Asymmetric Dimethylarginine Predicts Cardiovascular Events in Patients With Type 2 Diabetes

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OBJECTIVE — Circulating concentrations of an endogenous inhibitor of nitric oxide synthase, asymmetric dimethylarginine (ADMA), are elevated in patients with increased cardiovascular risk. We hypothesized that ADMA predicts cardiovascular events and enhances risk prediction independent of established risk markers in patients with type 2 diabetes.

RESEARCH DESIGN AND METHODS — This prospective cohort study included 125 patients with type 2 diabetes. ADMA, L-arginine, high-sensitivity C-reactive protein (CRP), and routine clinical parameters were determined at baseline. First occurrence of cardiovascular events (myocardial infarction, percutaneous coronary intervention, coronary artery bypass graft, stroke, carotid revascularization, or all-cause mortality) was defined as a composite end point.

RESULTS — During a median follow-up of 21 (interquartile range 11–27) months, 84 events occurred in 48 patients. According to multivariate Cox regression analysis, patients with baseline ADMA or CRP in the highest tertile had a significantly increased hazard ratio for incident cardiovascular events compared with those with ADMA or CRP in tertile 1 (2.37 [95% CI 1.05–5.35], $P = 0.038$, and 3.63 [1.59–8.28], $P = 0.002$). Assessing the joint effect of ADMA and CRP revealed that patients with either ADMA or CRP or both in the highest tertile had increased hazard ratios for cardiovascular events compared with patients with neither ADMA nor CRP in the highest tertile before and after adjustment for possible confounders (hazard ratio 4.59 [95% CI 2.07–10.15], $P < 0.001$).

CONCLUSIONS — ADMA predicted cardiovascular events and enhanced the predictive role of CRP in patients with type 2 diabetes. ADMA therefore could improve cardiovascular risk assessment in patients with type 2 diabetes.

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Type 2 diabetes is associated with a considerably increased risk for development of cardiovascular disease (1,2). Aggressive risk factor management is an important mean for reducing the cardiovascular morbidity in this patient group, which implies accurate risk stratification. Poorly controlled blood pressure and glycemia seem to be significantly involved in the development process of cardiovascular disease in patients with type 2 diabetes (3,4). Besides traditional risk markers, the acute-phase protein C-reactive protein (CRP) has turned out to be an important cardiovascular risk predictor in the general population and patients with type 2 diabetes (5,6). To further improve the identification of patients with type 2 diabetes, who are exposed to a particularly high cardiovascular risk, detection of additional independent risk markers might be useful.

Nitric oxide (NO), which is synthesized by NO synthases, is an important antiatherogenic molecule (7). As initially described by Vallance et al. (8) an endogenous inhibitor of NO synthase, asymmetric dimethylarginine (ADMA), occurs in significant amounts in peripheral blood. Since this discovery, numerous clinical studies have been performed that found elevated circulating ADMA concentrations in humans suffering from diseases associated with increased cardiovascular risk. Different prospective cohort studies in selected patient groups suggested ADMA as an independent predictor of cardiovascular morbidity (9–12). In a cross-sectional study, ADMA was associated with macrovascular disease in patients with type 2 diabetes (13). However, the predictive role of ADMA for the occurrence of cardiovascular events in type 2 diabetes has not been examined prospectively until now.

We hypothesized that ADMA predicts cardiovascular events in patients with type 2 diabetes. Further, we sought to investigate if measurement of circulating ADMA concentrations adds to the predictive value of the well-established risk marker CRP in patients with type 2 diabetes. It recently was reported that long-term supplementation of the precursor of NO, L-arginine, improves endothelial function, oxidative stress, glucose metabolism, and insulin sensitivity in patients with type 2 diabetes (14,15). We determined L-arginine plasma concentrations to explore if L-arginine is related to cardiovascular risk in type 2 diabetes.

