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SDMA is a marker of detrimental outcome in the acute phase after ischemic stroke: Role of renal function.

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Abstract

Methylarginines have been shown to interfere with nitric oxide (NO) formation by inhibiting NO synthase (asymmetric dimethylarginine, ADMA) and cellular L-arginine uptake into the cell (ADMA, and symmetric dimethylarginine, SDMA). In a recent study elevation of SDMA was related to long term mortality in patient recruited 30 days after a stroke event. In the present study we aimed to investigate the association of SDMA and adverse clinical outcome in the early phase (first 30 days) after acute ischemic stroke. 137 patients were recruited immediately upon admission to the emergency unit with an acute ischemic stroke. Plasma levels of methylarginines were determined by a validated LC/MS-MS method. Patients were prospectively followed for 30 days. 25 patients (18.2 %) experienced the primary composite endpoint (death, recurrent stroke, MI, rehospitalisation). SDMA plasma levels were significantly higher in patients with compared to patients without event (0.89 ± 0.80 vs. 0.51 ± 0.24 $\mu\text{mol/l}$; $p < 0.001$). SDMA levels were significantly correlated with markers of renal function. Kaplan-Meier survival analysis demonstrated that cumulative survival decreased significantly with ascending tertiles of SDMA ($p < 0.001$).

Our study provides first data indicating that SDMA is strongly associated with adverse clinical outcome during the first 30 days after ischemic stroke. Our results strengthen the prognostic value of renal function in patients with stroke and confirms the hypothesis that SDMA is a promising marker for renal function.

Introduction

Dimethylarginines are endogenously occurring structural analogues of the amino acid L-arginine which contain two methyl groups. Asymmetric dimethylarginine (ADMA) has been known as an endogenous inhibitor of nitric oxide (NO) synthases, whilst symmetric dimethylarginine (SDMA) does not interfere with NO synthase activity. Both dimethylarginines are formed during the dimethylation of proteins by protein arginine methyltransferases (PRMTs), a process involved in posttranslational protein modification. After hydrolytic protein degradation, both ADMA and SDMA are carried from cytoplasm to the extracellular space by the hCAT-2B amino acid transporter. ADMA is largely cleaved to L-citrulline and dimethylamine by the enzyme dimethylarginine dimethylaminohydrolase (DDAH) and excreted by the kidneys to a minor amount (about 20%) [1], whilst SDMA was reported to be eliminated almost exclusively by the renal route [2]. Oxidative stress and inflammation, both processes that also occur in acute cerebral ischemia, upregulate PRMTs [3] and inhibit DDAH [4], resulting in increased production of ADMA and SDMA and reduced degradation of ADMA.

ADMA has been characterized, in prospective clinical studies, as a novel cardiovascular risk marker for major cardiovascular events and mortality in patients with low, medium, and high cardiovascular risk (for review cf. [5]). SDMA, by contrast, has not been studied to a comparative extent. However, the few published studies suggest that SDMA may equally be associated with cardiovascular disease events [6]. Specifically, we have recently reported that SDMA was strongly associated with total mortality during long-term follow-up after ischemic stroke [7]. In that study, 394 patients were included who had already survived the first 30 days after acute ischemic stroke (i.e., the acute phase), and during a median 7.4 years of follow-up, elevated SDMA was associated with a 2.4-fold elevated risk of death of any cause. In 3,229 individuals suffering from coronary artery disease (CAD) of the Ludwigshafen Risk and Cardiovascular Health Study (LURIC) SDMA strongly correlated with renal function and was independently associated with all-cause and cardiovascular mortality [8]. These two studies suggest that the predictive value of SDMA is underestimated, may be different from ADMA, and needs further investigation

The present study was designed as a precursor study to investigate the relationship of the dimethylarginines, ADMA and SDMA, with cardiovascular events and death during the acute phase of ischemic stroke.

Patients and Methods

Subjects and Study Design

Between May 2007 and May 2008, we included 137 consecutive patients of both sexes who have been admitted to the stroke unit of the Department of Neurology at the Asklepios Clinic Hamburg-Harburg with the symptoms of an acute stroke. The study was approved by the Ethics Committee at the Hamburg Board of Physicians, and all participants provided informed consent.

