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ORIGINAL ARTICLE

Association of homoarginine and methylarginines with liver dysfunction and mortality in chronic liver disease

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Abstract Previous studies on arginine metabolites reported an association of asymmetric dimethylarginine (ADMA) and symmetric dimethylarginine (SDMA) with liver dysfunction and an inverse relation of homoarginine (hArg) with cardiovascular risk. The aim of the present study was to investigate the relationships between hArg, ADMA, SDMA, and the dimethylarginine score (DAS, i.e., ADMA + SDMA) and liver dysfunction and survival in chronic liver disease. In 94 consecutive cirrhotic patients admitted to our outpatient liver clinic, serum levels of hArg, ADMA, and SDMA were measured by HPLC at baseline. Patients were followed with respect to mortality. In the entire study cohort (age 58.5 ± 11.2 years; 31 % females), the serum concentrations were 1.94 ± 0.90 μM for homoarginine, 0.90 ± 0.22 μM for ADMA, and 0.70 (0.60–0.93) μM for SDMA. ADMA correlated with both Child–Pugh and MELD scores, while SDMA, DAS, and hArg correlated with MELD score only. Thirty patients (32 %) died during a median follow-up of 3.5 years. Age- and sex-adjusted Cox proportional hazard ratios (HR) per μM (with 95 % confidence intervals) showed that hArg was associated with decreased mortality [HR 0.59 (0.37–0.96)], whereas mortality was increased in patients with higher ADMA [HR 3.78 (0.98–14.60)], SDMA [HR 6.54 (3.15–13.59)] and DAS [HR 4.13 (2.26–7.56)]. Only SDMA and DAS remained significantly associated with mortality after additional adjustments for either Child–Pugh stage or MELD score. In conclusion, in cirrhotic patients seen in an outpatient liver clinic, hArg as well as the dimethylarginines ADMA and SDMA was related to long-term mortality. In particular, SDMA predicts mortality independently of both Child–Pugh stage and MELD score.

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Abbreviations

ADMA	Asymmetric dimethylarginine
AGAT	Arginine:glycine amidinotransferase
ANOVA	Analysis of variance
CV	Coefficient of variation
DAS	Dimethylarginine score (i.e., ADMA + SDMA)
DDAH	Dimethylarginine dimethylaminohydrolase
hArg	Homoarginine
HPLC	High-performance liquid chromatography
HR	Hazard ratio
MELD	Model for end-stage liver disease
NO	Nitric oxide
PRMT-1	Protein arginine N-methyltransferase 1
ROC	Receiver operating characteristic
SDMA	Symmetric dimethylarginine

Introduction

Asymmetric dimethylarginine (ADMA) and symmetric dimethylarginine (SDMA) are dimethylarginines that may be relevant to the pathophysiology and outcome of patients with cirrhosis (Mookerjee et al. 2007a; Richir et al. 2008; Siroen et al. 2005; Laleman et al. 2005; Tsikas et al. 2003; Kasumov et al. 2011; Vizzutti et al. 2007). ADMA and its stereoisomer SDMA are released by proteolysis of methylated proteins. ADMA, but not SDMA, is hydrolyzed to dimethylamine (DMA) and citrulline by dimethylarginine dimethlyaminohydrolases (DDAH), which are highly expressed in the liver (Mookerjee et al. 2007a; Richir et al. 2008; Siroen et al. 2005; Laleman et al. 2005; Tsikas et al. 2003; Kasumov et al. 2011; Vizzutti et al. 2007). ADMA and SDMA are endogenous inhibitors of nitric oxide (NO) synthase (Tsikas et al. 2000a, b) and can thereby contribute to intrahepatic NO deficiency, an important factor for the development of portal hypertension (Laleman et al. 2005; Vizzutti et al. 2007). Previous studies suggest that liver dysfunction is associated with excess ADMA levels due to both, increased ADMA production by high protein methylation and decreased ADMA degradation by low hepatic DDAH activity (Mookerjee et al. 2007a; Richir et al. 2008; Siroen et al. 2005; Laleman et al. 2005; Tsikas et al. 2003, 2007; Kasumov et al. 2011; Vizzutti et al. 2007; Becker et al. 2009). By contrast, SDMA levels are considered to be mainly determined by renal SDMA excretion; however, findings in 24 patients undergoing hepatic resection suggest that the liver may also contribute to SDMA elimination (Siroen et al. 2005). SDMA may affect NO metabolism by interference with membrane transport of arginines (Bode-Böger et al. 2006).

