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Endogenous Nitric Oxide Synthase Inhibitors in Sickle Cell Disease: Abnormal Levels and Correlations with Pulmonary Hypertension, Desaturation, Hemolysis, Organ Dysfunction and Death

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Abstract

Pulmonary hypertension (PH) in patients with sickle cell disease (SCD) is linked to intravascular hemolysis, impaired nitric oxide bioavailability, renal dysfunction, and early mortality. Asymmetric dimethylarginine (ADMA), an endogenous inhibitor of nitric oxide synthases (NOS), is associated with vascular disease in other populations. We determined the plasma concentrations for several key arginine metabolites and their relationships to clinical variables in 177 patients with SCD and 29 control subjects: ADMA, symmetric dimethylarginine (SDMA), NG-monomethyl L-arginine (L-NMMA), N-omega-hydroxy-L-arginine (NOHA), arginine and citrulline. The median ADMA was significantly higher in SCD than controls (0.94 vs. 0.31 $\mu\text{mol/L}$, $p < 0.001$). Patients with homozygous SCD had a remarkably lower ratio of arginine to ADMA (50 vs. 237, $p < 0.001$). ADMA correlated with markers of hemolysis, low oxygen saturation and soluble adhesion molecules. PH was associated with high levels of ADMA and related metabolites. Higher ADMA level was associated with early mortality, remaining significant in a multivariate analysis. Subjects with homozygous SCD have high systemic levels of ADMA, associated with PH and early death, implicating ADMA as a functional NOS inhibitor in these patients. These defects and others converge on the nitric oxide pathway in homozygous SCD with vasculopathy.

Keywords

Sickle Cell Disease; ADMA; SDMA; NOHA; Arginine; Pulmonary Hypertension

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Clinical Trials Registration: <http://clinicaltrials.gov/ct2/show/NCT00011648>

INTRODUCTION

Sickle cell disease (SCD), caused by homozygosity for a single amino acid substitution within the β -subunit of hemoglobin or by compound heterozygosity for the sickle and another β -globin mutation, is associated with a range of clinical complications. Intracellular polymerization of the abnormal hemoglobin results in rigid red cells that flow poorly through the microvasculature, promoting tissue ischemia and infarction. Pulmonary hypertension (PH), cutaneous leg ulceration, and priapism constitute a distinct constellation of sickle cell complications associated with particularly severe intravascular hemolysis (Kato, *et al*, 2007). This process releases cell-free hemoglobin and arginase into plasma, correlating with depletion of nitric oxide (NO) and arginine, the obligate substrate of the NO synthases (NOS) (Morris, *et al*, 2005, Reiter, *et al*, 2002). These pathophysiologically related complications have been called sickle vasculopathy or the hemolysis-endothelial dysfunction subphenotype, involving a decreased bioavailability of NO, a gas that normally maintains vascular homeostasis (Kato, *et al*, 2007). Additional factors that disrupt the normal production of NO may also contribute to vasculopathy in SCD.

Asymmetric dimethylarginine (ADMA) is a naturally occurring modified arginine found in plasma (Boger, 2004). It originates from hydrolysis of proteins bearing methylated arginine residues, a known post-translational modification (Teerlink, 2005). ADMA, normally metabolized by dimethylarginine dimethylaminohydrolases I and II (DDAH I and II), is an endogenous inhibitor of NOS isoforms. Elevated systemic levels of ADMA have been associated with atherosclerosis, particularly in patients with renal insufficiency (Boger and Zoccali, 2003). ADMA has been found at higher levels in idiopathic and chronic thromboembolic PH (Kielstein, *et al*, 2005, Skoro-Sajer, *et al*, 2007), PH related to congenital heart disease (Loukanov, *et al*, 2008), and in a rodent model of PH (Sasaki, *et al*, 2007). Elevation of ADMA in DDAH deficient mice is associated with endothelial dysfunction and elevated systemic and pulmonary blood pressure (Leiper, *et al*, 2007). ADMA has been found to be elevated in the plasma of patients with SCD, and associated with soluble vascular cell adhesion molecule-1 (sVCAM-1), a marker of endothelial activation normally suppressed by NO (Landburg, *et al*, 2008a, Schnog, *et al*, 2005). ADMA was also associated with lower hemoglobin, suggesting a correlation with more severe intravascular hemolysis. We hypothesized that elevated systemic levels of ADMA contribute to the hemolysis-vascular dysfunction subphenotype found in patients with SCD.