Patients were included if they had clinical symptoms of an acute stroke, the diagnosis "acute ischemic stroke" had been confirmed by cranial computed tomography, and if they had given their informed consent to participate in the study. From patients who were comatose or aphasic and therefore could not consent to participate on day 1, blood samples were collected during routine venipuncture, and samples were only included in the analysis after the patients were able to consent to participate. Patients who had had a myocardial infarction within the preceding 2 weeks, who had

undergone major surgery within the preceding 3 months, who had experienced cardiogenic shock within the last 12 months or who suffered from decompensated heart failure or malignancy, or women who were pregnant or lactating, were excluded.

Hypertension was defined according to current WHO criteria and/or the use of antihypertensive drugs.

A fasting venous blood sample was taken within 24 hours after the acute cerebral event and was used to determine laboratory parameters; plasma was separated after centrifugation and stored frozen at -80°C until analysis. Patients were followed daily during the time of their inpatient treatment and, after discharge, by telephone until day 30 after stroke for cardiovascular events and mortality. The primary endpoint was a combined endpoint of death, non-fatal recurrent stroke, non-fatal myocardial infarction, and rehospitalisation. The secondary endpoint was defined as a combined endpoint of death, non-fatal recurrent stroke, and non-fatal myocardial infarction.

Laboratory Analyses

Circulating levels of L-arginine, ADMA and SDMA were determined by liquid chromatography - tandem mass spectrometry (LC-MS/MS) as described elsewhere [9]. We used a validated high throughput LC-MS/MS assay [10] developed in our laboratory which is now commercially available (DLD Diagnostika, Hamburg, Germany). In brief, proteins were precipitated with methanol using 96-well $0.20\text{-}\mu\text{m}$ microfiltration plates pre-coated with L- $[\text{}^2\text{H}_7]$ -arginine, $[\text{}^2\text{H}_6]$ -ADMA and $[\text{}^2\text{H}_6]$ -SDMA as internal standards. After centrifugation, the microfiltrates were dried and analytes were converted to their butyl ester derivatives. Subsequent analyses were performed using a Chirobiotic T, $20 \times 1.0 \text{ mm i.d.}$, microbore guard column (Astec, Whippany, NJ, USA) connected to a Varian 1200L Triple Quadrupole MS (Varian, Walnut Creek, CA, USA) in the positive electrospray ionisation (ESI+) mode. The sample run time was 1.6 min with an intra-assay and inter-assay precision of 2.2% and 4.7%, (CV), respectively.

Calculations and Statistical Analyses

Associations between variables were assessed by Spearman correlation coefficients. Differences between two unrelated groups were compared using unpaired Student's *t*-tests. Differences between categorical data were analysed by χ^2 -test. Univariate associations between tertiles of ADMA, SDMA, L-arginine and renal function and mortality were assessed using Kaplan–Meier survival analysis with significance determined using the log rank test. The association between ADMA, SDMA and L-arginine and mortality was determined using multivariate Cox regression analyses including variables which were shown to be significantly associated with the endpoint in a univariate cox-regression model. A probability of $p < 0.05$ was considered significant. All statistical analyses were performed using SPSS for Windows v15.0 (SPSS Inc.).

For the estimation of glomerular filtration rate we used the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation.

Results

Baseline Patient Characteristics

137 patients (56.9 % women) with acute ischemic stroke were included in this study. Their mean age was 70 ± 11 years; 30 patients had atrial fibrillation, 16 patients had had a previous myocardial infarction and 78.8% of all included patients were hypertensive. Demographic and anthropometric patient characteristics as well as risk factor profiles are given in Table 1. Mean L-arginine, ADMA and SDMA plasma levels were 59.4 ± 24.7 $\mu\text{mol/l}$, 0.58 ± 0.12 $\mu\text{mol/l}$ and 0.58 ± 0.42 $\mu\text{mol/l}$, respectively.

