Asymmetric Dimethylarginine Is a Marker of Poor Prognosis and Coronary Calcium in Systemic Lupus Erythematosus

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ABSTRACT. ***Objective.*** To determine the association of serum asymmetric dimethylarginine (ADMA) with clinical features, laboratory tests, treatment, cardiovascular risk factors, and subclinical atherosclerosis in patients with systemic lupus erythematosus (SLE).

Methods. Serum ADMA concentrations were determined by ELISA, using purified ADMA as a standard. Coronary calcium was measured by helical computerized tomography.

Results. Two hundred patients with SLE participated. Patients had a mean age of 44.3 ± 11.4 years and were 92% female, 61% Caucasian, 34% African American, 2% Asian, and 2% Hispanic; 18% had elevated ADMA levels. The mean ADMA was 0.31. Significantly higher ADMA levels were found in African Americans ($p < 0.001$), and were correlated with anti-dsDNA ($p < 0.001$), anti-Sm ($p = 0.005$), anti-ribonucleoprotein ($p = 0.002$), low C4 ($p = 0.004$), and high erythrocyte sedimentation rate ($p < 0.001$). ADMA was negatively associated with total cholesterol ($p = 0.004$). Elevated ADMA was associated with the presence of coronary calcium ($p = 0.02$).

Conclusion. Elevated ADMA is strongly associated with African American ethnicity, anti-dsDNA, low complement, and prednisone use, all markers of poor prognosis in SLE. It is negatively associated with hyperlipidemia, but positively associated with coronary calcium. Thus, it identifies a subset of SLE patients with normal lipid levels who are at risk for atherosclerosis. (J Rheumatol 2007;34:1502–5)

Key Indexing Terms:
ASYMMETRIC DIMETHYLARGININE
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Nitric oxide (NO), released from endothelial cells, is a strong vasodilator that regulates vascular tone. Decreased bioavailability of NO results in endothelial dysfunction, one of the earliest steps in the process of atherosclerosis[1]. NO regulates tissue blood flow, and inhibits platelet aggregation and leukocyte adhesion on the endothelial surface. It is synthesized by stereospecific oxidation of the terminal guanidinonitrogen of the amino acid l-arginine by a family of NO synthases (NOS)[2]. Asymmetric dimethylarginine (ADMA) is an endogenous NOS inhibitor. After posttranslational methylation from proteins involved in RNA processing and transcriptional control, ADMA is released by endothelial cells[3,4].

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High ADMA concentrations are seen in chronic heart failure, diabetes mellitus, and hypertension[5-7]. In a study of healthy Japanese, ADMA was associated with age, hypertension, and carotid artery intima-media thickness. ADMA and C-reactive protein (CRP) levels emerged as the sole independent predictors of progression of carotid intimal lesions during a followup period of 1 year[8]. In a prospective, nested case–control study of middle-aged, nonsmoking men in Finland, plasma levels of ADMA in the highest quartile were associated with a 3.9-fold increase in risk for acute coronary events[9]. In the CARDIAC study, an ADMA concentration > 1.75 μM/l led to a 6- to 7-fold increase in cardiovascular disease[10].

In a study of 107 patients with systemic lupus erythematosus (SLE), mean plasma ADMA levels were higher in those with cardiovascular events, including coronary artery disease, ischemic cerebrovascular events, and peripheral artery disease. Multivariate analysis showed that high SLE Disease Activity Index activity scores, high Systemic Lupus International Collaborating Clinics/American College of Rheumatology damage index, high anti-dsDNA titers, and low serum high density lipoprotein (HDL) were also significantly associated with high plasma ADMA levels[11]. ADMA has not been previously studied in subclinical atherosclerosis in SLE.

MATERIALS AND METHODS

Patients. Two hundred patients with SLE gave informed consent to participate in the Lupus Atherosclerosis Prevention Study. The study was approved by the Johns Hopkins University School of Medicine Institutional Review Board. Patients with a history of an atherosclerotic event (angina or myocardial infarction) were excluded. Any patient with a low density lipoprotein (LDL) level > 190 mg/dl or with a triglyceride level > 500 mg/dl was also excluded. However, 33.5% had a cholesterol above 200 mg/dl, 8% had a cholesterol above 240, 10% had elevated triglycerides, and 7% had low HDL.

All patients were part of the Hopkins Lupus Cohort. Demographic, clinical, laboratory, and treatment data were obtained from the prospective cohort database.