RESEARCH DESIGN AND METHODS

— Clinical investigations were approved by the institutional review board and were conducted in accordance with the guidelines of the Declaration of Helsinki. Informed consent was obtained from the study participants. This study was designed as prospective cohort study. Patients with type 2 diabetes duration of at least 2 years attending a speciality outpatient department for diabetes between September 2003 and March 2004 were included in this cohort study. All subjects were on stable antidiabetes therapy for at least 6 months. No lifestyle restrictions were imposed, but all patients were stimulated to adhere to an appropriate nutrition program, weight control, and to

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Abbreviations: ADMA, asymmetric dimethylarginine; CRP, C-reactive protein.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

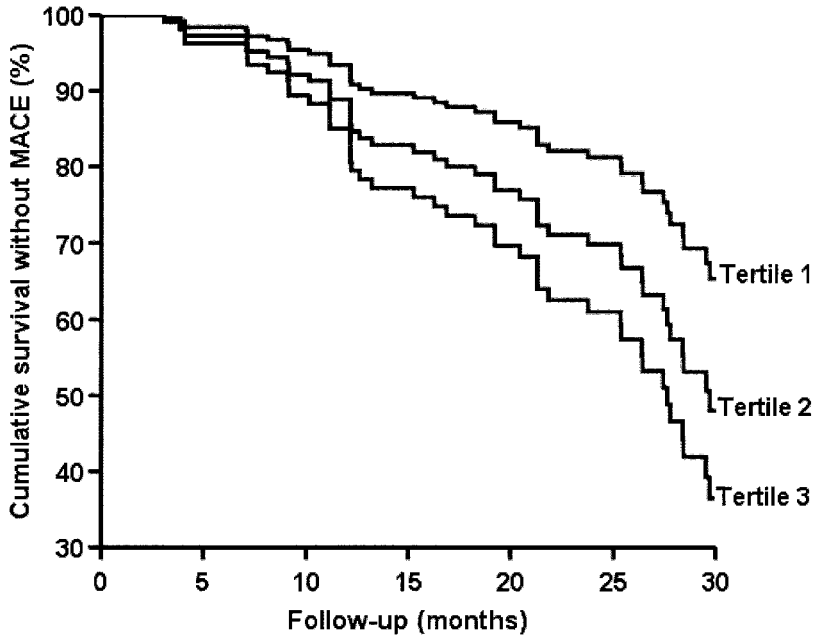

Figure 1—*Cumulative survival without cardiovascular events in patients with type 2 diabetes according to tertiles of ADMA adjusted for age, sex, history of macrovascular disease, and glomerular filtration rate (tertile 1: ≤0.53 μmol/l, tertile 2: >0.53 and ≤0.63 μmol/l, and tertile 3: >0.63 μmol/l).*

perform regular exercise. At the time of inclusion, demographic data, clinical characteristics, and current medication of the patients were recorded with special attention to cardiovascular risk factors and comorbidities. Age, sex, smoking habits, hyperlipidemia, arterial hypertension, coronary artery disease, history of myocardial infarction, and stroke were assessed. Arterial hypertension was diagnosed in patients with resting blood pressure values >140/90 mmHg and was assumed to be present in patients with a history of hypertension taking antihypertensive drugs. Hyperlipidemia was defined as present lipid-lowering therapy or elevation of blood lipids (LDL cholesterol >130 mg/dl, triglycerides >200 mg/dl). Subjects with end-stage renal disease were not included.

Laboratory investigations

Venous blood was drawn after an overnight fast for determination of ADMA, L-arginine, CRP, and routine clinical parameters. Blood levels of creatinine; A1C; blood glucose; total, HDL, and LDL cholesterol; triglycerides; and high-sensitivity CRP were measured by standard laboratory methods. Glomerular filtration rate was calculated with the Modification of Diet in Renal Disease Study Group formula (16). For measurement of ADMA and L-arginine, plasma was subjected to cation exchange solid-phase extraction and analyzed by high-performance liquid chromatography (17,18). The coefficients of variation for inter- and intra-assay variations tested with a pooled plasma sample were <3% for all analytes. The detection limit for dimethylarginines was 0.04 μmol/l.

Study end point and follow-up

The occurrence of the first cardiovascular events (myocardial infarction, percutaneous coronary intervention, coronary artery bypass graft, stroke, carotid revascularization, and all-cause mortality) was defined as the composite end point. Patients were reexamined at 2- to 6-month intervals until June 2006. At these visits, routine adjustment of medication was performed to optimize glycemia, blood lipids, and blood pressure in all patients. The occurrence of cardiovascular events was confirmed by review of hospital records.