Cross-sectional Association of Dimethylarginines with Risk Factors for Stroke

L-Arginine, ADMA and SDMA were each significantly correlated with a number of demographic and hemostatic factors in univariate analyses (as shown in Table 2). Variables associated with SDMA included age, serum creatinine, eGFR, INR, hematocrit, serum C-reactive protein and systolic blood pressure. ADMA correlated significantly with the NIHSS and hematocrit whereas L-arginine only correlated with the NIHSS.

During 30 days of follow-up after the onset of acute ischemic stroke, 25 patients experienced the primary endpoint. Of these, 5 patients died, 9 experienced a recurrent stroke, 3 experienced a myocardial infarction and 8 patients were rehospitalised for various causes. Patients who experienced the primary endpoint were slightly older and showed a higher NIHSS. In addition, these patients had higher C-reactive protein and lower hematocrit levels. Their renal function was worse and SDMA plasma concentrations were higher than in patients who did not reach the primary endpoint. All other parameters were not significantly different between both groups (Table 3).

Prospective Analysis of the Relationship between L-Arginine, Dimethylarginines and Event Rates after Stroke

As a first step to assess the relation of L-arginine, ADMA and SDMA with total mortality, Kaplan-Meier curves for tertiles of the variables were plotted (Figure 1). Cumulative survival significantly decreased with ascending tertiles of SDMA: 6.4% of patients in the lowest tertile of SDMA, 12.5% in the middle tertile, and 30.4% of patients in the highest tertile of SDMA experienced the primary endpoint ($p = 0.005$). By contrast, neither ADMA nor L-arginine showed any significant association with the primary or secondary endpoint. At this point it is interesting to mention that incidence rates for each component of the primary endpoint increased with increasing SDMA tertiles. Low absolute numbers of events precluded multivariable adjustments (data not shown).

The Kaplan-Meier curve for GFR tertiles showed decreasing cumulative survival with impaired renal function. 60% of the patients reaching the primary endpoint were in the lowest GFR tertile (< 63 ml/min per 1.73m^2), 28% were in the middle GFR tertile (63 to 87 ml/min per 1.73m^2), and 12% were in the highest GFR tertile (> 87 ml/min per 1.73m^2).

In a second step, a univariate cox-regression analysis of the primary endpoint for clinical and laboratory parameters was performed (table 4). Age, the NIHSS, eGFR, hematocrit, CRP and SDMA were significantly associated with an increased hazard ratio. In a third step, a multivariate cox-regression model including all significant parameters from the univariate analysis was performed. Only the NIHSS and CRP

remained significantly associated with an increased hazard ratio for reaching the primary endpoint (table 5).

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Discussion

The current study has the following major results: 1) It provides evidence that SDMA and not ADMA is a marker for major cardiovascular events and death in the acute phase of ischemic stroke. 2) The association of SDMA and outcome was highly related to renal function supporting the important role of renal function in patients suffering from ischemic stroke and the evidence of SDMA being a valuable marker for renal function. 3) The association of SDMA and CRP levels suggests a pathomechanistic link between ischemic stroke and inflammation.

