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Assessment of Asymmetrical Dimethylarginine metabolism in Patients with Critical Illness.

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Abstract

Background: Critically ill patients experience metabolic disorders including hypercatabolic state and hyperglycaemia and these are associated with poor outcome. Hyperglycaemia and asymmetric dimethylarginine (ADMA) are reported to have significant influences on endothelial dysfunction. The aim of the present study was to examine the relationship between plasma asymmetric dimethylarginine (ADMA) and related arginine metabolism in patients with critical illness.

Materials and Methods: Two venous blood samples (EDTA) (104 patients), on admission and follow up sample in the last day in ICU (died or discharge sample median 7, IQR 6-8, range 5-15). Plasma ADMA, arginine, homoarginine and symmetrical dimethylarginine (SDMA) were measured by high-performance liquid chromatography (HPLC).

Result: ADMA (p<0.01) and SDMA (p<0.05) were elevated and homoarginine was decreased (p<0.05) in non-survivors and were directly associated with predicted mortality rate (p<0.05 and p<0.001), SOFA (p<0.05, p<0.001), ICU stay (p<0.05, p<0.001) and mortality (p<0.01, p<0.05). ADMA was directly associated with SDMA (p<0.001), albumin (p<0.05), ICU stay and mortality (p<0.01). SDMA was directly associated with creatinine (p<0.001) and APACHE II score (p<0.001). In the follow up measurements there was a significant decrease in SOFA score (p<0.01), homoarginine (p<0.01), ALT (p<0.01), Lab-Glucose (p<0.01), and albumin (p<0.01). In contrast, there was an increase in arginine ((p<0.01), ADMA (p<0.01), ADMA:SDMA ratio (p<0.01), and the norepinephrine administration (p<0.01).

Conclusion: In the present longitudinal study ADMA metabolism was altered in patients with critical illness and was associated with disease severity and mortality.

Key words: Homoarginine; arginine; asymmetric dimethylarginine; symmetrical dimethylarginine; critical illness.

Key messages:

1. The significant elevation of ADMA and SDMA in patients with critical illness were directly associated with disease severity and mortality.
2. Plasma homoarginine concentrations were significantly inversely associated with C-reactive protein suggesting an effect of inflammation on homoarginine synthesis and/or metabolism.
3. Plasma homoarginine concentrations were lower in patients with critical illness and were also associated with disease severity and mortality.

Introduction

Patients with critical illness are often hypercatabolic and hyperglycaemic, and these metabolic disorders have been shown to have a significant influence on patient outcome [1;2]. Furthermore, several studies have reported a beneficial effect of controlling hyperglycaemia on morbidity and mortality [1;3]. However, the mechanisms by which these beneficial effects are brought about remain unclear. One possible mechanism is the amelioration of endothelial dysfunction related to endogenous nitric oxide synthase (NOS) inhibitors [1;4;5].

Endothelial dysfunction has been reported in association with insulin resistance and increased concentrations of the NOS inhibitor asymmetric dimethylarginine (ADMA) which may lead to a reduction in the bioavailability of nitric oxide (NO) (**Figure 1**) [6;7]. ADMA has been implicated in the endothelial dysfunction accompanying a broad range of clinical disorders such as obesity, cardiovascular disease, chronic renal failure, diabetes and hypertension [7-9]. Indeed, a significant increase in plasma ADMA concentration has been reported in individuals with impaired glucose tolerance [10], diabetes [11] and in the overweight, in whom weight loss was associated with a reduction in ADMA and improved markers of insulin sensitivity [10]. It is of interest that, in patients with acute illness, increased ADMA concentration has been associated with increased mortality and several studies have reported ADMA concentration as an independent risk factor for adverse outcomes in patients with critical illness. For example, in the intensive care unit (ICU) and in severe sepsis [1;12-14] and in patients presenting to the emergency department [15]. Siroen reported that there was a significant reduction in ADMA concentrations in critically ill patients who received intensive insulin treatments compared with patients who were treated conventionally [1]. This reduction was associated with a significant improvement in mortality [1;4]. In the critically ill patients higher concentrations of ADMA have been associated with increased mortality [10]. Furthermore improved outcomes have been reported with intensive insulin therapy, with amelioration of high ADMA concentrations a possible mechanism for this beneficial effect [1;4], possibly through preservation of dimethylaminohydrolase (DDAH) activity, the key enzyme involved in ADMA degradation. Moreover, insulin may decrease the degradation of arginine and thereby reduce the production of ADMA [16]. In contrast, however, Iapichino (2008) reported that intensive insulin treatments, while achieving tight glucose control, did not reduce ADMA levels in patients with septic shock who were fed with no more than 25 kCal/kg per day [17].