ADMA. Serum ADMA concentrations were measured using a commercial sandwich ELISA kit (Cardiovasics, Palo Alto, CA, USA), according to the manufacturer's instructions. All samples were measured in duplicate. ADMA concentrations (in μM) were determined using a standard curve prepared in parallel on each plate, using purified ADMA. ADMA ≥ 0.5 μM was considered high.

Image acquisition and evaluation. Coronary calcification was assessed on helical computerized tomography with a Siemens Volume Zoom Scanner (Siemens Medical Solutions, Malvern, PA, USA), using a 2.5 mm collimation and a slice width of 3 mm. Data were reloaded into a Siemens Leonardo workstation, using the Siemens calcium scoring software. The software calculated a calcium score, as described by Budoff, *et al*[12].

Carotid duplex was performed using high resolution linear transducers (5–10 MHz; Acuson 128XP, Hewlett Packard Image Point or ATL HDI-3000) in the General Clinical Research Center at Bayview Medical Center. Images were acquired of the distal common carotid arteries, carotid bulb, and proximal internal carotid arteries in the sagittal plane. Carotid intima-media thickness was measured at the near and far walls of the distal common carotid artery and Doppler spectrum tracings were taken in the first 2 cm of the proximal internal carotid artery. Flow velocities of the common carotid artery and internal carotid artery were also obtained. If atherosclerotic plaque was identified, transverse images and measurements of plaque were recorded.

Statistical analysis. All results for continuous variables are expressed as means ± standard deviation, unless specified otherwise. Continuous variables were analyzed with a 2-sided t-test. Categorical variables were compared by the Pearson chi-squared or Fisher's exact test. One-way analysis of variance was performed for normally distributed variables. Statistical analysis was performed using JMP (v5.0.1, SAS Institute, Cary, NC, USA). A p value of 0.05 was taken as statistically significant.

RESULTS

Data were obtained on 200 subjects with SLE (92% female). The patients were 61% Caucasian, 34% African American, 2% Asian, 2% Hispanic, and 1% other ethnicity. The mean age was 44.3 ± 11.4 years. Cumulative clinical manifestations included malar rash 63%, discoid rash 23%, photosensitivity 60%, oral ulcers 54%, arthritis 80%, serositis 50%, renal disorder 40%, neurological disorder 9%, immunologic disorder 75%, and antinuclear antibody positivity 97%. Eighteen percent of the patients had a positive ADMA. The mean ADMA was 0.31 ± 0.20.

ADMA levels were strongly associated with different serologic measures of disease activity in lupus, including anti-dsDNA (< 0.0001), low C3 (p = 0.0233), and low C4 (p = 0.0043) (Table 1).

ADMA levels were not associated with traditional cardiovascular risk factors (Table 2). However, ADMA was associated with fibrinogen (401 ± 95 vs 349 ± 93 mg/dl; p = 0.003). It was not associated with homocysteine [10.1 ± 3.4 vs 9.8 ± 3.1 μmol/l; p = not significant (NS)] nor with high sensitivity-CRP (5.8 ± 5.0 vs 4.7 ± 6.19 ng/l; p = NS). ADMA was negatively associated with hyperlipidemia (Table 3).

Abnormal ADMA levels were associated with coronary calcium (0.28 ± 0.18 vs 0.34 ± 0.22; p = 0.02; Table 4). SLE patients with abnormal ADMA levels had a higher percentage of mild carotid plaque than those with normal ADMA (54% vs 39%), but this failed to reach statistical significance.

DISCUSSION

Endothelium-derived NO is a strong vasodilator and has antiatherogenic properties[8,13]. In animal models, a decrease in NO bioavailability leads to the development of atherosclerosis, and supplementation of L-arginine may reverse the process[13]. ADMA is an endogenous competitive inhibitor of NO synthase. High concentrations of ADMA are associated with risk factors for atherosclerosis, including hypercholesterolemia, aging, and hypertension[14,15]. ADMA levels generally predict future cardiovascular risk and death[16], although not in patients with chronic kidney disease[17].

Table 1. ADMA levels by presence or absence of variable. Data are mean ± SD.