In this study two endpoints were defined. The primary endpoint consists of death, myocardial infarction, recurrent stroke and rehospitalisation, and the secondary endpoint was defined as death, myocardial infarction and recurrent stroke. SDMA plasma levels were highly elevated in patients with the primary endpoint as compared to patients without any subsequent event, even if due to the low number a statistical analysis was not viable. Our finding that ADMA was unrelated to the primary and secondary endpoint in this study was unexpected, given that ADMA has been much more thoroughly characterized than SDMA as a risk marker in preceding studies (for an overview, cf.[5]). Sample size and power analysis warrant a cautious interpretation of this negative finding. However, our current findings are in agreement with two earlier studies which showed that after adjustment SDMA, is a predictor of all-cause mortality during long-term and 90 days follow-up after ischemic stroke [7, 11] Furthermore, a cross-sectional study of patients with acute cerebrovascular disease demonstrated that ADMA was only poorly associated with acute ischemic stroke, whereas SDMA but not ADMA was closely associated with the presence of cardio-embolic stroke [12]. In this respect, it is interesting to note that ADMA and SDMA apparently behave differently as risk markers in patients after myocardial infarction (where ADMA is a better predictor) or stroke (where SDMA is a better predictor). In general, stroke patients tend to be older than patients with myocardial infarction. Traditional risk factors are different for stroke and myocardial infarction, respectively. Whilst serum cholesterol and smoking are important risk factors for myocardial infarction, their role appears less prominent for stroke, whilst high blood pressure and atrial fibrillation are important risk factors for ischemic stroke, but not quite as prominently so for myocardial infarction [13-16]. The association of these risk factors with ADMA and SDMA may be different, but yet ill understood in detail. In keeping with this hypothesis, we found an association of SDMA with atrial fibrillation in our previous study in stroke patients [7], whilst ADMA was repeatedly shown to be related to cholesterol levels in various populations [17-18]. Several mechanisms have been suggested to explain the association of ADMA and/or SDMA and adverse outcome. Both ADMA and SDMA can influence the L-arginine – NO pathway. Whilst ADMA directly inhibits NO synthase (NOS) activity and thereby reduces the amount of NO released [1, 19], causing endothelial dysfunction, SDMA was shown not to inhibit NO synthesis rate. However, SDMA might influence NO formation by an indirect mechanism, as it interacts with the transport of L-arginine into the cell via the hCAT-2B transporter for cationic amino acids. SDMA is efficiently transported by this molecule and exchanged against intracellular L-arginine, which may lead to an intracellular depletion of L-arginine [20]. However, this effect was only observed at very high (millimolar) concentrations of SDMA.

The relationship of SDMA with events was strongly related to renal function, so that the association of SDMA with outcome became insignificant in models

including eGFR. Patients in our study had an average serum creatinine of 1.1 mg/dl which suggested to use the CKD-EPI equation for estimating the GFR. The CKD-EPI equation is more accurate especially in patients with an estimated GFR greater than 60 ml/min per 1.73 m² [21]. Especially in patients suffering from stroke of any cause the renal function seems to have a prognostic value for long term survival and the occurrence of cardiovascular events after the acute cerebral event [22]. Acute kidney injury which develops within 2 days after the acute event is prominent in almost 27% of patients suffering from stroke. Even if the acute kidney injury is mostly reversible when looking at the symptoms, it could cause endothelial damage, tubular inflammation, and activate pathways damaging renal structure. This might explain the strong association with renal function and worse outcome in patients with stroke.

Our study confirms the predictive value of renal function for survival after an acute cerebral ischemic event and strengthens the hypothesis that SDMA may serve as a valid and sensitive marker of renal dysfunction. However, when Schulze et al. included eGFR (as a measure of renal function) into their multivariate outcome models, SDMA remained an independent predictor of mortality. This implies that it would constitute an oversimplification to attribute the association of SDMA and adverse clinical outcome solely to the fact that SDMA constitutes a measure of renal function. With increasing age, renal dysfunction becomes more prominent, which may favor a more prominent role of SDMA in the stroke population. Indeed, a recent study by Cho et al. showed that impaired renal function is associated with the presence of cerebral microbleeds in acute ischemic stroke independently of other risk factors [23].

Interestingly, recent data suggests that SDMA may affect vascular homeostasis by other, NO-independent mechanisms. SDMA apparently modulates store-operated calcium channels in monocytes, resulting in an increased Ca²⁺ entry from the extracellular milieu, thereby stimulating the formation of reactive oxygen species. These pro-inflammatory effects were not seen with ADMA [24], which, in contrast, upregulates endothelial adhesion molecule expression. Thus, both dimethylarginines may stipulate endothelial-monocyte interaction via different mechanisms and by targeting different cell types. This hypothesis could be partly supported by the correlation of SDMA and CRP found in this study. Further, besides NIHSS CRP remained an independent predictor for any event in this study. There is increasing evidence that inflammation plays a crucial role in the progression of acute ischemic stroke. Recently an increase in CRP levels was found to be associated with infarct volume in 45 patients suffering from acute ischemic stroke [25]. It can be speculated whether interaction of SDMA with calcium homeostasis might also interfere with myocardial electrophysiology, contributing to mortality after stroke. Therefore, SDMA might be one pathogenic link between renal dysfunction and vascular events, inflammation and death after stroke. Clearly, this hypothesis and the various potential new molecular mechanisms which may explain the association of SDMA with cardiovascular events beyond renal function need further experimental verification.