It has been reported during acute inflammatory response of elective surgery plasma ADMA concentration decreases rapidly in the first 48 hours [18]. It has also been shown that inflammatory markers, such as interleukin-6 (IL-6), C-reactive protein (CRP) and tumour necrosis factor α are inversely associated with ADMA concentrations and the ADMA:SDMA ratio, and this has been proposed to be the result of increased (DDAH-mediated) catabolism induced by an inflammatory response [13].

The aim of the present study was to examine the relationships between the concentrations of plasma ADMA and related compounds (homoarginine, arginine, SDMA), length of stay and mortality in patients with critical illness.

Methods

Patients and study design

Patients in the intensive care unit (ICU) of the Royal Infirmary, Glasgow who were ≥ 18 years old, and had evidence of the systemic inflammatory response syndrome as per 1992 consensus criteria [19, 20] were studied. There were no other exclusion criteria. The study was approved by the ethics committees of the North Glasgow NHS Trust and Multicentre Research Ethics Committee (MREC) Scotland. Where patients were unable to give signed informed consent, consent was obtained from the patients' next of kin or welfare guardian in accordance with the requirements of

the Adults with Incapacity Scotland (2000) Act.

Blood samples for population references values were obtained from laboratory staff, from local health centres and from people attending a cardiovascular risk clinic. None of the subjects were taking any vitamin supplements or had evidence of a systemic inflammatory response (serum C-reactive protein <10 mg/ l).

Data collection.

Baseline demographics including age, ICU admission cause, and ICU length of stay were extracted from ICU computer using Carevue programme. Such data was collected as part of routine clinical care including laboratory blood glucose, C-reactive protein (CRP), white cell count, albumin, creatinine, alanine aminotransferase (ALT) and liver function tests. Drugs and their dosages that may have influenced blood glucose were also recorded such as insulin, epinephrine, norepinephrine, dobutamine and hydrocortisone. APACHE II score and predicted hospital mortality and SOFA scores were recorded.

ICU insulin protocol

The blood glucose concentration were targeted at 4.4-6.9 mmol/l (80-124 mg/dl) using a standardised insulin infusion protocol using insulin infusion with a concentration of 50 units of insulin in 50 ml 0.9% saline.

There were several enteral feeding preparations used in the ICU such as Jevity (1 Kcal/ml), Jevity Plus (1.2 Kcal/ml), Jevity 1.5 (1.5 Kcal/ml) and Osmolite (1 Kcal/ml), Osmolite Plus (1.2 Kcal/ml), Peptisorb (1 Kcal/ml), Perative (1.31 Kcal/ml) and Nepro (2 Kcal/ml). Patients routinely received 2L of one of these preparations per day, amounting to an intake of 2000-2500 calories per day.

Analytical methods

Collection and preparation of blood samples.

Venous blood samples (EDTA) were withdrawn on admission (day 1) as well as a follow up sample on the last day of the ICU admission whether the patient died or was discharged (median 7, IQR 6-8, range 5-15 days) for the analysis of plasma ADMA, arginine, homoarginine and SDMA. Blood samples were centrifuged (500 g, 10 minutes), the plasma removed and stored at -70°C until analysis. Blood samples that formed part of patients' clinical care were handled in the usual way according to established standard operating procedures in the hospital biochemistry laboratory.

Laboratory analysis

ADMA, arginine, homoarginine and SDMA were measured using isocratic high performance liquid chromatography (HPLC) with fluorescence detection as previously described [21]. The coefficients of variation (CV) were approximately 3% for the analytes. Reference values for the studied analytes were derived from 86 healthy people [21]. In the present study the control group

was not age and sex matched to the ICU patients rather they were to provide a "normal" reference range for the analytes measured in the patients with critical illness.

Blood glucose, CRP, white cell count, albumin, creatinine and alanine aminotransferase (ALT) were measured in accordance with the manufacturer's instructions on an automated analyzer (Architect; Abbott Diagnostics, Abbott Park, Chicago, IL) in the routine accredited biochemistry laboratory (Clinical Pathology Accreditation UK). For CRP, the limit of detection was 0.5 mg/L. The interassay CV was < 5 % over the sample concentration range for all of the analytes measured.