There are also other forms of modified arginine found in plasma. L-monomethylarginine (L-NMMA) is also a NOS inhibitor. Symmetric dimethylarginine (SDMA) is an isomer of ADMA, does not inhibit NOS (Leiper and Vallance, 1999), although it is not clear if it could inhibit arginine transport or other relevant aspects of the arginine-NO pathway. N-omega-hydroxyarginine (NOHA) is a metabolic intermediate of NOS activity (Morris, 2007). Herein we measured the plasma levels of these modified arginines in a large cohort of patients with SCD and examined their relationship with clinical characteristics and adverse outcomes in subjects with SCD. We find high plasma levels of ADMA in SCD patients are associated with the presence of PH, with its associated markers, and increased mortality. We also find a new, significant association of high NOHA levels with renal dysfunction.

MATERIALS AND METHODS

Patients

The patient and control populations in this study have been described previously (Gladwin, *et al*, 2004). The control group was approximately one quarter the size of the patient population and equivalent in ethnic, age and sex distribution. All patients provided informed consent under a protocol approved by the Institutional Review Board at the National

Institutes of Health (clinicaltrials.gov identifier NCT00011648). Their baseline characteristics are summarized in Supplemental Table 1. They provided a medical history, and underwent physical examination, blood sampling, and Doppler echocardiography as previously described in detail (Gladwin, *et al*, 2004, Taylor, *et al*, 2008). Consistent with our previous validation data in this population (Gladwin, *et al*, 2004), PH was defined as a tricuspid regurgitant jet velocity (TRV) ≥ 2.5 m/sec, and moderate-to-severe PH was defined as TRV ≥ 3.0 m/sec. This is a research definition, and was used for individual patient diagnosis and management only in conjunction with clinically indicated right heart catheterization studies.

Laboratory measurements

Standard clinical laboratory assays were performed at the Department of Laboratory Medicine at the Clinical Center at the National Institutes of Health. Soluble vascular cell adhesion molecule-1 and soluble E-selectin levels were measured as previously described (Kato, *et al*, 2005), Arginase assays were performed as previously described (Morris, *et al*, 2005).

Amino acid assays

Plasma samples were stored at -80°C until analyses. After thawing and brief vortex mixing, aliquots (100 μL) were supplemented with [$^{13}\text{C}_6$]arginine internal standard, and subsequently quantified by liquid chromatography electrospray ionization tandem mass spectrometry using an ABI 365 triple quadrupole mass spectrometer (Applied Biosystems Inc., Foster City, CA, USA) equipped with Ionics EP 10+ upgrade (Concord, Ontario, Canada) and Aria LX4 series multiplexed HPLC system with Flux pumps interface (Cohesive Technologies, Franklin, MA). Mass spectrometric analyses were performed in the positive ion mode with multiple reaction monitoring (MRM) using unique characteristic parent \rightarrow daughter ion transitions for each analyte, as previously described (Nicholls, *et al*, 2007). Each analyte monitored demonstrated near quantitative recovery, good linearity over multiple orders of magnitude in concentration range, and intra- and inter-assay coefficients of variance of $<10\%$.

Statistical analysis

Due to skewed distributions of the arginine metabolites, nonparametric analyses were used. Group comparisons were performed with the Mann-Whitney test, or the Kruskal-Wallis test where appropriate. Associations between continuous variables were assessed using the Spearman rank correlation coefficient. Survival analysis was performed using proportional hazards (Cox) regression. Survival data were current as of October 1, 2007. For the 124 homozygous SCD patients included in the survival analysis, median follow-up was 5.3 years for the 102 survivors and 3.3 years for the 22 patients who died. Two-sided p-values reported; $p < 0.05$ was considered statistically significant. The authors had full access to the data and take responsibility for its integrity. All authors have read and agree to the manuscript as written.