Statistics

Continuous data are presented as medians (interquartile range). Categorical data are given as counts (percentages). Mann-Whitney *U* test was applied for univariate comparison of continuous data and Spearman rank correlation for assessment of associations between continuous variables. Univariate and multivariate Cox proportional hazards analysis was used to assess the independent effect of ADMA

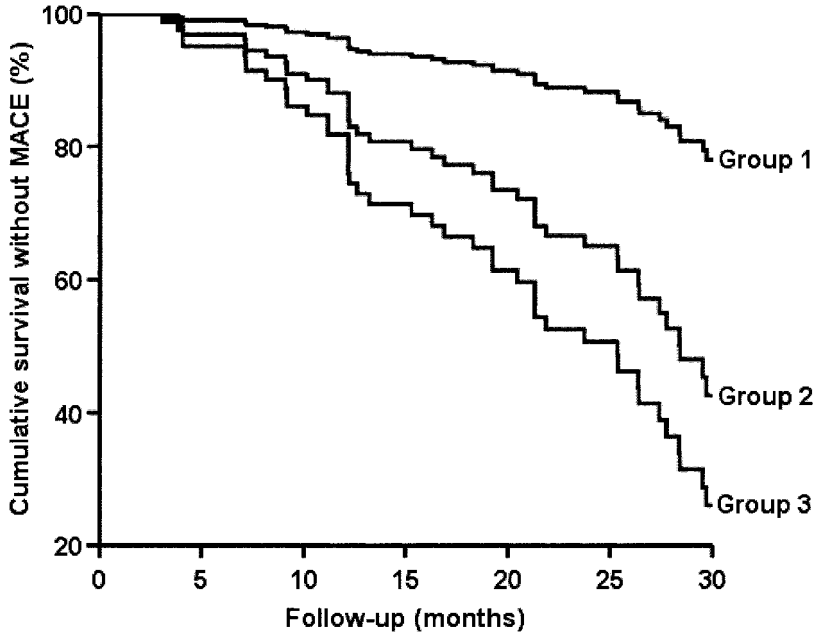

Figure 2—*Cumulative survival without cardiovascular events in patients with type 2 diabetes according to joint effects of ADMA and CRP adjusted for age, sex, history of macrovascular disease, glomerular filtration rate, and HDL cholesterol (group 1: neither ADMA nor CRP in the highest tertile, group 2: ADMA or CRP in the highest tertile and the other parameter in the lowest tertile, and group 3: ADMA and CRP or one of them in the highest tertile and none in the lowest).*

Table 1—*Baseline characteristics and Spearman correlation coefficients (P value) for ADMA and CRP and continuous variables of patients with type 2 diabetes (n = 125)*

		ADMA	CRP
Age (years)	64 (58–73)	0.142 (0.114)	0.041 (0.656)
BMI (kg/m2)	29.1 (26.5–33.7)	−0.123 (0.174)	0.167 (0.064)
Systolic blood pressure (mmHg)	150 (136–164)	−0.104 (0.251)	−0.075 (0.410)
Diastolic blood pressure (mmHg)	80 (74–90)	−0.214 (0.084)	0.039 (0.755)
Diabetes duration (years)	10 (4–16)	0.079 (0.384)	−0.111 (0.225)
Glucose (mg/dl)	150 (119–197)	0.027 (0.772)	−0.020 (0.828)
A1C (%)	7.6 (6.8–8.6)	0.006 (0.948)	−0.062 (0.496)
Glomerular filtration rate (ml/min per 1.73 m^2)	71.9 (54.5–94.3)	−0.320 (<0.001)	−0.016 (0.863)
Triglycerides (mg/dl)	177 (136–274)	−0.064 (0.482)	0.154 (0.219)
Cholesterol (mg/dl)	184 (159–220)	−0.043 (0.638)	−0.078 (0.393)
LDL cholesterol (mg/dl)	98 (77–125)	−0.030 (0.744)	−0.020 (0.835)
HDL cholesterol (mg/dl)	46 (39–53)	0.067 (0.460)	−0.263 (0.003)