Statistical analysis

As the data was not normally distributed it was presented as median (median, IQR) and non parametric testing used as appropriate. The relationship between patient characteristics and plasma arginine, homoarginine ADMA and SDMA concentrations were carried out with the use of the Mann-Whitney *U* test. Correlations between variables in the control and critically-ill groups were carried out using the Spearman rank correlation. Data from different time points in the patient groups were tested for statistical significance with the use of the Wilcoxon signed rank test. Due to the number of unpaired and paired statistical comparisons carried out a *P* value of <0.01 was considered to be significant. With reference to ICU mortality data were analysed by univariate binary logistic regression analysis and only variables at the *P*<0.05 significance levels were included in multivariate analysis. Analysis was performed with the use of SPSS software (version 19; SPSS Inc., Chicago, IL).

Results

In total, one hundred and four adult patients with critical illness (medical n=44, surgical n=60) admitted to Glasgow Royal Infirmary ICU in the period between September 2006 to March 2008 were studied. The characteristics of the patients with critical illness on admission are shown in **Table 1**. The majority of patients were male (69%) and had median age of 61 years. The median APACHE II score was 21, predicted hospital mortality rate was 39% and SOFA score was 7. All patients had sepsis and in particular, 10 and 5 patients were treated for severe sepsis and septic shock respectively. Compared with controls (n=86), patients with critical illness had lower median arginine and homoarginine concentrations and higher ADMA and SDMA concentrations (all $p<$ 0.001). Also, critically ill patients had lower ADMA: SDMA and arginine: ADMA ratios and higher total dimethylarginine (sum of ADMA and SDMA) concentrations (both $p<$ 0.001).

The relationship between patient characteristics, plasma ADMA concentrations, other parameters and ICU death is shown in **Table 2**. Non-survivors had significantly higher SOFA scores (p < 0.001), higher ADMA, SDMA and total dimethylarginine concentrations (all p < 0.05), lower homoarginine concentrations (p < 0.028) and lower albumin concentrations (p < 0.017). They also had a longer ICU length of stay (p < 0.001).

Univariate and multivariate binary logistic regression analysis of clinical characteristics and dimethylarginine concentrations on admission to ICU and ICU death in critically ill patients is shown in **Table 3**. On univariate binary logistic regression analysis of the significant parameters identified in

comparison of survivors and non-survivors, only SOFA scores (p <0.01), ICU length of stay (p<0.01), homoarginine (p<0.05), ADMA (p< 0.01), ADMA+SDMA (p<0.05) and daily fluid balance were significantly associated with ICU mortality. On multivariate binary logistic regression analysis of these significant parameters, only SOFA score (p <0.01) was independently associated with ICU mortality. When SOFA score was removed from the multivariate analysis only ADMA (OR= 23.7, p<0.01) and ICU length of stay (OR= 1.04, p< 0.01) were independently associated with ICU mortality.

Of the 104 patients who were admitted into the ICU, 33 patients had a second blood sample measurement of plasma ADMA concentrations (**Table 4**) prior to discharge (n=17) or death (n=16). The remaining patients did not have a blood sample due to discharge (n = 61) or death (n = 10). The former patients spent longer in ICU (median 16 days) than the latter patients (median 3 days) and therefore were more likely to have a second blood sample. The median time between admission and the last sample was 7 (range 5-15, IQR 6-8) days. There was a significant decrease in SOFA score (p < 0.02), arginine (p<0.008), homoarginine (p<0.001), ALT (p < 0.012), Lab-Glucose (p<0.012), and albumin (p<0.012). There was a significant increase in ADMA (p<0.009), ADMA: SDMA ratio (p< 0.003), and the administration of norepinephrine (p< 0.017).

Discussion

Plasma dimethylarginines, especially ADMA, have been previously reported to be associated with mortality in critically ill patients, and have been speculated to be a cause of endothelial dysfunction and multiple organ failure in this patient group [1;13;14;22]. In the present study it was shown that plasma concentrations of ADMA and SDMA were significantly higher in critically ill patients compared with healthy controls and that both were significantly associated with disease severity and mortality. The strength of association between ADMA and mortality was striking (odds ratio ~ 30 and was directly associated with organ dysfunction (SOFA), most closely associated with mortality [13]. When SOFA scores were removed from the analysis, the admission ADMA was independently associated with ICU mortality. This association supports the hypothesis that ADMA metabolism may play a causative role in endothelial dysfunction through impairment of NOS activity. However, further studies of ADMA metabolism are warranted in patients with critical illness.