Limitations of the study

Several limitations should be pointed out in the current study. This study was intended as a pilot study to pick up a signal, hence sample size and event numbers were small. This precluded detailed multivariate analyses as well as detailed subgroup analyses.

Kidney function was estimated using serum creatinine-based formulas. The measurement of urinary albumin or 24-hour urine collections may have improved the

sensitivity of the assessment of renal function. Additionally there were no pre-stroke SDMA and serum creatinine levels available. It is known that SDMA increases within the first 6 hours with a peak at 24 hours after the acute event [26]. In this study the measured SDMA plasma levels may reflect the peak after 24 hours. However our data confirms other studies suggesting SDMA as a promising marker of renal function. The median NIHSS of 4.0 and the exclusion of patients with worse outcome (due to the missing consent) indicates that in this study only patients with mild stroke are involved and that our results cannot be generalized to the overall stroke population.

Conclusion

Our study demonstrates that SDMA is associated with adverse clinical outcome during 30 days after acute ischemic stroke. This association is apparently linked to the association of SDMA and renal function which is also associated with outcome in this cohort. Our results strengthen the prognostic value of renal function in patients with stroke and confirms the hypothesis that SDMA is a promising marker for renal function. Additionally the causes for acute and long-term mortality after stroke may differ widely and SDMA and renal function may be only one part besides other predominant laboratory parameters.

Disclosures

Drs Böger, Maas, Schwedhelm are named as inventors on patents relating to analytical assays for methylarginines and receive royalties from them.

Table 1
Demographic and clinical characteristics of patients

Subjects (n)	137
Age [years]	70 (\pm 11)
Male, n (%)	59 (43)
Smokers, n (%)	48 (35)
Cardiovascular disease n (%)	17 (12.4)
Diabetes, n (%)	33 (24.1)
Hypertension, n (%)	108 (78.8)
Myocardial infarction n (%)	16 (11.7)
Atrial fibrillation n (%)	30 (21.9)
Diastolic blood pressure [mmHg]	83.9 (\pm 10.4)
Systolic blood pressure [mmHg]	143.2 (\pm 16.6)
Hyperlipidemia, n (%)	73 (53.3)
CRP [mg/L]	15.5 (\pm 28.7)
NIHSS at admission	4.0 (3.0-7.0)
INR	1.1 (\pm 0.5)
Partial thromboplastin time [sec]	30.2 (\pm 28.0)
Hematocrit [%]	40.8 (\pm 4.7)
Triglyceride [mg/dl]	159.2 (\pm 290.9)
Cholesterol [mg/dl]	191.3 (\pm 44.4)
Leukocytes [/nl]	8.6 (\pm 2.7)
Thrombocytes [/nl]	232.5 (\pm 67.1)
S-creatinine [mg/dl]	1.1 (\pm 0.9)

Data presented as mean (\pm SD) or median (25th – 75th percentile); NIHSS: National Institutes of Health Stroke Scale; INR: International normalized ratio

Table 2

Variables associated with L-arginine, SDMA and ADMA in cross-sectional analyses.

	L-arginine	SDMA	ADMA
Age	-0.068	0.455**	0.103
S-creatinine [mg/dl]	0.034	0.613**	0.106
GFR [ml/min/1.73m ²]	0.062	-0.666**	-0.091
NIHSS	-0.185*	-0.056	0.196*
INR	-0.123	0.192*	0.097
Partial Thromboplastin Time [sec]	-0.062	0.019	0.012
Hematocrit [%]	-0.031	-0.366**	-0.178*
CRP [mg/l]	-0.096	0.260**	0.147
Triglycerides [mg/dl]	0.050	-0.094	0.090
Total cholesterol [mg/dl]	0.064	-0.134	-0.044
Atrial fibrillation, n (%)	-0.025	0.080	-0.132
Systolic blood pressure [mmHg]	0.052	0.215*	0.149
Diastolic blood pressure [mmHg]	-0.090	0.079	0.026

Data given as Spearman correlation coefficients

* p<0.05

** p<0.001

NIHSS: National Institutes of Health Stroke Scale; INR: International Normalized Ratio; GFR: Glomerular filtration rate

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Table 3

Comparison of clinical and laboratory parameters between patients who experienced the primary endpoint and those who did not.