Regarding NO, evidence is accumulating that hArg, a cationic amino acid formed from lysine, may modulate NO synthesis and thereby improve endothelial dysfunction (Valtonen et al. 2008; Meinitzer et al. 2011a; Atzler et al. 2015). This hypothesis is strengthened by recent data that low hArg concentrations are associated with increased mortality and fatal cardiovascular events in patients undergoing coronary angiography derived from the LURIC (Ludwigshafen RIsk and Cardiovascular health) study as well as in diabetic dialysis patients derived from the 4D (Die Deutsche Diabetes Dialyse) study (Meinitzer et al. 2011a, b; März et al. 2010; Pilz et al. 2011a, b; Drechsler et al. 2011). The association between low hArg and increased mortality has also been confirmed in population-based studies (Atzler et al. 2014; Pilz et al. 2014). It must, however, be stressed that it is still not entirely clear whether the net effect of hArg on NO metabolism translates into increased or decreased NO levels (Atzler et al. 2015; van der Zwan et al. 2013). In this context, it should also be considered

that hArg concentrations and hArg affinity to NO synthase are several fold lower than those of Arg.

Data on the association of hArg with liver dysfunction are sparse and the prognostic value of hArg in patients with cirrhosis is unknown. On the other hand, the prognostic value of ADMA, SDMA, and DAS has been demonstrated for short-term mortality in severe liver failure associated with alcoholic liver disease (Mookerjee et al. 2007a). The aim of the present study was to evaluate the association of hArg, ADMA, SDMA, and DAS with (1) liver dysfunction as assessed by the Child–Pugh and model for end-stage liver disease (MELD) scores and (2) long-term mortality in a cohort of cirrhotic patients with a broad spectrum of liver dysfunction.

Materials and methods

Study population

Our study population consisted of 94 consecutive patients with cirrhosis who attended our outpatient liver clinic at the Medical University of Graz, Austria, and were enrolled between July 2007 and February 2009. Diagnosis of cirrhosis was based on typical clinical, biochemical, and radiological features and/or on liver histology. Child–Pugh and MELD scores were used for staging of cirrhosis. Specifically, the UNOS modification of MELD was obtained using the Mayo Clinic website calculator (http://www.mayoclinic.org/meld/mayomodel6.html). Informed consent was obtained in accordance with the Declaration of Helsinki. After baseline examination, patients were followed up in the liver clinic with respect to mortality.

Laboratory methods

Blood samples were drawn in the morning after an overnight fast and routine laboratory parameters were immediately measured. The remaining serum samples were stored at −80 °C until analysis of hArg and dimethylarginines. Creatinine, albumin, and total bilirubin were measured by the Jaffé method, bromcresol green method, and DPD method, respectively (Roche Diagnostics, Mannheim, Germany) on a Modular instrument. Aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), gamma-glutamyl transferase (GGT), and alkaline phosphatase were measured enzymatically and C-reactive protein (CRP) by immunoturbidimetry (Roche Diagnostics, Mannheim, Germany). Prothrombin time (PT) was determined using the Thromborel S reagent (Siemens Healthcare Diagnostics, Marburg, Germany) on a BCS analyzer. International normalized ratio (INR) was calculated as (patient PT/mean normal PT)[ISI].

Serum hArg was determined by high-performance liquid chromatography (HPLC) method, according to a previously

described method (März et al. 2010; Pilz et al. 2011a, b; Drechsler et al. 2011; Meinitzer et al. 2007). Intra- and inter-day coefficients of variation (CV) were 2.2–4.7 and 6.8–7.9 %, respectively. ADMA and SDMA were also measured in serum by means of a reverse-phase HPLC method (Meinitzer et al. 2007, 2011b; Teerlink et al. 2002). Within-day and between-day CV for SDMA were 4.6 % (0.60 μM) and 1.9 % (1.0 μM), and 9.8 % (0.60 μM) and 6.1 % (1.0 μM), respectively. Within-day and between-day CV for ADMA were 3.1 % (0.62 μM) and 1.0 % (2.0 μM), and 9 % (0.62 μM) and 1.5 % (2.0 μM), respectively.

Statistical analysis

According to serum concentrations of hArg, ADMA, and SDMA, baseline characteristics are presented for patients stratified into groups with levels above and below the median. According to their distribution, continuous variables are either presented as mean ± standard deviation (normally distributed variables) or as medians with interquartile range (skewed variables). Categorical variables are presented as percentages. All variables following a non-normal distribution were log(e) transformed before use in parametric procedures. Between-group comparisons were calculated by Student's t test and by analysis of variance (ANOVA) with P for trend for continuous variables and by Chi-square test or Fisher's exact test for categorical variables. Furthermore, we performed Pearson correlation analyses of hArg, ADMA, SDMA, and DAS with each other and both Child–Pugh and MELD scores.