RESULTS

Arginine metabolite levels in patients with SCD

Patients with SCD at steady state had significantly higher plasma levels of methylated arginines, including ADMA, SDMA and L-NMMA, compared to healthy African-American control subjects ($p < 0.001$ for all analytes) (Table 1). In contrast, patients with SCD had lower levels of plasma L-arginine ($p < 0.001$), NOHA ($p = 0.002$), and citrulline ($p = 0.03$) (Table 1). When these comparisons were repeated according to SCD genotype subgroup,

homozygous sickle cell disease patients had the most dramatic and significant abnormalities in levels of arginine metabolites, and these overlapped the values seen in hemoglobin S β° -thalassemia patients, as expected parallel to the known clinical severity of each phenotype. Most prominently, the median ADMA level in patients with homozygous SCD was three times higher than in African American healthy control subjects ($p < 0.001$), and median plasma L-arginine was 42% lower ($p < 0.001$). As expected, differences from controls were smaller, but significant, in hemoglobin SC and hemoglobin S β^+ thalassemia compound heterozygotes (Table 1). The ratio of arginine to ADMA, a marker of vascular disease risk in other disorders, was remarkably lower in SCD compared to controls (median 50 vs. 237, $p < 0.001$). In order to exclude confounding effects due to different SCD genotypes, only patients with homozygous SCD were included for the remainder of the analysis.

Relationship of methylated arginine levels to renal function

The methylated arginine levels showed significant relationships to markers of renal function. Homozygous SCD patients with moderate or severe renal insufficiency (serum creatinine $> 220 \mu\text{mol/L}$, $n=6$) had levels of methylated arginines that were very significantly different from levels in patients without renal insufficiency ($n=123$), including lower levels of ADMA and higher levels of L-NMMA, SDMA and NOHA (Table 2). Serum citrulline levels also were much higher in patients with renal insufficiency. The inverse relationship of ADMA to creatinine is a consistent finding when all SCD patient data are analyzed as a continuous variable (linear regression $r^2 = 0.21$, $p < 0.001$ using log-transformed values), so it is not due simply to outlier characteristics of the small renal failure cohort. Qualitatively similar correlations were seen with glomerular filtration rate as estimated by the Cockcroft-Gault formula (data not shown). Most strikingly, in homozygous SCD patients linear regression analysis demonstrated very strong links of serum creatinine to NOHA ($r^2=0.91$, $p < 0.0001$) and citrulline ($r^2=0.72$, $p < 0.0001$). The levels of the arginine-related amino acids in renal insufficiency patients were thus outliers in many respects, and were excluded from the remainder of the analysis.

Clinical correlates of arginine metabolites in homozygous SCD

Among patients with homozygous SCD and relatively normal renal function, the levels of three arginine metabolites varied significantly with respect to degree of pulmonary hypertension. Levels of ADMA, SDMA and NOHA were significantly higher in patients with PH than those without (Table 3). ADMA and SDMA levels also correlated with the PH marker amino-terminal brain-type natriuretic propeptide (NT-proBNP)(Table 4), further supporting their link to PH. Several clinical indices of accelerated hemolysis (low hemoglobin levels, high reticulocyte counts, and high serum levels of lactate dehydrogenase or indirect bilirubin) showed significant correlations to ADMA, and to a lesser extent, with SDMA and L-NMMA (Table 4). In addition, ADMA was correlated with plasma arginase activity, which also has been linked to intravascular hemolysis. Low transcutaneous oxygen saturation correlated significantly with high levels of ADMA, SDMA, L-NMMA and the NOS intermediate NOHA (Table 4). Homozygous SCD patients taking hydroxyurea tended to have slightly lower levels of ADMA, but this was not significant.