	Patients without event	Patients with event	p
Subjects, n (%)	112	25	
Age, [years]	69 (±11)	75 (±10)	0.45
Hypertension, n (%)	86 (76.8)	22 (88.0)	0.21
Atrial fibrillation, n (%)	22 (19.6)	8 (32.5)	0.18
Myocardial infarction, n (%)	14 (12.5)	2 (8.0)	0.53
Diabetes mellitus, n (%)	29 (25.9)	4 (16.0)	0.30
Smokers, n (%)	43 (38.4)	5 (20.0)	0.08
S-creatinine [mg/dl]	1.0 (±0.7)	1.7 (±1.6)	<0.001
eGFR [ml/min/1.73m ²]	75.3 (±24.4)	54.5 (±29.5)	<0.001
NIHSS at admission	4.6 (±3.2)	8.6 (±4.4)	<0.001
INR	1.1 (±0.6)	1.0 (±0.1)	0.38
Partial Thromboplastin time [sec]	28.3 (±20.1)	38.6 (±48.8)	0.10
Hematocrit [%]	41.3 (±4.7)	38.8 (±5.9)	0.025
CRP [mg/l]	10.6 (±18.3)	37.9 (±50.3)	<0.001
ADMA [µmol/l]	0.58 (±0.12)	0.59 (±0.12)	0.89
SDMA [µmol/l]	0.51 (±0.24)	0.89 (±0.80)	<0.001
L-Arginine [µmol/l]	60.4 (±25.4)	54.8 (±21.0)	0.32

Data presented as mean (±SD)

Proportions are given referring to the number of patients in the two groups

NIHSS: National Institutes of Health Stroke Scale; INR: International Normalized Ratio; GFR: Glomerular filtration rate

Table 4

Univariate cox-regression analysis of clinical and laboratory parameters of the primary endpoint

Variable	HR (95% CI)	p-value
Age	1.05 (1.01 – 1.10)	0.03
Hypertension	2.02 (0.60 – 6.74)	0.26
Myocardial infarction	0.66 (0.16 – 2.80)	0.57
Atrial fibrillation	1.74 (0.75 – 4.04)	0.20
eGFR	0.98 (0.96 – 0.99)	0.001
NIHSS at admission	1.22 (1.12 – 1.33)	< 0.001
INR	0.53 (0.11 – 2.47)	0.42
Partial Thromboplastin time	1.01 (0.10 – 1.01)	0.16
Hematocrit	0.92 (0.86 – 0.99)	0.03
CRP	1.02 (1.01 – 1.03)	<0.001
ADMA	0.97 (0.04 – 27.29)	0.99
SDMA	2.10 (1.37 – 3.21)	0.001
L-Arginine	0.99 (0.97 – 1.01)	0.32

HR: Hazard Ratio; NIHSS: National Institutes of Health Stroke Scale; INR: International Normalized Ratio; GFR: Glomerular filtration rate

Table 5

Multivariable cox-regression model of the primary endpoint adjusted for parameters significant in the univariate cox-regression analysis (Table 5)

