Increased levels of symmetric dimethyl-arginine are associated with all-cause mortality in patients with symptomatic peripheral arterial disease

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Objective: Recent interest has focused on the role of the methyl-arginines, endogenous inhibitors of nitric oxide, as adverse prognostic indicators. To date, few studies have assessed the role of symmetric dimethyl-arginine (SDMA) in patients with peripheral arterial disease. We aimed to determine the relationship, if any, of SDMA to all-cause mortality and disease severity as assessed by the ankle-brachial index (ABI) in patients with symptomatic peripheral arterial disease (PAD).

Methods: In 238 patients with symptomatic PAD and an ABI of <0.8, L-arginine, asymmetric dimethyl-arginine (ADMA) and SDMA levels were measured by hydrophilic-interaction liquid chromatography-electrospray tandem mass spectrometry.

Results: The median follow-up was 6 years 11 months (interquartile range [IQR], 4 years 5 months-7 years 10 months). SDMA and ADMA levels were higher in those who died compared with those who survived (0.51 [IQR, 0.44-0.66] μmol/L vs 0.46 [IQR, 0.39-0.55] μmol/L, $P \leq .001$; and 0.48 [IQR, 0.41-0.55] μmol/L vs 0.45 [IQR, 0.39-0.50] μmol/L, $P = .007$, respectively). L-arginine levels were similar in the two groups. On multivariate analysis, SDMA and ADMA as continuous variable were significantly associated with mortality ($P = .001$). For SDMA and ADMA, the highest compared with the lowest quartile levels were significantly associated with mortality (SDMA: hazard ratio, 3.855; 95% confidence interval, 1.625-9.143; $P = .002$; ADMA: hazard ratio, 2.277; 95% confidence interval, 1.114-4.654; $P = .024$). ADMA and SDMA showed a negative correlation with severity of PAD as assessed by ABI ($r = -0.236$, N = 216, $P < .001$; $r = -0.209$, N = 208, $P = .002$, respectively).

Conclusions: The novel finding of this study is that SDMA levels were predictive of all cause-mortality and correlated with disease severity. Further studies should assess the role of nitric oxide donors in patients with high levels of SDMA. (J Vasc Surg 2015;61:1292-8.)

Peripheral artery disease (PAD) is a common condition that is associated with considerable morbidity and mortality, mainly from cardiovascular causes.[1-4] The endothelial and platelet dysfunction that occurs in patients with PAD has been implicated in the development and progression of atherosclerosis.[5] In normal vessels, endothelial cells protect the vessel by regulating blood flow, blood content, and blood pressure. They exert this function via nitric oxide (NO), a potent vasodilator that is synthesized from the amino acid L-arginine by a family of enzymes called NO syntheses.[6]

NO production from its substrate L-arginine is autoregulated by endogenous proteins called methyl-arginines.

Symmetric dimethyl-arginine (SDMA), a structural analog of asymmetric dimethyl-arginine (ADMA), influences NO formation by inhibiting the cellular uptake of L-arginine.[7] ADMA, which is a by-product of monomethyl-arginine, inhibits NO syntheses and thus reduces levels of NO. Increased levels of these inhibitors have been implicated in the reduced NO synthesis observed in patients with PAD and their increased cardiac morbidity and mortality.[8]

The one prior study involving primary care patients with symptomatic or asymptomatic PAD found that SDMA was not associated with all-cause mortality.[9] In contrast, high SDMA levels are associated with all-cause mortality[10-15] in patients with cerebrovascular and coronary disease. High ADMA plasma concentrations were shown to be an independent predictor of major adverse cardiac events and all-cause mortality in the general population, in patients with PAD, and in those undergoing coronary angioplasty.[10,11,16]

In this study, we aimed to assess the relationship, if any, of SDMA to all-cause mortality and severity of disease as determined by the ankle-brachial index (ABI) in patients with symptomatic PAD.

METHODS

The Grampian Research Ethics Committee granted ethical approval for this study, and informed written consent was obtained.

