ADMA and SDMA were recently described in different clinical settings, with the highest concentration of ADMA being found in septic patients. Although a correlation between ADMA and organ failure has been described, the data are still controversial (15-17). Moreover, little attention has been paid to the role of SDMA and adverse outcome in critically ill patients. We aimed to investigate the trend of ADMA and SDMA in high-risk patients with severe sepsis and septic shock, and to elucidate the relationship between dimethylarginines, organ failure, sepsis status and ICU mortality.

PATIENTS AND METHODS

The study was approved by the hospital ethics committee and informed written consent was obtained from each patient or a first-degree relative. Consecutive patients with severe sepsis or septic shock (18) were enrolled between December 2004 and March 2007 in 3 general adult Italian ICUs.

Patients were treated according to the Surviving Sepsis Campaign (19). On the day of admission, artificial nutrition (enteral, mixed or parenteral), intended to deliver 25 kcal/kg/day as maximum level of nutritional support was started in all patients. Blood glucose level was not allowed to exceed 180 mg/dL.

Blood sampling was performed immediately after enrolment (T0) and on the third (T3), sixth (T6) and twelfth (T12) day. ADMA and SDMA were determined in plasma by extraction and high performance liquid chromatography (HPLC) assay as previously described (20). Briefly, 0.1 mL plasma supplemented with L-homoarginine as internal standard (50 µL; 1 nmol) was applied to a cation-exchange solid-phase extraction cartridge (Phenomenex STRATA SCX 100 mg/mL, Chemtek Analitica, Bologna, Italy) previously activated with methanol (1 mL) and trichloroacetic acid (TCA) (2%, 2mL). After the column washings (TCA 2%, 150 mmol/L phosphate buffer pH 8.0, methanol), the amino acids were eluted with 1 mL of 2% triethylamine solution in methanol:water; 70:30; v:v). The eluate was dried under nitrogen, redissolved in 0.1 mL bidistilled water, and derivatized with ortho-phthaldialdehyde reagent (1:1; v:v, 1 min) before analysis. The HPLC device was equipped with a fluorescent detector (λ 340 nm, λ_{em} 455 nm) and an Ultrasphere ODS (250x4.6mm, 5 m) column. Baseline separation of the methylated arginines was achieved with a gradient between mobile phase A (sodium citrate buffer, 50 mmol/L, pH 6.2) and B (distilled water:acetonitrile: methanol; 1:2:2; v:v:v).

We collected the Simplified Acute Physiology Score II (SAPS II) (21) at ICU admission, and, at any sampling time, Sequential Organ Failure Assessment (SOFA) score (22), sepsis status (18), standard blood parameters (creatinine, bilirubin, platelets, white blood cells, mean daily glucose concentration, pH) and ICU outcome. ADMA and SDMA levels were compared to normal values. The average daily level of both amino acids during ICU stay was compared between patients who developed septic shock and those who did not. For each time point, we checked the relationship between ADMA/SDMA levels and preexisting comorbidities, diagnosis, SAPS II at admission, and daily values of SOFA score, sepsis status and blood parameters. Moreover, we compared at each time point the daily values of dimethylarginines registered in deceased vs surviving patients. We checked also which of these variables were associated with ICU mortality.

Statistical analysis

All data are expressed as mean ± standard deviation (SD). We used Anova with Scheffe's correction and Student's *t*-test to assess differences between groups. Relation-

ship was assessed by linear regression analysis. For each time point we built a multivariate regression model with variables selected by univariate analysis.

We tested a general linear mixed model for repeated measures based on every single patient for ICU mortality. The variables associated with ICU mortality assessed by means of univariate logistic regression analysis were included with the stepwise technique into a multivariate logistic regression model. Odds ratios (OR) for each variable were computed. The ability of the final model to predict outcome was assessed by the area under the receiver operating characteristic (ROC) curve. Calibration ability was measured with the Hosmer-Lemeshow goodness-of-fit test.

We considered $p < 0.05$ to be statistically significant. Statistical analysis was performed using the Stata statistical software, release 9.2 (Stata Corporation, College Station, TX, USA).

RESULTS

Twenty-nine consecutive patients were enrolled in the study. The demographics of the study population are given in Table I. Only T0 and T3 blood samples were available for analysis in 2 patients; 8 patients had blood samples until T6 and 19 patients had blood samples available for the 4 time points.

ADMA (0.98 ± 0.48 µmol/L) and SDMA (2.1 ± 1.05 µmol/L) levels at T0 were 2 and 5 times higher, respectively, than those found in healthy volunteers (77 healthy volunteers, 38 male, age 41 ± 16 years, creatinine value 0.76 ± 0.13 mg/100 mL) and did not decrease during treatment (Tab. II). Sixteen patients who suffered septic shock during ICU stay had a higher average value of daily ADMA (1.15 ± 0.51 µmol/L, n = 56 vs 0.91 ± 0.33 µmol/L, n = 48, p = 0.0084) and SDMA (2.71 ± 1.52 µmol/L, n = 56 vs 1.72 ± 0.89 µmol/L, n = 48, p = 0.0001) than patients with only severe sepsis.