Homoarginine is an amino acid largely generated from lysine and may promote endothelial function as a substrate for NO synthesis [23], although it may be present at too low a concentration for this to be important, given the relative concentration of arginine is about 30 times greater [21]. A potentially more important role that has received some attention is in the inhibition of the enzyme arginase with consequent preservation of arginine for NOS [24]. Indeed, low homoarginine concentrations have been recently shown to be associated with mortality and cardiovascular events [25]. Furthermore, increased homoarginine concentrations during normal pregnancy are associated with increases in brachial artery diameter and flow-mediated dilatation [26]. In the present study, homoarginine was positively associated with arginine (r_s = 0.641, p<0.001), consistent with the above concept. Indeed, homoarginine was significantly inversely associated with C-reactive protein (r_s= -0.349, p= 0.001) suggesting an effect of inflammation on homoarginine synthesis and/or metabolism.

In the present study SDMA was also associated with increased mortality. However, there was a strong association between SDMA and creatinine concentrations on admission (n= 104, r_s= 0.62, p<0.001) and on follow-up (n= 60, r_s= 0.76, p<0.001) and therefore plasma SDMA concentrations, in the main, reflect renal function, which is perhaps not surprising given that its main route elimination is renal. It has previously been speculated that by competing with arginine uptake at cationic amino acid transporters (CAT) SDMA can impair the delivery of the substrate arginine to NOS [27]. However, it has not been established whether the concentrations encountered *in vivo* are sufficient to have a significant effect. Therefore, it may be that the association of SDMA with mortality simply reflects the effect of renal impairment.

ADMA and SDMA are generated by the methylation of arginine residues in proteins by type 1 protein arginine methyltransferases (type 1 PRMTs) and from type 2 protein arginine methyl transferases (type 2 PRMTs) respectively. Daily production of ADMA is approximately 300 µmol with about 10% of this amount excreted unchanged into the urine. The majority is metabolized by dimethylarginine dimethylaminohydrolase (DDAH) to citrulline and dimethylamine. In contrast, SDMA is mainly excreted unchanged in urine and is increasingly being regarded as a sensitive marker of renal function [11;28].

In the present study the ADMA: SDMA ratio was significantly lower in critically-ill patients compared with controls. The ADMA: SDMA ratio has previously been shown to be reduced in patients with acute infections, severe sepsis, post-operatively and in rats following lipopolysaccharide administration; it has been speculated that the reduction in ratio reflects an inflammation-induced increase in ADMA clearance through DDAH activity [13;29-31]. However, in no such study has DDAH activity *in vivo* been assessed, and therefore no conclusion can be reached about this at the present time; indeed in the present study there was no direct association between CRP and ADMA concentration. Inflammatory cytokines have been shown to both increase and decrease DDAH activity in different models [32;33], but the net effect *in vivo*, taking into account the confounding effects of oxidative stress and NO itself, is unclear.

A novel aspect of the present study was the longitudinal measurement of ADMA metabolism. In the present study approximately one third of patients had follow-up analysis. The mortality was greater in those patients with a follow-up sample compared to those who did not (48% vs 25%). Moreover, length of ICU stay was greater in this group (median 16 days vs 3 days). Therefore, this subsample is likely to represent a group of sicker patients. Nevertheless, the ADMA and arginine concentrations increased on subsequent sampling whereas SOFA scores, homoarginine and glucose concentrations fell. In contrast, C-reactive protein concentrations were not altered. Therefore, from these results and from the foregoing discussion it is clear ADMA metabolism in the patient with critical illness is complex. However, a similar pattern has been reported in patients undergoing elective surgery who had a significant inflammatory response, although in those patients this was following a significant reduction in ADMA concentration from baseline [18]. In these patients there was no change in SDMA concentrations, indicating differential handling of the dimethylarginines. However, there was no clear evidence of increased ADMA metabolism, and it may be have been that ADMA transport via CATs was the more important factor [18;34]. This is an area which is worthy of further study in critical illness, as it is becoming clear that the plasma concentrations may not reflect the pathophysiologically important tissue concentrations, which even in health are up to 10 – 20 times that found in plasma [2;32].

There are two distinct isoforms of DDAH. DDAH-1 is widely expressed, particularly in tissues such as liver, renal cortex, lung and immune tissues and cells. There are large arterio-venous gradients across organs such as the liver and kidney, and DDAH-1 has been described as the "guardian" of circulating ADMA concentration [35]. In contrast, DDAH-2 is mainly found in tissues which express endothelial nitric oxide synthase (eNOS) and inducible nitric oxide synthase (iNOS) such as endothelium and smooth muscle cells of cardiovascular system [36;37]. As such, DDAH-2 appear to be more involved in the regulation of local endothelial responses [35]. Consequently, ADMA concentrations in plasma are regulated within fairly narrow limits in health, and it is clear that DDAH-mediated metabolism is the critical step in this regulation [37;38].