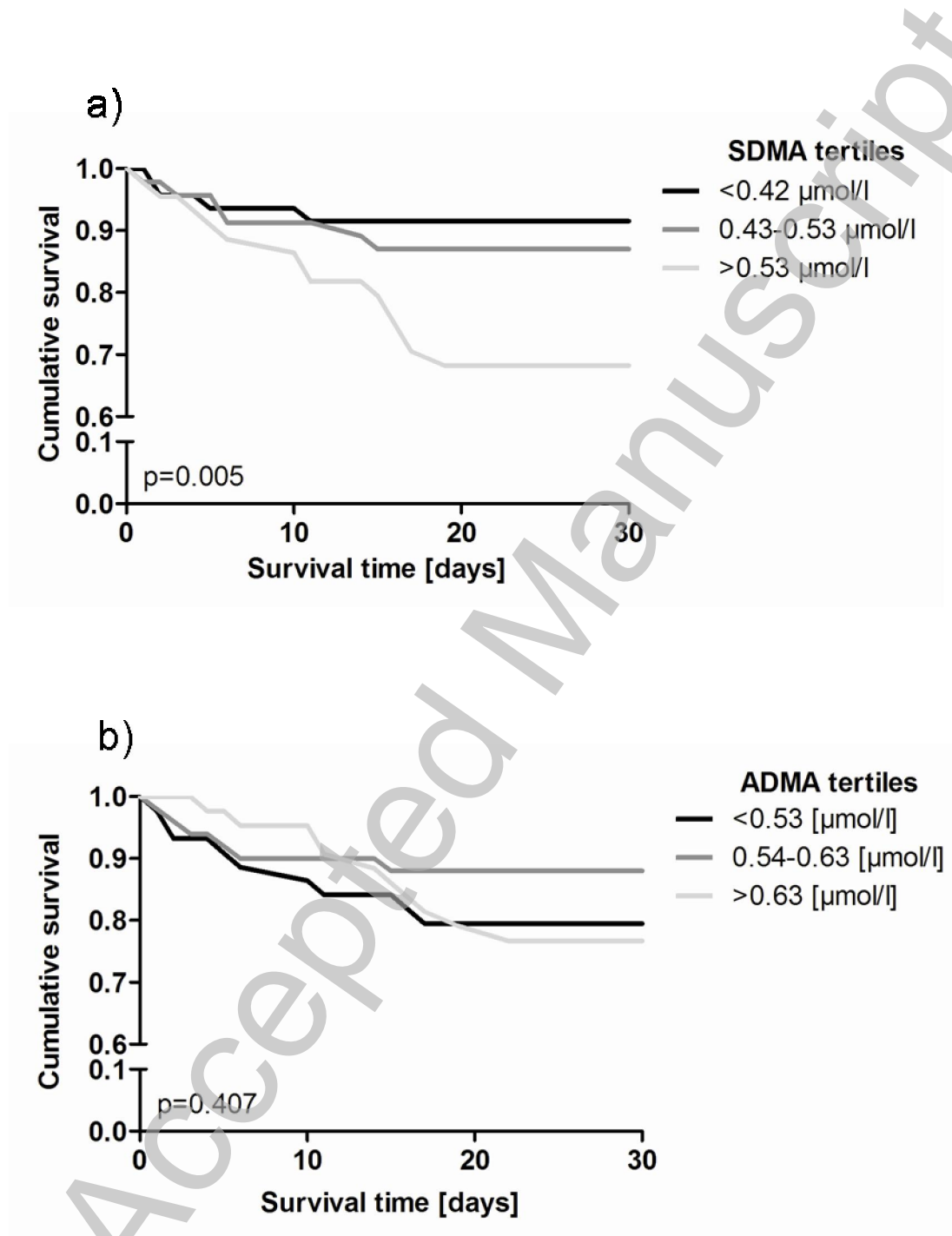
Variable	HR (95% CI)	p-value
SDMA	0.80 (0.23 – 2.80)	0.73
Age	1.01 (0.95 – 1.06)	0.84
NIHSS at admission	1.26 (1.12 – 1.42)	<0.001
eGFR	0.99 (0.96 – 1.01)	0.24
Hematocrit	1.00 (0.89 – 1.12)	0.99
CRP	0.80 (0.23 – 2.80)	<0.001