ADMA was significantly related to arterial pH (T0: b = -1.85 R2 = 0.35 p = 0.028; T3: b = -4.51 R2 = 0.587 p = 0.003). SDMA was related at T0 (R2 = 0.295) to diabetes mellitus (b = 1.104 p = 0.016), SAPS II (b = 0.32 p = 0.049), and arterial pH level (b = -4.3 p = 0.031). Moreover, SDMA was related at T3 (R2 = 0.715), T6 (R2 = 0.690) and T12 (R2 = 0.980) to daily SOFA score (T3: b = 0.163 p = 0.006; T6: b = 0.2 p = 0.027) and creatinine (T3: b = 0.403 p = 0.001; T6: b = 0.466 p = 0.009; T12: b = 0.826 p = 0.007).

The SDMA concentration was significantly higher in dead than alive patients at T3, T6 and T12 as represented in Figure 1. Independent variables predicting ICU mortality in the linear mixed model were SAPS II on admission (OR = 1.08 per point, p = 0.017), mean SOFA score during ICU stay (OR = 1.39 per point, $p < 0.0001$) and SDMA (OR = 2.25, p = 0.002). The area under the ROC curve was 0.912. Calibration ability gave a p = 0.9625.

TABLE I - DEMOGRAPHICS OF STUDY POPULATION

Number of patients	29
Gender male/female	21/8
Age (mean ± SD)	62 ± 11
Admission day	
Type: Medical / Surgical	23/6
Pneumonia	15 (57.69%)
Peritonitis	6 (22.22%)
Urinary infection	5 (19.23%)
Fournier's syndrome	1 (3.44%)
Osteomyelitis	1 (3.44%)
Soft tissue infection	1 (3.44%)
SAPS II (points)	43.9 ± 12.0
Septic shock	14/29 (48.3%)
SOFA score (points)	9.3 ± 3.7
SOFA score (organ system ≥ 3 points)	
Respiratory failure	21/29 (72.4%)
Coagulation failure	6/29 (20.7%)
Hepatic failure	3/29 (10.3%)
Cardiovascular failure	18/29 (62.1%)
Neurological failure	1/29 (3.5%)
Renal failure	5/29 (17.24%)
Whole ICU stay	
Worst SOFA score	9.9 ± 3.2
Septic shock	16/29 (55.2%)
Length of stay (days)	16.3 ± 10.6
Mortality	7/29 (24.1%)

SAPS II, Simplified Acute Physiology Score II; SOFA, Sequential Organ Failure Assessment; ICU, intensive care unit

TABLE II - DIMETHYLARGININE LEVELS BY TIME POINTS

	Number	ADMA	SDMA
Healthy volunteers	77	0.46 ± 0.12 (0.19-0.73)	0.43 ± 0.99 (0.19-0.66)
Patients			
T0	29	0.99 ± 0.49 (0.41-2.48)	2.10 ± 1.06 (0.81-4.09)
T3	29	1.06 ± 0.47 (0.52-2.43)	2.31 ± 1.20 (0.60-5.39)
T6	27	1.09 ± 0.49 (0.54-2.73)	2.26 ± 1.47 (0.57-6.79)
T12	19	1.03 ± 0.32 (0.68-1.8)	2.38 ± 1.84 (0.75-8.16)

Mean values of ADMA and SDMA in mol/L at each time point in study population and healthy volunteers. All data $p < 0.001$ vs normal values

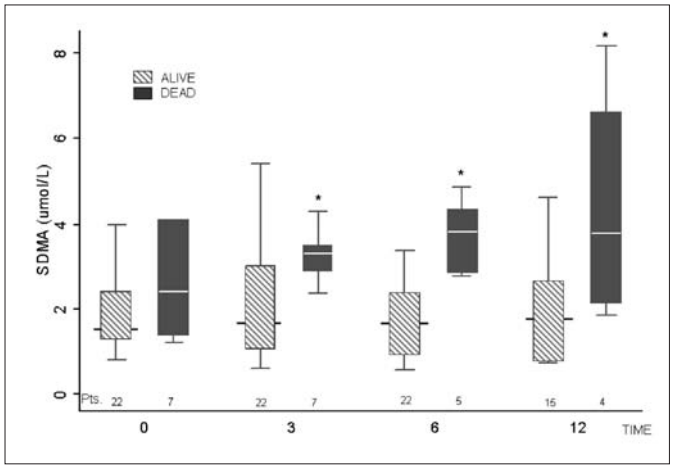

Fig. 1 - *SDMA concentration in dead or alive patients during ICU stay. Values in µmol/L are depicted as median, interquartile range and upper and lower reference range.*
**p < 0.01. Pts., number of patients*

DISCUSSION

There are at least 2 interesting reasons for studying methylarginines in critically ill patients. Methylarginines could be a marker of protein breakdown like other methylated amino acids (23) but of greater importance because specifically deriving from nuclear proteins (2, 3). Moreover, ADMA and SDMA could have a metabolic role that is closely related to the development of systemic inflammatory reactions (8-10).