The activity of dimethylaminohydrolase (DDAH) is highly dependent on a cysteine residue at its catalytic site. This is susceptible to oxidation as a consequence of oxidative stress and may provide a mechanism that results in increased ADMA concentration and reduced NO formation [37]. This has been observed with hyperglycaemia and hyperhomocystinaemia [37]. Therefore, interaction between dimethylaminohydrolase (DDAH), ADMA and NOS may present a common pathway of several risk factors affecting the vascular endothelium.

Indeed, a recent study in critically ill rabbits showed that tissue DDAH activity was a stronger determinant of plasma than tissue ADMA concentration, suggesting that CAT-mediated exchange of ADMA between compartments might be even more important than changes in DDAH activity itself [39]. Previous work has documented increased fractional excretion of ADMA by the liver during endotoxaemia [30], but, again, this does not differentiate between an increase in CAT-mediated uptake and a true increase in DDAH activity itself. In this regard, it has also been shown that methylarginine uptake into endothelial cells and macrophages increases with cytokine stimulation [34]. With current limitations in knowledge, caution should be exercised when interpreting the ADMA: SDMA ratio that might not be useful as a simple surrogate marker of DDAH activity for these reasons.

Limitations of the present study were principally that it was a single centre study and there was no validation cohort. The cohort was a mix of surgical and medical patients and there was considerable variation in the severity of illness. Furthermore, there were significant attrition of patients on follow up and this limits interpretation of the data and the conclusions that can be reached.

In summary, plasma ADMA and SDMA concentrations were higher in patients with critical illness and were associated with disease severity and mortality. In contrast, plasma homoarginine concentrations were lower in patients with critical illness and were also associated with disease severity and mortality. These results suggest that ADMA metabolism is perturbed with likely knock on effects on NOS and endothelial function. There is a need for further work on in vivo DDAH activity in critical illness and the effect of critical illness on the CAT-mediated exchange of ADMA between intra and extra-cellular compartments.

Conflict of interest statement: None of the authors had personal or financial conflicts of interest.

Ethical approval: The study was approved by the ethics committees of the North Glasgow NHS Trust and Multicentre Research Ethics Committee (MREC) Scotland. Where patients were unable to give signed informed consent, consent was obtained from the patients' next of kin or welfare guardian in accordance with the requirements of the Adults with Incapacity Scotland (2000) Act.

Authors' contributions: The author's responsibilities were as follows— DT, JK and DCM conceived the idea and funded the study; RG and SR carried out the laboratory analysis of the blood samples; clinical information was collected by RG, LW and JK; RG and DCM performed the statistical analysis; all authors contributed to the drafts and final version of the paper and are the guarantors.

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Abbreviations

ICU: Intensive Care Unit.
Lab-Glucose: Laboratory Glucose.
CRP: C-reactive protein.
ALT: aminotransferase.
NOS: nitric oxide synthase.
APACHE II: Acute physiology and Chronic Health Evaluation II score.
SOFA: Sequential Organ Failure Assessment.
HPLC: high-performance liquid chromatography.
CV: coefficients of variation.
ADMA: Asymmetrical Dimethylarginine.
SDMA: Symmetrical Dimethylarginine.
DDAH: dimethylarginine dimethylaminohydrolase.
CAT: cationic amino acid transporters.
IQR: interquartile range

Figure 1: Interactions between oxidative stress, dimethylarginine dimethylaminohydrolase (DDAH), asymmetric dimethylarginine (ADMA) and nitric oxide synthase (NOS). SDMA, symmetric dimethylarginine; PRMTs, protein arginine methyltransferases; TNF-a, tumour necrosis factor-a (Blackwell, 2010)