HR: Hazard Ratio; NIHSS: National Institutes of Health Stroke Scale

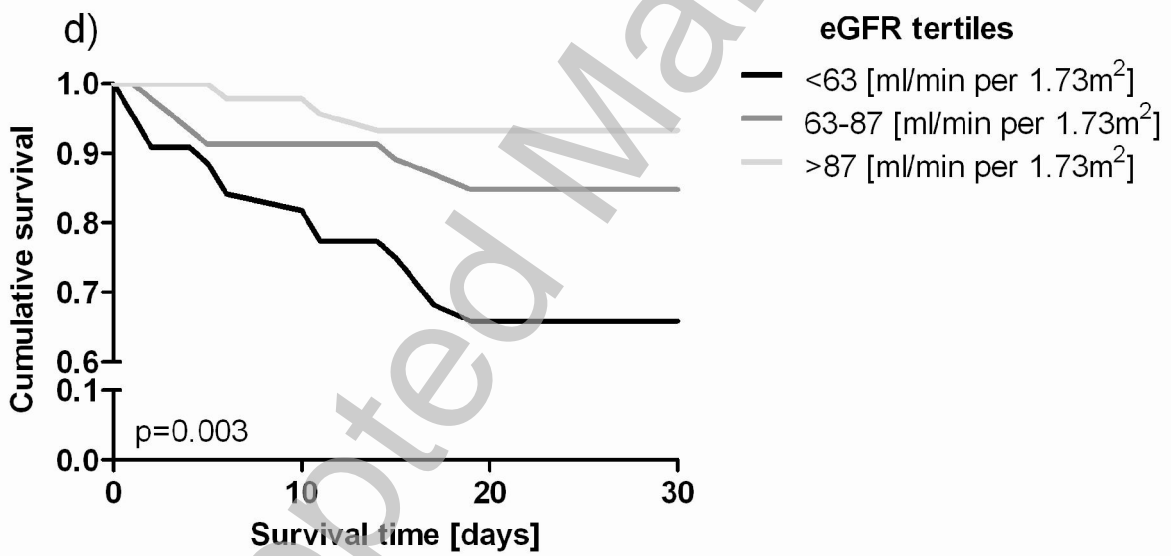
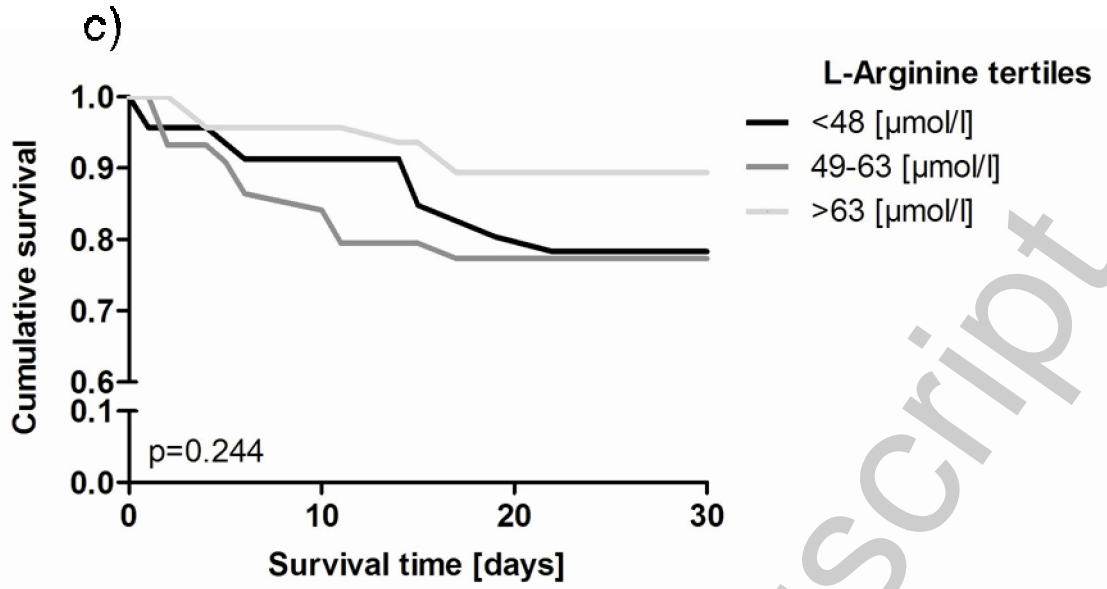
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Figure 1
Kaplan-Meier curve displaying association of SDMA (a), ADMA (b), L-Arginine (c) plasma levels and eGFR (d) with total mortality in patients after acute ischemic stroke. The population was divided into optimized tertiles for each variable analysed.



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