Associations of hArg, ADMA, SDMA, and DAS groups with survival were evaluated by Kaplan–Meier curves followed by log-rank test. In addition, we calculated Cox proportional hazard ratios (HR) (with 95 % confidence intervals) for survival according to groups and according to continuous values for hArg, ADMA, SDMA, and DAS, respectively. We calculated unadjusted HRs and HRs adjusted for age (years), sex (females: yes/no), Child–Pugh stage (stage A, B and C), and MELD score as indicated. In addition, receiver operating characteristic (ROC) curves were also constructed to evaluate the prognostic value of hArg, ADMA, SDMA, and DAS as well as MELD score for the prediction of mortality. A P value below 0.05 was considered statistically significant and all statistical tests were two sided. All analyses were performed using SPSS version 19.0.

Results

Baseline characteristics and their relation to arginine metabolites

Our study population consisted of 65 males and 29 females with a mean age of 59 ± 11 years. In the entire study cohort, the serum concentrations were 1.94 ± 0.90 μM for

homoarginine, 0.90 ± 0.22 μM for ADMA, and 0.70 (0.60 to 0.93) μM for SDMA. The main etiology of cirrhosis was alcohol ($n = 53$), followed by chronic hepatitis C ($n = 20$), genetic liver disease ($n = 6$), non-alcoholic fatty liver disease ($n = 5$), and other etiologies ($n = 10$). Comparing patients with cirrhosis of alcoholic origin ($n = 53$) with the remaining study cohort ($n = 41$), we observed no significant differences for hArg (1.93 ± 0.95 vs. 1.95 ± 0.84 μM; $P = 0.890$), ADMA (0.89 ± 0.18 vs. 0.91 ± 0.27 μM; $p = 0.601$), and SDMA [0.77 (0.60–0.94) vs. 0.67 (0.60–0.98) μM; $P = 0.582$] serum concentrations. The baseline characteristics are presented stratified by hArg, ADMA, and SDMA levels above and below the median (Table 1) and by groups according to Child–Pugh stage (Table 2).

Correlation of arginine metabolites with scores of liver dysfunction

Correlation analyses of hArg, ADMA, SDMA, and DAS with each other as well as with liver function parameters, Child–Pugh, and MELD score are shown in Table 3. ADMA levels correlated significantly with both Child–Pugh and MELD scores, whereas hArg, SDMA, and DAS were found to correlate with MELD but not Child–Pugh score.

Association of arginine metabolites with survival

Thirty patients (32 %) died during a median follow-up of 3.5 years. Kaplan–Meier curves, followed by log-rank test, showed a significantly increased mortality risk in patients with hArg levels below the median and with ADMA, SDMA, and DAS levels above the median (Fig. 1a–d). These associations with mortality were also significant in crude and age- and sex-adjusted Cox proportional hazard analyses (Table 4). Additional adjustments for Child–Pugh stage or MELD score generally attenuated these associations; however, SDMA and DAS remained significantly associated with mortality despite adjustments for either Child–Pugh stage or MELD score (Table 4).

Prognostic value of arginine metabolites and MELD score

ROC curves with respect to mortality were constructed for arginine metabolites and MELD score (Fig. 2). The area under the ROC curve was highest for MELD score, followed by DAS, SDMA, hArg, and ADMA (see legend to Fig. 2). In an additional ROC analysis for estimated glomerular filtration rate according to the Modification of Diet in Renal Disease (MDRD) equation, the area under the curve was 0.44 (95 % CI: 0.31 to 0.58) and thus less predictive for mortality compared to SDMA.