Characteristic	Factor Absent	Factor Present	p
Demographics			
Ethnicity (African American)	0.23 ± 0.17	0.42 ± 0.20	< 0.0001
Serologies			
Anti-dsDNA	0.23 ± 0.20	0.35 ± 0.19	< 0.0001
Anti-Sm	0.29 ± 0.20	0.41 ± 0.20	0.0052
Anti-RNP	0.28 ± 0.20	0.39 ± 0.18	0.0019
Low C3 (mg/dl)	0.27 ± 0.19	0.34 ± 0.21	0.0233
Low C4 (mg/dl)	0.27 ± 0.18	0.35 ± 0.22	0.0043
Inflammatory markers			
ESR (≥ 25 mm/h)	0.21 ± 0.16	0.34 ± 0.21	< 0.0001
hs-CRP (mg/l)	4.7 ± 6.19	5.8 ± 5.0	0.37
Treatment			
Prednisone use	0.25 ± 0.19	0.32 ± 0.20	0.0470
Hydroxychloroquine use	0.28 ± 0.23	0.30 ± 0.19	0.64

Table 2. ADMA levels by presence or absence of non-lipid traditional cardiovascular risk factors. Data are mean ± SD.

Risk Factor	Factor Absent	Factor Present	p
Hypertension	0.29 ± 0.20	0.31 ± 0.19	0.64
Obesity	0.31 ± 0.19	0.29 ± 0.21	0.56
Smoking	0.30 ± 0.20	0.30 ± 0.19	0.97
Diabetes mellitus	0.30 ± 0.20	0.32 ± 0.20	0.66

Table 3. ADMA and lipid levels. Data are mean ± SD.

Lipid	Normal ADMA	Abnormal ADMA, ≥ 0.5 μM	p
Total cholesterol (mg/dl)	190 ± 37	169 ± 37	0.0035
LDL cholesterol (mg/dl)	106 ± 30	93 ± 30	0.0190
HDL cholesterol (mg/dl)	61 ± 18	55 ± 15	0.0705

LDL, low density lipoprotein; HDL, high density lipoprotein.

A previous smaller study also found that ADMA was associated with anti-dsDNA in SLE[11]. For the first time, we have found that ADMA is also associated with anti-Sm, anti-RNP, low complement, and high sedimentation rate. In our study, ADMA was associated with multiple markers of poor prognosis in SLE, including African American ethnicity, anti-dsDNA, low complement, and prednisone use.

In the general population, ADMA is associated with hyperlipidemia[18]. However, in patients with SLE, we have shown a negative association of ADMA with lipids. Moreover, in our study, there was no association with other traditional cardiovascular risk factors, such as obesity, hypertension, and smoking.

ADMA has been independently associated with carotid intima-media thickness[19], early coronary atherosclerosis in men[20], stroke, and transient ischemic attack[21,22] in the general population. We now report that ADMA is associated with coronary calcium in patients with SLE. These results are consistent with studies in the general population that have shown higher ADMA levels in those with coronary artery disease[9,10]. We measured ADMA once; we recognize that coronary calcium accumulated over a long period of time. Fluctuation of ADMA levels could not be ascertained in this study. In SLE, ADMA is more of a risk factor for coronary atherosclerosis than for carotid atherosclerosis. In our study, SLE patients with abnormal ADMA were also more likely to have "mild" carotid plaque, but this failed to reach statistical significance. A differential effect of risk factors on coronary versus carotid atherosclerosis is not a new concept. For example, in the Atherosclerosis Risk In Communities Study (ARIC), CRP was associated with coronary, but not carotid, atherosclerosis[23].

Recent data have suggested that oxidized LDL cholesterol or native LDL may increase ADMA accumulation[24,25]. Oxidized LDL is known to be increased in SLE and may be one explanation for higher ADMA levels[26]. ADMA levels were significantly reduced in one 6-week trial of rosuvastatin in the general population[18], but not in 2 other statin trials[27,28]. Thus, it is not certain whether statins will be an effective intervention for ADMA.

ADMA is a novel marker of "poor prognosis" in SLE, in terms of both clinical SLE and coronary atherosclerosis. ADMA thus might become the second "lupus factor" along with proinflammatory HDL[29] to identify patients with SLE having normal lipids at risk for atherosclerosis.

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Table 4. ADMA and subclinical atherosclerosis.

Indicator	Normal ADMA	Abnormal ADMA, ≥ 0.5 μM	Odds Ratio	95% CI	p
Coronary calcium, %	38	60	2.39	1.13–5.0	0.02
Carotid plaque, %	86	77	0.54	0.22–1.34	0.18

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