NOS pathway

Patients with homozygous SCD and relatively normal renal function ($n = 124$) demonstrated significantly lower median plasma arginine levels than healthy African-American control subjects ($n=29$) (45 vs. 78 $\mu\text{mol/L}$, Mann-Whitney $p < 0.001$)(Figure 1), presumably due in large part to plasma arginase, as indicated by a low ratio of arginine to ornithine as previously published (Morris, *et al*, 2005). Homozygous SCD patients have higher median ADMA (1.02 vs. 0.31 $\mu\text{mol/L}$, $p < 0.001$), lower median NOHA (1.8 vs. 2.5 $\mu\text{mol/L}$,

$p < 0.001$), and lower median citrulline (16 vs. 20 $\mu\text{mol/L}$, $p < 0.01$), potentially compatible with decreased flux through the NOS pathway.

While only a limited sample size ($n = 6$), a distinctly different profile emerged in the homozygous SCD patients with marked renal insufficiency, indicated by a serum creatinine $> 220 \mu\text{mol/L}$. In these patients, median citrulline was 38-fold higher than in control subjects ($n = 29$) (97 vs. 2.5 $\mu\text{mol/L}$, $p < 0.001$), and median arginine was again low (50 vs. 78 $\mu\text{mol/L}$, $p < 0.01$), consistent with a defect in conversion of citrulline to arginine, due to renal insufficiency (Supplemental Figure 1). Interestingly, in homozygous SCD patients with renal insufficiency, the NOS intermediate NOHA was elevated to very high levels compared to healthy control subjects (median 34 vs. 2.5 $\mu\text{mol/L}$, $p < 0.001$).

Survival

Follow up was available for 124 homozygous SCD patients with serum creatinine $< 220 \mu\text{mol/L}$. Patients with ADMA above the group median value ($> 1.02 \mu\text{mol/L}$, $n = 62$) had significantly shorter survival than patients with ADMA at or below the median value ($\leq 1.02 \mu\text{mol/L}$, $n = 62$, $p = 0.02$, Cox regression) (Figure 2). When analyzed as a continuous variable, higher ADMA was significantly associated with mortality ($p = 0.04$), remaining significant ($p = 0.02$) in a multivariable model with creatinine ($p < 0.001$) as a covariate. However, ADMA lost significance ($p = 0.08$) when PH was added to the model, consistent with the association between ADMA and PH (Table 3).

DISCUSSION

Our data from a large cohort indicate that patients with SCD have very high levels of the endogenous NOS inhibitor, ADMA - three times that of healthy African-American controls. This confirms and extends the preliminary observations of Schnog and colleagues on 12 patients with SCD (Schnog, *et al*, 2005). As might be predicted, the levels are the most abnormal in patients with homozygous SCD and hemoglobin $\text{S}\beta^0$ thalassemia, and elevated to an intermediate degree in hemoglobin SC and hemoglobin $\text{S}\beta^+$ thalassemia. ADMA levels are linked to multiple markers of hemolytic severity, including low hemoglobin, and high levels of LDH, bilirubin, reticulocytes, fetal hemoglobin and arginase. ADMA is typically generated during proteolysis of arginine-methylated proteins (Teerlink, 2005), and it is tempting to speculate that in SCD, ADMA might be produced from the breakdown of proteins contained in sickle erythrocytes, which turn over at a rate up to twenty times normal (Crosby, 1955). Alternatively, there might be a hemolysis-linked inhibition of DDAH, the enzyme that hydrolyzes methylarginine (Ito, *et al*, 1999). In addition, endothelial shear stress induced protein arginine methyltransferase activity (Landburg, *et al*, 2008a), which reportedly is highest in patients with lowest hemoglobin levels (Osanai, *et al*, 2003), may contribute. Even though peripheral tissue oxygenation is often near normal due to the low affinity of HbS for oxygen, hypoxia induced down regulation of DDAH specifically in the pulmonary vasculature (10) could also contribute. However, it is not immediately apparent what factor might be contributing to the accumulation of plasma ADMA. In fact, this is the converse of what might have been expected, since DDAH activity is contained in red cells, and might be released during intravascular hemolysis (Kang, *et al*, 2001). Clearly hemolysis is not necessary for ADMA elevation in other vasculopathies without hemolysis, such as idiopathic PH (Kielstein, *et al*, 2004), but their epidemiologic association in SCD is found in our study and by Schnog and colleagues (Schnog, *et al*, 2005).