Table 1 Baseline characteristics according to the hArg, ADMA, and SDMA groups

	HArg		ADMA		SDMA	
	≤1.82 μM	≥1.83 μM	≤0.88 μM	≥0.89 μM	<0.70 μM	≥0.70 μM
Females (%)	36	26	28	34	21*	41*
hArg (μM)	1.21 ± 0.32**	2.67 ± 0.67**	2.10 ± 0.93	1.78 ± 0.85	2.31 ± 0.88**	1.56 ± 0.75**
ADMA (μM)	0.93 ± 0.21	0.86 ± 0.23	0.73 ± 0.10**	1.06 ± 0.18**	0.82 ± 0.23**	0.98 ± 0.18**
SDMA (μM)	0.86 (0.66–1.17)**	0.62 (0.56–0.77)**	0.62 (0.53–0.80)**	0.86 (0.68–1.12)**	0.61 (0.54–0.65)**	0.94 (0.84–1.22)**
MELD score	15 ± 5*	11 ± 4*	11 ± 3**	15 ± 5**	12 ± 4*	14 ± 5*
Child–Pugh score	8 (6–9)*	6 (5–8)*	6 (5–7)**	8 (6–9)**	6 (5–8)*	8 (6–9)*
Bilirubin (mg/dL)	1.7 (1.3–3.6)	1.6 (1.1–2.8)	1.3 (0.9–2.5)**	2.1 (1.5–4.0)**	1.6 (1.1–2.8)	1.7 (1.2–3.6)
INR	1.39 ± 0.32*	1.21 ± 0.17*	1.20 ± 0.21	1.40 ± 0.29	1.27 ± 0.27	1.32 ± 0.27
Creatinine (mg/dL)	1.01 (0.74–1.33)	0.88 (0.75–1.01)	0.90 (0.72–1.05)	0.93 (0.78–1.32)	0.80 (0.67–0.96)**	1.14 (0.90–1.41)**
Albumin (g/dL)	3.5 ± 0.6	3.6 ± 0.6	3.7 ± 0.6*	3.4 ± 0.5*	3.6 ± 0.6	3.5 ± 0.5
ASAT (U/L)	54 (38–71)	50 (44–86)	50 (39–70)	54 (41–73)	56 (46–74)	46 (36–72)
ALAT (U/L)	31 (21–47)	34 (26–44)	34 (23–47)	31 (22–47)	38 (26–47)	29 (22–43)
GGT (U/L)	94 (55–141)	86 (46–166)	95 (47–179)	81 (50–130)	87 (38–165)	97 (56–133)
Alkaline phosphatase (U/L)	123 (91–167)	105 (83–134)	111 (87–154)	121 (87–155)	102 (84–150)*	121 (91–162)*
Platelet count (G/L)	126 ± 70	114 ± 57	136 ± 67*	103 ± 57*	108 ± 47	131 ± 76
CRP (mg/L)	5.5 (2.7–11.0)*	3.6 (1.2–6.3)*	3.6 (1.2–6.4)*	5.3 (2.7–8.8)*	3.0 (1.2–7.7)*	5.5 (2.9–9.7)*

Continuous data are presented as mean ± standard deviation and as medians with interquartile ranges, and categorical data are shown as percentages

Student's *t* test and Chi-square test were used

* $P < 0.05$

** $P < 0.001$

Table 2 Clinical and laboratory characteristics according to Child–Pugh stage

Variable	Child–Pugh A	Child–Pugh B	Child–Pugh C	P value
Numbers	39	38	17	
Age (years)	58 ± 14	60 ± 9	58 ± 9	NS
Females (%)	31	40	12	NS
hArg (μM)	2.05 ± 0.64	1.94 ± 1.03	1.68 ± 1.10	NS
ADMA (μM)	0.83 ± 0.23	0.93 ± 0.20	0.98 ± 0.21	<0.05
SDMA (μM)	0.66 (0.58–0.82)	0.81 (0.63–1.07)	0.86 (0.61–1.11)	NS
MELD score	10 ± 3	14 ± 4	18 ± 4	<0.001
Child–Pugh score	5 (5–6)	8 (7–9)	11 (10–12)	<0.001
Bilirubin (mg/dL)	1.2 (0.9–1.6)	1.8 (1.4–3.0)	4.9 (3.2–7.9)	<0.001
INR	1.15 ± 0.19	1.34 ± 0.22	1.55 ± 0.33	<0.001
Creatinine (mg/dL)	0.97 (0.78–1.10)	0.94 (0.82–1.34)	0.74 (0.66–0.94)	NS
Albumin (g/dL)	4.0 ± 0.4	3.4 ± 0.5	2.9 ± 0.5	<0.001
ASAT (U/L)	47 (37–72)	50 (38–72)	58 (49–76)	NS
ALAT (U/L)	35 (26–58)	31 (22–43)	32 (22–47)	NS
GGT (U/L)	114 (50–182)	95 (52–131)	59 (20–109)	<0.05
Alkaline phosphatase (U/L)	102 (83–152)	120 (90–152)	123 (97–171)	NS
Platelet count (G/L)	124 ± 56	123 ± 71	102 ± 64	NS
CRP (mg/L)	2.3 (1.3–4.7)	5.1 (1.7–8.8)	10.2 (6.2–19.1)	<0.001

Continuous data are presented as mean ± standard deviation and as medians with interquartile ranges, and categorical data are shown as percentages

ANOVA with *P* for trend and Fisher's exact test were used

NS not significant

Table 3 Correlations of arginine metabolites with parameters of hepatic dysfunction