A population of highly stressed critically ill patients is a prerequisite to testing these hypotheses. For this purpose we chose to study patients with severe sepsis (18). The patients selected for our study were really at high risk (24): about 80% had pneumonia and peritonitis, and more than 50% had septic shock. The severity of their condition and their organ failures (more than 60% of patients were admitted with severe respiratory and cardiovascular dysfunction) resulted in a considerable mortality rate and a quite long ICU stay (Tab. I).

In this setting, ADMA and SDMA plasma levels were 2 and 5 times higher, respectively, than in healthy volunteers on the day of admission and all along the ICU stay. The ADMA levels in our study were similar to those recently reported in the only study on septic patients (17) but were higher than those reported in less critically ill patients (15, 16). In fact, in addition to increased production and reduced elimination (organ failure), oxidative stress and cytokine release may cause reduction of dimethylarginine dimethylaminohydrolase activity in liver, kidney and pancreas, further contributing to the increase in ADMA levels (25, 26). Concerning SDMA, we observed a greater accumulation compared with data reported in the only study on critically ill patients where SDMA was evaluated (15). Unfortunately, SDMA evaluation is lacking in the study by O'Dwyer et al on patients with septic shock (17).

The main determinants of dimethylarginine concentrations at ICU admission were preexisting diabetes (SDMA and a trend for ADMA), as already observed (27), blood acidosis (SDMA and ADMA), and severity of acute organ failure (SDMA), previously described by O'Dwyer only for ADMA. The impact of diabetes, severity of clinical conditions, and acidosis on dimethylarginines could be explained by an increased chronic and acute body protein catabolism due to a stress state.

In the following ICU days, during a full-blown stress reaction we found a close relationship between SDMA (and not ADMA as reported in the literature by Nijveldt et al [15] and O'Dwyer et al [17]) and the number and severity of organ failure. Moreover, SDMA concentration was strongly and independently related to the presence of renal failure. This is not surprising since urinary excretion is the main eliminating process in humans (6, 7).

In conclusion, ADMA but particularly SDMA seem to be reliable markers of derangements of vital functions in critical conditions. We registered overall ICU stay ADMA and SDMA concentrations that were 20% and 63% higher, respectively, in patients who developed septic shock during ICU stay in comparison to patients with severe sepsis as the highest level of septic status. Moreover, SDMA levels were higher in dead than in alive patients during the entire ICU stay (Fig. 1).

Dimethylarginines could play a role in organ damage in sepsis. Overproduction of NO as part of the inflammatory/immune response to infection is implicated in the genesis of sepsis (28). In animal and human studies the nonselective inhibitors of both constitutive and inducible isoforms of NOS gave evidence that inhibition of the constitutive NOS is unfavorable (13, 28-30). High dimethylarginine concentrations, through a reduction of arginine cell transport and/or a nonselective block of constitutive NOS, may contribute to pathological changes in the vascular wall, resulting in impaired organ blood flow, endothelial damage, interstitial edema, and immunological changes interfering with physiological functions of NO. These features subsequently impair the already compromised organs and place other organs at risk of taking part in the cascade of failing organs.

Interestingly, available studies in critically ill patients show that ADMA is an independent predictive factor of ICU mortality for the most severe patients (highest quartile of statistical distribution - 15) or that an ADMA increase between day 0 and day 2 and between day 7 and the last day of ICU stay are significantly related to ICU

mortality (16). By contrast with literature data (15-17), in our patients ADMA appeared a less potent indicator both of organ failure and ICU survival, probably because of the high variability. SDMA, on the other hand, is an independent predictor of ICU mortality as well as robust clinical parameters like SAPS II and mean SOFA score of the whole ICU stay. The literature's hypothesis that dimethylarginines could play a specific role in the microvascular derangement associated with sepsis seems therefore reasonable.

CONCLUSIONS

We found elevated levels of dimethylarginines in high-risk critically ill patients with sepsis syndrome. Taken together, our results strongly suggest that dimethylarginines are not only markers of body catabolism but may have a high metabolic activity, tightly related to the risk of adverse outcome of sepsis syndrome.

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