There is substantial evidence that intravascular hemolysis with SCD induces a multifactorial decrease in nitric oxide bioavailability. Intravascular hemolysis releases cell-free plasma hemoglobin that scavenges nitric oxide (Reiter, *et al*, 2002), and cell-free plasma arginase that is correlated with depletion of the NOS substrate arginine (Morris, *et al*, 2005). Markers

of this process are linked to nitric oxide resistance in blood flow physiology assays and pulmonary vasoconstriction in humans and animals with SCD (Aslan, *et al*, 2001, Eberhardt, *et al*, 2003, Kaul, *et al*, 2000, Nath, *et al*, 2000, Reiter, *et al*, 2002). A similar pathophysiology is observed in other animal models of hemolysis and other human hemolytic diseases (Gramaglia, *et al*, 2006, Hsu, *et al*, 2007, Minneci, *et al*, 2005, Rother, *et al*, 2005). Thus, hemolysis-linked accumulation of the NOS inhibitor ADMA adds to the list of insults to NO bioavailability in patients with SCD.

Our data also **confirm and extend** that ADMA levels in patients with SCD are linked to a vasculopathic phenotype, specifically PH (as defined by TRV \geq 2.5 m/sec, and also associated with a high NT-proBNP level), and corroborated by the endothelial activation markers soluble VCAM-1 and soluble E-selectin. These same patients also manifest lower oxygen saturation, which speculatively might be related to ventilation-perfusion mismatch due to pulmonary vascular tone dysregulation. Interestingly, the high ADMA level also is linked in our data with two additional markers previously correlated to PH in SCD, higher serum alkaline phosphatase and direct bilirubin level (De Castro, *et al*, 2008, Gladwin, *et al*, 2004, Onyekwere, *et al*, 2008). Our linkage of ADMA levels to increased mortality risk in SCD adds to the list of disease conditions with similar associations between ADMA and death (Meinitzer, *et al*, 2007, Nijveldt, *et al*, 2003, Ravani, *et al*, 2005, Schnabel, *et al*, 2005, Zoccali, *et al*, 2001).

One very unusual observation in our patients with SCD is the inverse relationship of ADMA levels to serum creatinine. In the general population, ADMA levels are positively correlated with serum creatinine levels, implying that ADMA is partly excreted by the kidney and accumulates during renal dysfunction (Xiao, *et al*, 2001). However, we find that in SCD patients, the relationship is inverted; ADMA levels are inversely related to serum creatinine levels, and fall as renal dysfunction progresses. Presumably, this paradoxical relationship must be induced by some aspect of SCD pathophysiology that impacts upon ADMA formation or clearance.

The other methylarginine derivatives provide a few interesting observations. First, the relationship of NOHA to serum creatinine level is very striking. This suggests that NOHA and creatinine are handled by a highly overlapping mechanism in the kidney. To our knowledge, such a relationship of plasma NOHA levels to renal function has not been previously reported in any patient cohort. NOHA is considered a transient intermediate of NOS activity, and its accumulation in renal insufficiency up to 20-fold was unexpected. More investigation is needed to understand the mechanism and physiologic implications of the close correlation of NOHA to creatinine. Second, the levels of the other endogenous NOS inhibitor, L-NMMA, are lower than ADMA, though it shares some of the epidemiologic correlations to ADMA, especially to cholestatic hepatic dysfunction and endothelial activation. Third, the pattern of SDMA correlations to markers of hemolysis, endothelial activation and PH overlaps that of ADMA, even though it has the converse relationship with renal function shown by ADMA. Lastly, the strong relationship of citrulline levels, renal dysfunction and PH in SCD invites speculation whether impaired renal regeneration of arginine from citrulline may influence the bioavailability of arginine to NOS for NO production, which is unclear from our limited data but merits further research. A recent study also supports the predictive value of elevated creatinine for poor survival in PH (Shah, *et al*, 2008). At the time of submission of this manuscript, another report of ADMA elevation in sickle cell pulmonary hypertension is in press (Landburg, *et al*, 2008b).