Variable	HArg	ADMA	SDMA	DAS
Creatinine	−0.15 (NS)	0.13 (NS)	0.45 (<0.001)	0.40 (<0.001)
Bilirubin	−0.11 (NS)	0.31 (<0.05)	0.13 (NS)	0.22 (<0.05)
INR	−0.24 (<0.05)	0.42 (<0.001)	0.25 (<0.05)	0.36 (<0.001)
MELD score	−0.26 (<0.05)	0.46 (<0.001)	0.52 (<0.001)	0.57 (<0.001)
Child–Pugh score	−0.11 (NS)	0.25 (<0.05)	0.13 (NS)	0.20 (NS)
hArg	n/a	−0.17 (NS)	−0.36 (<0.001)	−0.34 (<0.05)
ADMA	−0.17 (NS)	n/a	0.44 (<0.001)	0.73 (<0.001)
SDMA	−0.36 (<0.001)	0.44 (<0.001)	n/a	0.94 (<0.001)
DAS	−0.34 (<0.05)	0.73 (<0.001)	0.94 (<0.001)	n/a

Data are presented as Pearson correlation coefficients (with *P* values)

DAS dimethylarginine score (ADMA + SDMA)

*NS* not significant, *n/a* not applicable

Discussion

In a cohort of 94 consecutive cirrhotic outpatients, we have shown that serum levels of the arginine metabolites, hArg, ADMA and SDMA, are related to the degree of liver dysfunction as estimated by Child–Pugh stage. Also, all arginine metabolites measured were found to correlate with MELD, the current standard liver prognostic score with respect to liver transplantation. In prospective analyses, low hArg and higher levels of ADMA, SDMA, and DAS were all predictive of increased long-term mortality (Meinitzer et al. 2011b; Atzler et al. 2014; Mookerjee et al. 2007a). Adjustments for either Child–Pugh stage or MELD score attenuated the associations of all arginine metabolites with survival, but SDMA and DAS remained significantly predictive of mortality independently of both Child–Pugh stage and MELD score.

Our results are in line with previous studies that reported on increased ADMA levels in patients with liver diseases (Mookerjee et al. 2007a, b; Richir et al. 2008; Siroen et al. 2005; Laleman et al. 2005; Becker et al. 2009; Kasumov et al. 2011; Vizzutti et al. 2007; Lluch et al. 2004, 2006; Nijveldt et al. 2003a, 2004; Karakurt et al. 2009). The present work extends the current knowledge on ADMA and liver dysfunction by confirming previous data on the association of ADMA with Child–Pugh score (Lluch et al. 2004), and by demonstrating for the first time that ADMA is positively correlated with the MELD score. These latter results are likewise the consequence of liver dysfunction related to reduced DDAH activity and probably also increased arginine methylation by type 1 protein arginine *N*-methyltransferase (PRMT-1) activity, which both contribute to higher circulating ADMA levels (Richir et al. 2008; Siroen et al. 2004, 2005; Nijveldt et al. 2003b; Tsikas et al. 2007; Becker et al. 2009). Elevated ADMA levels in chronic liver disease may have deleterious consequences via inhibition of NO synthesis and consecutive

intrahepatic NO deficiency contributing to portal hypertension as well as systemic NO deficiency, which may in turn increase cardiovascular risk (Vizzutti et al. 2007; Meinitzer et al. 2011b; Nijveldt et al. 2003a; Karakurt et al. 2009). It should be noted that ADMA levels were found to predict cardiovascular events and mortality in various cohorts of patients without significant liver disease (Meinitzer et al. 2011b; Nijveldt et al. 2003a). However, in our cohort of cirrhotic patients, the relationship of ADMA with mortality was completely abolished after adjustment for Child–Pugh score or MELD score. This is probably due to the close relationship of ADMA with the degree of liver dysfunction. Accordingly, it can be hypothesized that ADMA is either just a marker of liver dysfunction or that the poor outcome in cirrhotic patients can at least partially be attributed to adverse effects of high ADMA levels. Hence, ADMA-lowering therapeutic approaches may represent a promising strategy to reduce complications in patients with advanced liver disease. As such, experimental studies indicate that novel farnesoid X receptor agonists may enhance ADMA metabolism by increasing DDAH-1 expression (Li et al. 2009).