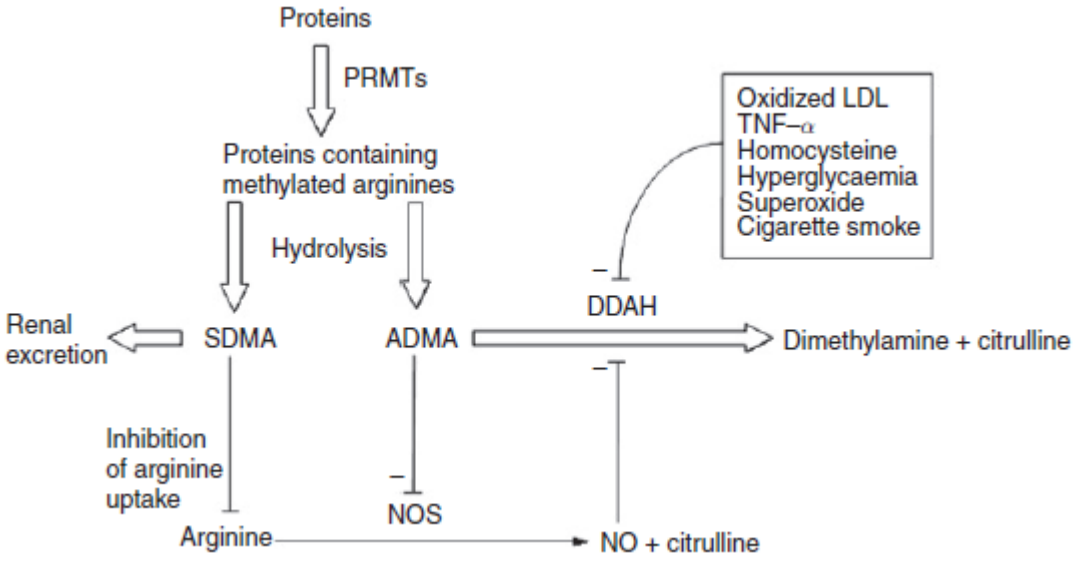

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Table 1. Admission characteristics and arginine, homoarginine, ADMA and SDMA concentrations in normal subjects and critically-ill patients (n=104).

	Reference Intervals (Control) (n=86)	Patients with critical illness (n=104)	P-value*
Age (Year)	43 (20-65)	61 (47-71)	
Gender (Male/Female) (%)	44 (51)/ 42 (49)	72 (69)/32 (31)	
Admission cause (Surgical/Medical) (%)		60 (58)/44 (42)	
APACHE II		21 (17-27)	
Predicted mortality rate (%)		38.9 (17.6-60.4)	
SOFA score		7.0 (4.0-9.0)	
Severe sepsis/septic shock (%)		10 (10)/5 (5)	
Arginine (µmol L^{-1})	65.7 (48.8-79.9)	18.4 (12.9-29.1)	<0.001
Homoarginine (µmol L^{-1})	1.90 (1.38-2.49)	0.58 (0.32-1.18)	<0.001
ADMA(µmol L^{-1})	0.45 (0.41-0.52)	0.54 (0.41-0.64)	<0.001
SDMA (µmol L^{-1})	0.38 (0.34-0.43)	0.75 (0.52-1.15)	<0.001
ADMA/SDMA ratio	1.20 (1.10-1.36)	0.73 (0.43-1.02)	<0.001
Arginine/ADMA ratio	125.7 (101.0-171.5)	36.7 (26.0-56.6)	<0.001
Total dimethylarginine (µmol L^{-1})	0.82 (0.76-0.88)	1.2 (1.0-1.83)	<0.001
Creatinine (umol/L)[§]	68-118	117 (78-171)	
Total Bilirubin (umol/l) [§]	5.1–17.0	10.5 (7.0-21.0)	
ALT (U/L) [§]	10-40	37 (18-115)	
Lab-Glucose (mmol/l) [§]	3.5-5.5	6.4 (5.5-8.1)	
C-reactive protein (mg/l) [§]	<10	119 (41-216)	
White cell count (10^9/L) [§]	4-11 x10^9	11.4 (7.9-18.5)	
Albumin (g/l) [§]	35-55	16 (12-21)	
Daily fluid balance (ml)		1034 (393-2343)	
Insulin (U hour $^{-1}$) [‡]		0.9 (0.0-9.3)	
Norepinephrine (mg $hour^{-1}$) [‡]		0.5 (0.0-6.0)	
Epinephrine (mg $hour^{-1}$) [‡]		0.1 (0.0-2.7)	
Dobutamine (mg $hour^{-1}$) [‡]		2.5 (0.0-76.4)	
Hydrocortisone (mg $hour^{-1}$) [‡]		1.9 (0.0-16.0)	
ICU length of stay (Days)		5.1 (2.0-13.6)	
ICU death No/Yes		78 (75%)/26 (25%)	

Median (interquartile range, IQR), [§] reference interval, [‡] Mean (range), * Mann-Whitney *U*.

Table 2 Admission patient characteristics and measurements between survivors and non-survivors (n=104).