There are several limitations to this study. Our findings are limited by the purely correlative nature of this investigation, and they do not prove causation. However, other recently published data from a DDAH-deficient mouse convincingly suggests a causal relationship of

ADMA with vasculopathy (Leiper, *et al*, 2007). A larger sample size might clarify some of the trends seen in our population, but our cohort already exceeds by **over two-fold** the previously published cohort for ADMA in SCD (Schnog, *et al*, 2005), and our analysis adds the other five arginine metabolite levels and their correlations with pulmonary hypertension and death, providing a more comprehensive picture. Due to the registry nature of this study, patients received a variety of clinically indicated or investigational treatments, and there is insufficient statistical power in the cohort to determine relationships to ADMA levels.

The correlation of elevated ADMA levels in PH in SCD suggests that this risk factor for atherosclerosis in the general population may also be a risk factor for the development of vasculopathy and PH in the SCD population. We propose that in sickle cell vasculopathy, the known prominent risk factor of hemolysis-associated reduction in nitric oxide bioavailability in SCD patients is modified by other established vascular disease risk factors, such as ADMA levels.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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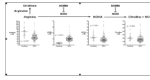


Figure 1. Relationship of the NOS inhibitor ADMA to arginine metabolism in sickle cell disease
 Nitric oxide synthase (NOS) converts L-arginine to the intermediate NOHA, which is then converted to citrulline and nitric oxide. In patients with sickle cell disease with relatively normal renal function (serum creatinine < 220 $\mu\text{mol/L}$, $n = 161$), plasma arginine levels are lower than controls ($n = 29$), consistent with previously published effects of plasma arginase activity. Plasma levels of the NOS inhibitor ADMA are much higher than controls, and the downstream products of NOS activity are lower than controls, including both NOHA and citrulline. Horizontal bars indicate median values, and p value is calculated by the Mann-Whitney test.

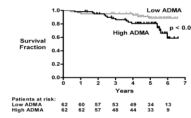


Figure 2. Higher ADMA levels associated with early mortality

Kaplan-Meier plot of survival fraction in 124 patients with homozygous sickle cell disease with serum creatinine $< 220 \mu\text{mol/L}$, divided into those with ADMA plasma levels above or below the median for this group ($1.02 \mu\text{mol/L}$). Patients with higher ADMA levels have earlier mortality ($p = 0.02$, Cox regression).

Arginine Metabolites in SCD vs. controls. Values indicate median plasma levels at steady state in micromolar. Results are compared for control subjects compared to all SCD patients, and then the same SCD patients categorized by genotype.

Table 1

Arginine Metabolite	Control (n=29)	All SCD (n=177)	SS (n=130)	Sf ⁰ -thal (n=3)	SC (n=34)	Sf ⁺ -thal (n=8)
ADMA	0.31	0.94 ***	0.99 ***	1.48 ***	0.82 ***	0.78
SDMA	0.83	1.02 ***	1.05 **	1.04	0.92	0.98
L-NMMA	0.13	0.18 ***	0.18 ***	0.23	0.15	0.17
L-Arginine	78.3	46.4 ***	45.5 ***	28.5 *	51.5 **	42.7 **
NOHA	2.50	1.93 **	1.80 *	2.15	2.23	2.11
Citrulline	20.1	17.7 *	17.0 *	19.1	21.1	20.6

* p<0.05 compared to control

** p<0.01

*** p<0.001; Mann-Whitney test for all SCD compared to control subjects; Kruskal-Wallis test with Dunnett's post-test for comparisons of genotype categories to control subjects.