Apart from our findings on ADMA, we herein demonstrate that SDMA is related to MELD score, but not to Child–Pugh score. This seemingly inconsistent association of SDMA with liver dysfunction may be related to the inclusion of creatinine in the MELD, but not Child–Pugh score. In this context, it should be noted that renal dysfunction is a main determinant of SDMA serum levels (Meinitzer et al. 2011b; Lluch et al. 2006; Nijveldt et al. 2003b). This is supported by previous data on significant associations of SDMA with cystatin C levels and reduced glomerular filtration rate (Meinitzer et al. 2011b; Lluch et al. 2006; Nijveldt et al. 2003b). Our findings are consistent with the association of SDMA levels with renal dysfunction, while the impact of liver dysfunction as assessed by the Child–Pugh score seems to be minor. Experimental
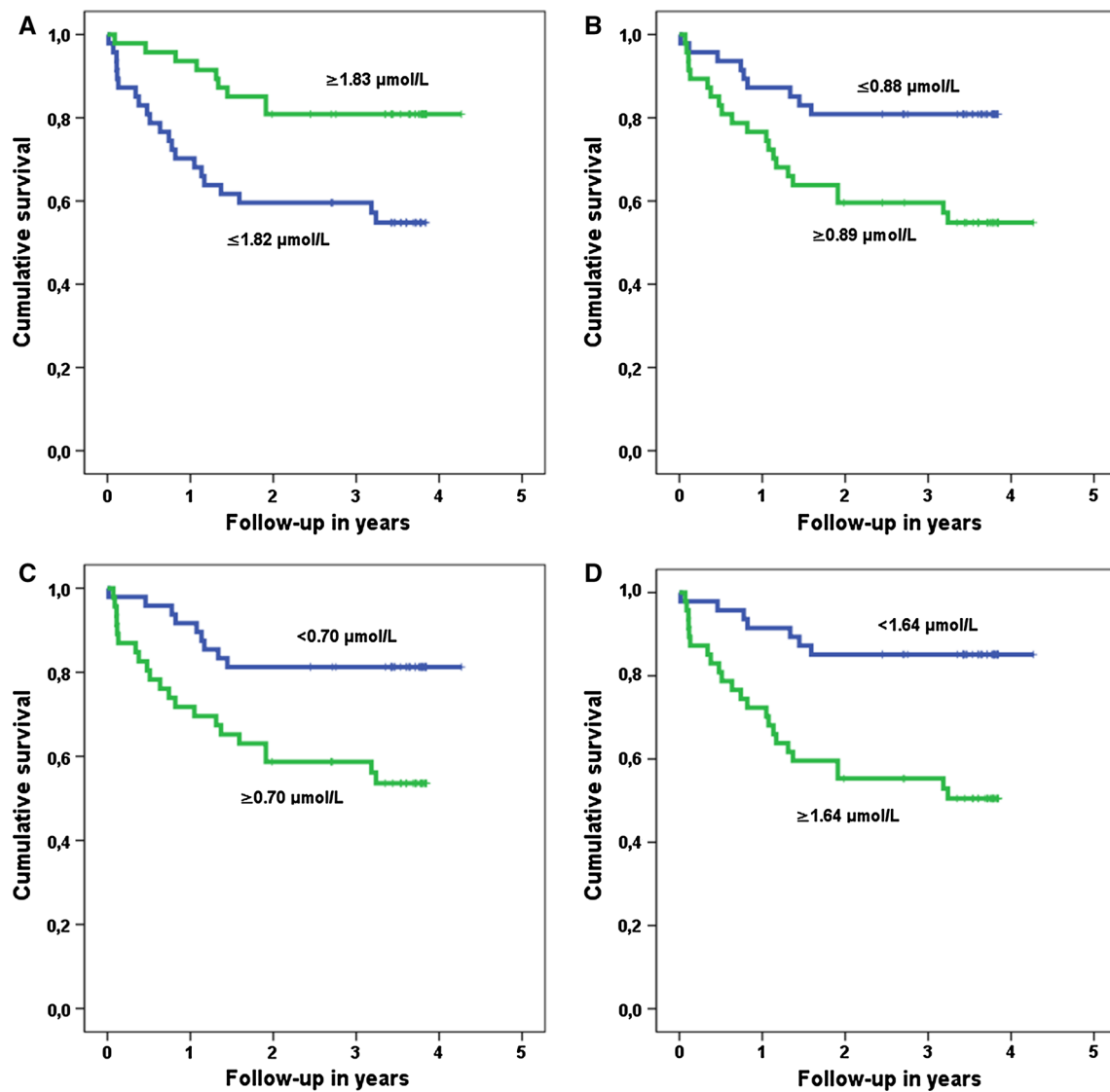
Fig. 1 Kaplan–Meier curves for mortality according to the hArg (**a**), ADMA (**b**), SDMA (**c**), and DAS (**d**) groups. Mortality was significantly higher in patients with baseline levels of hArg ($\leq$1.82 μM, $P = 0.005$), ADMA ($\geq$0.89 μM, $P = 0.009$), SDMA ($\geq$0.70 μM, $P = 0.005$), and DAS ($\geq$1.64 μM, $P = 0.005$)