	Admission Survivors (n=78)	Admission Non-survivors (n=26)	P-value*
Age (Year)	60 (43-69)	67 (48-74)	0.175
Gender (Male/Female) (%)	55 (71)/23 (29)	17/9	0.625
Admission cause (Surgical/Medical) (%)	45 (58)/33 (42)	15/11	1.000
APACHE II	21 (16-26)	24 (18-31)	0.174
Predicted mortality rate (%)	35.3 (14.1-57.3)	45.6 (23.3-73.8)	0.061
SOFA score	5 (4-8)	8 (7-11)	0.001
Severe sepsis/septic shock (%)	7 (9)/5 (6)	3 (12)/0 (0)	
Arginine (μmol L^{-1})	17.5 (12.1-29.0)	19.6 (17.3-34.9)	0.281
Homoarginine (μmol L^{-1})	0.70 (0.35-1.30)	0.50 (0.26-0.77)	0.028
ADMA(μmol L^{-1})	0.49 (0.38-0.62)	0.59 (0.52-0.90)	0.003
SDMA (μmol L^{-1})	0.69 (0.50-1.10)	0.94 (0.57-1.72)	0.047
ADMA/SDMA ratio	0.72 (0.43-1.02)	0.79 (0.37-1.05)	0.866
Arginine/ADMA ratio	38.5 (25.7-59.2)	33.3 (26.0-49.2)	0.218
Total dimethylarginine (μmol L^{-1})	1.2 (0.92-1.68)	1.7 (1.2-2.5)	0.008
eGFR >30/ ≤30	10 (0-35)	11 (0-28)	0.747
Creatinine (umol/L)	115 (73-169)	118 (79-249)	0.605
Total Bilirubin (μmol/l)	10.5 (8-20)	10.5 (7-28)	0.980
ALT	38.5 (19-129)	37 (15-115)	0.556
Lab-Glucose (mmol/l)	6.4 (5.5-8.1)	6.8 (5.4-9.2)	0.642
C-reactive protein (mg/l)	108 (40-218)	125 (42-169)	0.870
White cell count (10^9/L)	10.8 (7.6-16.9)	16.3 (7.9-20.0)	0.223
Albumin (g/l)	17 (13-23)	13 (10-19)	0.017
Daily fluid balance (ml)	1006 (393-2166)	4793 (-7-5113)	0.167
Insulin (U hour $^{-1}$) ‡	0.9 (0.0-9.3)	0.7 (0.0-4.8)	0.874
Norepinephrine (mg hour-1) ‡	0.4 (0.0-2.5)	0.8 (0.0-6.0)	0.292
Epinephrine (mg $hour^{-1}$) ‡	0.1 (0.0-2.7)	0.1 (0.0-1.0)	0.894
Dobutamine (mg $hour^{-1}$) ‡	2.4 (0.0-76.4)	2.7 (0.0-50.0)	0.431
Hydrocortisone (mg hour-1) ‡	2.2 (0.0-16.0)	1.2 (0.0-8.0)	0.276
ICU length of stay (Days)	3 (1-10)	11.6 (6-19)	<0.001

Median (interquartile range, IQR), ‡ Mean (range), * Mann-Whitney *U*.

Table 3. The relationship between clinical characteristics and ADMA on admission to ICU mortality in patients with critical illness. Univariate and multivariate binary logistic regression analysis.

	Univariate analysis OR (95%CI)	P-value	Multivariate analysis OR (95%CI)	P-value
Age (years)	1.02 (0.99-1.05)	0.292		
Sex (male/female)	1.27 (0.49-3.25)	0.624		
Patients (medical/surgical)	1.00 (0.41-2.46)	1.000		
APACHE II score	1.05 (0.99-1.12)	0.138		
Predicted mortality rate (%)	1.02 (0.99-1.03)	0.081		
SOFA score	1.34 (1.12-1.61)	0.002	1.053 (1.104-2.120)	0.011
ICU length of stay (days)	1.05 (1.01-1.09)	0.007	0.951 (0.097-9.323)	0.965
Arginine (µmol L-1)	1.02 (0.99-1.05)	0.241		
Homoarginine (µmol L-1)	0.39 (0.15-0.99)	0.048	0.545 (0.105-2.837)	0.471
ADMA(µmol L-1)	31.31 (3.34-293.18)	0.003	0.793 (0.003-218.189)	0.936
SDMA (µmol L-1)	1.54 (0.90-2.64)	0.118		
ADMA/SDMA ratio	1.10 (0.30-4.06)	0.887		
Arginine/ADMA ratio	0.99 (0.97-1.01)	0.243		
ADMA+SDMA	1.66 (1.02-2.70)	0.041	2.653 (0.090-78.137	0.572
eGFR >30/ <30	0.99 (0.97-1.02)	0.771		
Creatinine umol/L	1.00 (0.99-1.00)	0.721		
Bilirubin (umol/l)	1.00 (0.99-1.01)	0.858		
ALT (u/l)	1.00 (1.00-1.00)	0.661		
Lab-Glucose mmol/l	1.05 (0.94-1.16)	0.413		
C-reactive protein (mg/l)	1.00 (0.99-1.01)	0.665		
Albumin (g/l)	0.92 (0.85- 1.00)	0.063		
Daily fluid balance (ml)	1.00 (1.00-1.00)	0.020	1.000(1.000-1.001)	0.427
Insulin (U hour -1)	0.93 (0.70-1.24)	0.623		
Norepinephrine (mg hour-1)	1.51 (0.95-2.40)	0.079		
Epinephrine (mg hour-1)	0.89 (0.25-3.23)	0.864		
Dobutamine (mg hour-1)	1.00 (0.96-1.05)	0.896		
Hydrocortisone (mg hour-1)	0.92 (0.800-1.06)	0.263		