Table 2

Arginine Metabolites in Renal Insufficiency

Values indicate median plasma levels of arginine metabolites (in $\mu\text{mol/L}$) at steady state. The comparison includes patients with homozygous sickle cell anemia with serum creatinine $< 220 \mu\text{mol/l}$ ($n=123$) or $> 220 \mu\text{mol/l}$ ($n=6$)(Mann-Whitney test).

Arginine Metabolite	Creatinine $< 220 \mu\text{mol/L}$		Creatinine $> 220 \mu\text{mol/L}$		p
	Median	Interquartile Range	Median	Interquartile Range	
ADMA	1.02	0.82, 1.24	0.45	0.23, 0.71	<0.001
SDMA	1.01	0.82, 1.28	3.64	3.09, 4.29	<0.0001
L-NMMA	0.18	0.14, 0.22	0.27	0.23, 0.33	<0.0001
L-Arginine	46	36, 57	50	45, 56	NS
NOHA	1.8	1.4, 2.4	34	16, 63	<0.0001
Citrulline	16	12, 21	97	64, 113	<0.0001

Table 3
Arginine Metabolites in Pulmonary Hypertension

Values indicate median plasma levels of arginine metabolites (in $\mu\text{mol/L}$) at steady state. Patients are categorized by Doppler echocardiographic measurement of tricuspid regurgitant jet velocity of < 2.5 m/sec (no PH, **n=81**), $2.5 - 2.9$ m/sec (mild PH, **n=26**), or > 2.9 m/sec (moderate – severe PH, **n=11**). The comparison includes patients with homozygous sickle cell disease with serum creatinine levels < 220 $\mu\text{mol/L}$ (Kruskal-Wallis test).

Arginine Metabolite	No PH	Mild PH	Mod-Severe PH	p
ADMA	0.95	1.18	1.14	0.02
SDMA	0.96	1.11	1.17	0.008
L-NMMA	0.18	0.19	0.16	NS
L-Arginine	45	40	52	NS
NOHA	1.7	1.7	2.9	0.005
Citrulline	16	16	21	NS

Table 4

Correlations of arginine metabolites to clinical markers

Values shown are the Spearman correlation coefficients of the respective arginine metabolites to the indicated clinical variables in patients with homozygous sickle disease without significant renal insufficiency. Blank cells indicate nonsignificant ($p>0.05$) correlations.

Category	Variable	n	ADMA	SDMA	L-NMMA	NOHA
<i>Pulmonary</i>	NT-proBNP	117	0.24**	0.30**	-	0.21*
	Oxygen saturation	69	-0.48***	-0.46***	-0.25*	-0.42***
<i>Blood pressure</i>	Systolic blood pressure	104	-	-	-	0.23*
	Diastolic blood pressure	104	-	-	-	0.24*
<i>Hematologic</i>	Leukocyte count	116	0.34***	-	0.25**	-
	Hemoglobin	116	-0.38***	-0.34***	-	-
	Reticulocyte count	109	0.39***	-	-	-
	Fetal Hemoglobin	117	-0.40***	-0.24**	-0.30*	-
	Lactate dehydrogenase	102	0.30**	0.29**	-	-
<i>Hepatic</i>	Indirect bilirubin	116	0.23*	-	-	-
	Alkaline phosphatase	116	0.24**	0.27**	0.22*	-
	Alanine aminotransferase	116	0.20*	-	0.22*	-
	Bilirubin, direct	116	0.39***	0.32**	0.20*	-
<i>Renal</i>	Creatinine	117	-	0.36***	-	0.61***
<i>Endothelial</i>	Soluble VCAM-1	116	0.37***	0.35***	0.29*	0.20*
	Soluble E-selectin	110	0.27**	0.28**	-	0.26***
	Plasma Arginase activity	91	0.27*	0.36***	-	-

* $p<0.05$ ** $p<0.01$ *** $p<0.001$