studies addressing this issue showed controversial data on the role of hepatocytes in the metabolism or extraction rate of SDMA (Siroen et al. 2005; Nijveldt et al. 2003b). Further in-depth studies are therefore needed to shed more light on the relationship of liver function and SDMA. Even though the link of SDMA to liver function remains unclear, it should be pointed out that our study is the first to show that SDMA predicts mortality in cirrhotic patients independently of both Child–Pugh stage and MELD score. This may suggest that SDMA levels reflect previously unrecognized pathophysiologic mechanisms in cirrhotic patients. In ROC curve analyses, the area under the curve of SDMA was similar compared to Child–Pugh and MELD score. We cannot answer the question on the direction of causality for

the association of SDMA and poor outcome in our cohort of cirrhotic patients. Nevertheless, our data indicate that SDMA carries additional prognostic information beyond the Child–Pugh and MELD scores, possibly reflecting the hemodynamic disturbances associated with cirrhosis. This finding should be tested in further studies in large cohorts of cirrhotic patients and, if confirmed, could enable construction of a new prognostic model incorporating SDMA.

Our data are consistent with the findings of Mookerjee et al. (2007a) who reported strong associations of ADMA, SDMA, and DAS with short-term mortality in patients with advanced alcoholic liver disease with or without superimposed alcoholic hepatitis. Our findings confirm and extend the prognostic value of these dimethylarginine

Table 4 Cox proportional hazard ratios (with 95 % CI) for mortality according to arginine metabolite levels

Model	Homoarginine ≥1.88 vs. ≤1.77 μM	ADMA ≥0.89 vs. ≤0.88 μM	SDMA ≥0.73 per ≤0.70 μM	DAS ≥1.64 per <1.64 μM
Unadjusted	0.34 (0.16–0.75)*	2.72 (1.24–5.93)*	2.94 (1.35–6.43)*	4.13 (1.77–9.65)*
Model 1	0.35 (0.16–0.76)*	2.69 (1.23–5.90)*	3.07 (1.36–6.91)*	4.19 (1.77–9.89)*
Model 2	0.48 (0.22–1.12)	1.72 (0.75–3.92)	2.40 (1.06–5.43)*	2.96 (1.23–7.15)*
Model 3	0.54 (0.23–1.23)	1.18 (0.48–2.89)	2.45 (1.09–5.48)*	2.21 (0.88–5.51)
Model	Homoarginine Per μM	ADMA Per μM	SDMA Per μM	DAS Per μM
Unadjusted	0.60 (0.37–0.96)*	3.47 (0.96–12.52)*	6.37 (3.12–13.01)*	4.10 (2.22–7.22)*
Model 1	0.59 (0.37–0.96)*	3.78 (0.98–14.60)*	6.54 (3.15–13.59)*	4.13 (2.26–7.56)*
Model 2	0.75 (0.48–1.17)	2.28 (0.40–12.86)	5.42 (2.47–11.91)*	3.26 (1.70–6.23)*
Model 3	0.82 (0.52–1.31)	0.82 (0.10–6.46)	2.96 (1.38–6.33)*	2.16 (1.13–4.13)*

Model 1, adjusted for age and sex

Model 2, adjusted for age, sex, and Child–Pugh stage

Model 3, adjusted for age, sex, and MELD score

* Significant results with $P < 0.05$
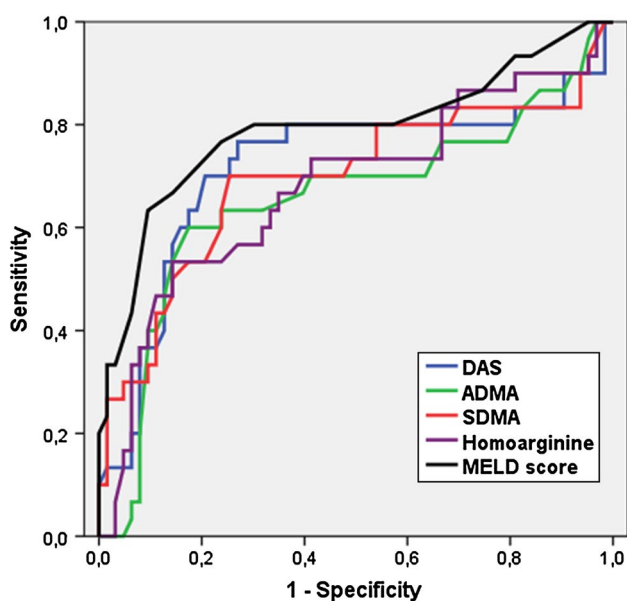
Fig. 2 ROC curves with respect to mortality for arginine metabolites and MELD score. Area under the ROC curves (95 % CI) in descending order: MELD 0.79 (0.68–0.90), DAS 0.72 (0.59–0.85), SDMA 0.69 (0.56–0.82), minus hArg 0.68 (0.55–0.81), and ADMA 0.66 (0.52–0.79)

levels with respect to intermediate/long-term prognosis in patients with less advanced chronic liver disease. However, while Mookerjee et al. (2007a) reported a better predictive utility of DAS compared to ADMA or SDMA per se, our findings do not suggest a marked superiority of DAS above SDMA for prediction of intermediate/long-term prognosis.