OR, odds ratio

Table 4. The relationship between patient characteristics and plasma arginine, homoarginine, ADMA, and SDMA concentrations in critically-ill patients on admission and (follow-*up)* last sample median day 7 (range 5-15) (IQR 6-8)

	Critically-ill patients Admission (n=33)	Critically-ill patients Follow-up (n=33)	P-value*
Age (yr)	64 (51-71)		
Sex (M/F) (%)	23 (70%) /10 (30%)		
Medical/ Surgical (%)	19 (58%) /14 (42%)		
APACHE II score	24 (18-29)		
Predicted mortality (%)	46.0 (21.8-71.9)		
SOFA score	8 (6-10)	6 (4-8)	0.002
Severe sepsis/septic shock	2/1		
Arginine (µmol L^{-1})	19.1 (12.8-31.8)	24.3 (21.5-35.6)	0.008
Homoarginine (µmol L^{-1})	0.56 (0.35-1.22)	0.33 (0.12-0.42)	<0.001
ADMA(µmol L^{-1})	0.60 (0.50-0.84)	0.71 (0.57-1.04)	0.009
SDMA (µmol L^{-1})	0.87 (0.53-1.44)	0.94 (0.51-1.49)	0.829
ADMA/SDMA ratio	0.75 (0.43-1.04)	0.89 (0.57-1.25)	0.003
Arginine/ADMA ratio	31.2 (16.3-44.2)	40.0 (27.0-46.3)	0.236
ADMA+SDMA	1.5 (1.04-2.44)	1.6 (1.16-2.52)	0.936
eGFR >30/ ≤30	20.0 (0.0-33.5)	17.0 (0.0-30.0)	0.604
Creatinine (umol/L)	126 (93-204)	167 (91-278)	0.626
Bilirubin (umol/l)	11 (7-20)	11 (6-27)	0.350
ALT (u/l)	57 (1-171)	42 (21-96)	0.012
Lab-Glucose mmol/l	7.4 (5.9-10.5)	6.2 (5.2-7.0)	0.012
C-reactive protein (mg/ l)	72 (13-156)	131 (56-173)	0.597
White cell count (10^9/L)	10.6 (7.4-18)	12.0 (9.2-20.8)	0.320
Albumin (g/ l)	15 (10-21)	13 (11-16)	0.012
Daily fluid balance (ml)	469 (-21-2000)	1491 (-275-2148)	0.778
Insulin (U hour $^{-1}$) ‡	1.0 (0.0-9.3)	2.1 (0.0-10.9)	0.148
Norepinephrine (mg $hour^{-1}$) ‡	0.4 (0.0-4.0)	0.5 (0.0-4.4)	0.017
Epinephrine (mg $hour^{-1}$) ‡	0.1 (0.0-2.7)	0.07 (0.0-1.6)	0.028
Dobutamine (mg $hour^{-1}$) ‡	3.9 (0.0-76.4)	4.5 (0.0-117)	0.028
Hydrocortisone (mg $hour^{-1}$) ‡	2.1 (0.0-16.0)	2.4 (0.0-16.0)	0.058
ICU length of stay (days)	16 (11-27)		
ICU death (no/ yes) (%)	17 (52)/16 (48)		

Median (interquartile range, IQR), ‡ Mean (range), * Wilcoxon signed rank test.