Table I. Patient demographics[a]

Patient characteristics	Total cohort (N = 238)	Alive (n = 138)	Dead (n = 99)	P value
Age, years	69 (62-75)	66 (60-74)	72 (64-76)	.004
Male gender	157 (66)	83 (60)	74 (75)	.027
Body mass index, kg/m^2	26.2 (23.5-29.4)	26.9 (24.3-29.7)	24.8 (22.0-27.7)	<.001
Hypertension	154 (65)	90 (65)	64 (65)	1.000
Diabetes mellitus	32 (14)	12 (9)	20 (20)	.019
Ischemic heart disease[b]	74 (31)	39 (28)	35 (35)	.348
Angina	23 (10)	16 (12)	7 (7)	.372
Drugs				
ACE inhibitor	63 (26)	37 (27)	26 (26)	1.000
β-Blocker	50 (21)	30 (22)	20 (20)	.905
ABI	0.56 (0.41-0.68)	0.59 (0.46-0.73)	0.50 (0.38-0.59)	<.001
ABI <0.5	78 (33)	37 (27)	41 (41)	.002
eGFR	65.6 (52.4-75.2)	63.7 (52.2-73.0)	67.3 (51.9-79.3)	.253
eGFR <60 mL/min	86 (36)	52 (38)	34 (34)	.700

ABI, Ankle-brachial index; *ACE*, angiotensin-converting enzyme; *eGFR*, estimated glomerular filtration rate; *IQR*, interquartile range.
[a]Statistical analysis: Continuous data were assessed with the Mann-Whitney U test and are presented as a median (IQR). Categorical data were assessed with the χ^2 test and are presented as number of patients (%).
[b]Defined as a history of myocardial infarction or a positive exercise test, current complaint of chest pain considered to be secondary to myocardial ischemia, use of nitrate therapy, or an electrocardiogram with pathologic Q waves.

Study design. This observational study recruited 238 patients with symptomatic PAD and an ABI of <0.8 attending the vascular clinic at Aberdeen Royal Infirmary during a 36-month period. Patients were monitored for a median of 6 years 11 months (interquartile range [IQR], 4 years 5 months-7 years 10 months). The patients involved in the study were the subject of previous publications.[17]

Inclusion criteria were patients with symptomatic PAD who were receiving appropriate risk factor management and able to give informed consent. Exclusion criteria were patients with cellulitis or wet gangrene at the time of entry into the study and patients who had undergone lower limb revascularization in the preceding 6 months.

Patient comorbidities including ischemic heart disease (defined as a history of myocardial infarction or a positive exercise test, a current complaint of chest pain considered to be secondary to myocardial ischemia, the use of nitrate therapy, or an electrocardiogram with pathologic Q waves), revised cardiac risk index, diabetes mellitus, hypertension, and renal function were recorded. All patients were receiving statin therapy and aspirin (75 mg) at the time of recruitment.

The ABI was measured at the time of patient recruitment by a fully trained vascular technician using the technique recommended by the Scottish Intercollegiate Guidelines Network.[18]

Venous blood samples were taken by a 21-gauge needle in the morning in fully rested patients at the time of recruitment. The blood was centrifuged, and plasma samples were frozen and stored at −80°C for subsequent analysis.

Assays. SDMA, ADMA, and L-arginine levels were measured by high-pressure liquid chromatography tandem mass spectrometry. A Thermo/Finnigan Surveyor HPLC (Thermo Scientific, San Jose, Calif) attached to TSQ Quantum Triple Quadrupole Mass Spectrometer (Thermo Scientific) system was used in this analysis. The intra-assay coefficient of variation (% CV) for L-arginine, ADMA, and SDMA was 2.8%, 3.9%, and 4.5%, and the interassay %CV was 5.7%, 2.2%, and 3.9%, respectively.

All cause mortality. Follow-up of the patients was completed through the Scottish National Health Service intranet system using the Community Health Index, which has been shown to be highly accurate.[19] The vital status of each patient was checked, and the date of death was recorded where appropriate. The follow-up period was calculated from the date of enrollment in the study until the date of the event.

Statistical analysis. Data are presented as medians with IQR, and significance was developed at the 5% probability level. Differences between two independent categorical values were tested with the χ^2 test, and differences between two continuous independent values were tested with the Mann-Whitney U test. Kaplan-Meier technique and Cox regression analysis were used for survival analysis. Multivariate logistical regression analysis was performed to determine the independent predictive value of SDMA. Analysis was done with IBM SPSS Statistics 21 software (IBM Corp, Armonk, NY).

RESULTS

The study included 238 patients with symptomatic PAD, of whom 132 had intermittent claudication (IC; Fontaine grade II), and 106 had severe limb ischemia (rest pain, tissue loss, or gangrene, or both; Fontaine III-IV). Patient demographics are reported in Table I. The median follow-up period for patients who survived was 7 years 6 months (IQR, 6 years 11 months-7 years 11 months), and the median time to death was 3 years 6 months (IQR, 1 year 6 months-5 years 9 months).