We also addressed the associations of hArg with liver dysfunction and mortality. Previous data from various patient cohorts and population-based studies showed that low hArg levels were strong and independent predictors of cardiovascular events and mortality (Meinitzer et al. 2011a; März et al. 2010; Pilz et al. 2011a, b; Drechsler et al. 2011; Kayacelebi et al. 2014; Atzler et al. 2015). Experimental studies suggest that hArg may increase NO synthesis from arginine by, e.g., serving as a substrate for NO synthase or inhibiting arginine degradation by the enzyme arginase, but there are also studies suggesting that hArg may decrease NO synthesis by interferences with arginine transport, because hArg is only a low-affinity substrate for NO synthase and its concentration is several fold lower compared to arginine (Valtonen et al. 2008; Meinitzer et al. 2011a; Atzler et al. 2015). In a previous study on various guanidino compounds in cirrhosis, circulating hArg levels did not differ between cirrhotic patients of various Child–Pugh stages and controls (Marescau et al. 1995). On the other hand, in our cohort we observed a significant association of hArg with MELD score. Similar to our findings on SDMA, it could be speculated that these associations may be driven by the link of hArg and renal function (Meinitzer et al. 2011a; Marescau et al. 1997; Ryan and Wells 1964). Accumulating evidence suggests that hArg deficiency emerges in patients with renal failure due to impaired renal synthesis of hArg (Meinitzer et al. 2011a; Marescau et al. 1997; Ryan and Wells 1964; Drechsler et al. 2013). In contrast, our results show that creatinine was only tentatively, but not significantly, elevated in patients with hArg levels below the median. It should, however, be noted that hArg and the hArg/ADMA ratio was inversely associated with SDMA

levels, which may also be considered a marker of renal dysfunction (Meinitzer et al. 2011b; Lluch et al. 2006; Nijveldt et al. 2003b). In this context, it is interesting to note that genome-wide association studies identified a key enzyme for the synthesis of hArg, arginine:glycine amidinotransferase (AGAT), as a gene locus related to the glomerular filtration rate (Köttgen et al. 2009; Chambers et al. 2010). AGAT is crucial for human energy metabolism by catalyzing the synthesis of guanidinoacetate, a precursor for creatine (Meinitzer et al. 2011a; Pilz et al. 2011b; Cullen et al. 2006). It is therefore of interest that AGAT expression was also found in the liver suggesting a role of hepatocytes in hArg metabolism (Cullen et al. 2006). Apart from this, it should also be mentioned that hArg has previously been shown to inhibit liver-type alkaline phosphatases, but mM-concentrations of hArg were required to show this effect (Rufo and Fishman 1972). Whereas the pathophysiologic relevance of this effect remains to be investigated, a significant association of hArg with serum alkaline phosphatase activity has been reported (Pilz et al. 2012, 2013). Regarding hArg and outcome, we have shown that high circulating hArg levels are significantly associated with decreased risk of mortality. This association was, however, attenuated toward a moderate trend after adjusting for Child–Pugh stage or MELD score. Using the proposed ratios of hArg and dimethylarginines, such as the hArg/ADMA ratio (Tsikas and Kayacelebi 2014), did not show materially different results compared to the use of dimethylarginines or hArg as single parameters (data not shown). Nevertheless, the observed relationship of hArg with liver dysfunction and mortality deserves further research to clarify the diagnostic and therapeutic potential of hArg metabolism in chronic liver failure.

Our results are limited due to the observational nature of our study design which precludes conclusions regarding causality. Furthermore, our findings were derived from a relatively small cohort of patients attending an outpatient liver clinic. Another drawback of our study is that we did not include a control group without liver diseases. It should, however, be noted that in comparison with another study in healthy controls at our laboratory, ADMA concentrations were higher in the present cohort of patients with liver cirrhosis (Meinitzer et al. 2007). Finally, our study population consisted of a Caucasian group of patients whose cirrhosis was mainly of alcoholic origin and our results can therefore not be simply extrapolated to other study populations.

In conclusion, high dimethylarginines as well as low hArg concentrations in serum are associated with long-term mortality of cirrhotic patients, but only the association of SDMA and DAS with mortality remains significant after adjustment for both Child–Pugh stage and MELD score. Further studies are needed to confirm the prognostic value of these arginine metabolites, in particular of SDMA, in

large cohorts of patients with different degrees of liver failure.

Conflict of interest The authors declare that they have no conflict of interest.

Ethical statement The study was approved by the local ethics committee.

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