Data are median (interquartile range) or *R* (*P* value).

and CRP on the composite end point and to adjust for potential confounders. To assess the joint effect of ADMA and CRP, the study cohort was stratified into three groups according to tertiles of ADMA and CRP (group 1: neither ADMA nor CRP in the highest tertile, group 2: ADMA or CRP in the highest tertile and the other parameter in the lowest tertile, and group 3: ADMA and CRP or one of them in the highest tertile and none in the lowest). Tertiles of continuous variables were included as confounders if these variables were associated with ADMA or CRP according to Spearman rank correlation coefficients. Categorical variables were entered into the model if they influenced ADMA or CRP according to univariate analysis. In addition, adjustment for age and sex was performed. For analysis of the joint predictive role of ADMA and CRP, confounders from both models were included. Results of the Cox proportional hazards model are presented as hazard ratios and 95% CI as well as survival curves. A two-sided $P < 0.05$ was considered as statistically significant. Considering previous studies in patients with type 2 diabetes, we estimated a cardiovascular event rate of 25% during the follow-up period. According to this estimate, a study in 100 patients has 80% power to detect a hazard ratio of 2.0 for the occurrence of cardiovascular events for tertiles of ADMA or CRP (two-sided hypothesis testing, $\alpha = 0.05$). Therefore, we aimed to include at least 100 patients. Calculations were performed with SPSS for Windows (Version 14.0; SPSS, Chicago, IL).

RESULTS — One hundred thirty-five subjects with type 2 diabetes were suitable for the study. Ten patients were excluded due to missing clinical follow-up data on cardiovascular events. The remaining 125 patients (93%) were included into the analysis.

Median ADMA, L-arginine, and CRP plasma concentration of all patients were 0.57 μmol/l (interquartile range 0.50–0.66), 63 μmol/l (49–76), and 4.0 mg/l (1.9–7.0), respectively. Other baseline parameters are presented in Table 1. ADMA and CRP both were elevated in patients with a history of macrovascular disease (Table 2). ADMA correlated negatively with glomerular filtration rate, and CRP was associated with HDL cholesterol (Table 1).

During a median follow-up of 21 months (interquartile range 11–27), 83 macrovascular events occurred in 48 (38%) patients, including 10 (12%) myocardial infarctions, 11 (13%) strokes, 38 (46%) percutaneous coronary interventions or coronary artery bypass grafts, 13 (16%) endovascular or surgical interventions in leg arteries, and 11 (13%) deaths. Patients with ADMA in the highest tertile had a significantly higher hazard ratio for macrovascular events compared with those in the lower tertiles, before and after adjustment for age, sex, history of macrovascular disease, and glomerular filtration rate (Fig. 1). Twenty-two (46%) end points were observed in patients with ADMA in the highest tertile. Patients with CRP in the highest tertile also had a significantly increased hazard ratio for macrovascular events before and after adjustment for age, sex, history of macrovascular disease, and HDL cholesterol (Table 3). Twenty (42%) end points occurred in patients with CRP in the highest tertile.

Patients with either ADMA or CRP or both in the highest tertile had a remarkably increased hazard ratio for cardiovascular events before and after adjustment for age, sex, history of macrovascular disease, glomerular filtration rate, and HDL cholesterol (Fig. 2). These were even higher than the hazard ratios found for ADMA and CRP alone (Table 4). Twenty-six end points (54%) were observed in this group.

L-arginine was not related to any categorical variable presented in Table 2 (data not shown) and correlated with ADMA ($R = 0.354$; $P < 0.001$), BMI ($R = -0.193$; $P = 0.042$), and glomerular filtration rate ($R = -0.237$; $P = 0.012$). L-arginine was not associated with the occurrence of macrovascular events with hazard ratios of 0.96 (95% CI 0.46–2.03) in tertile 2 and 0.94 (0.46–1.94) in tertile 3 compared with tertile 1 ($P = 0.921$ and $P = 0.872$, respectively).

Analysis of the associations of ADMA and CRP with a combined end point of death, myocardial infarction, and stroke revealed consistent results. Adjusted hazard ratios for increasing tertiles of ADMA and CRP for groups 2 and 3 compared with group 1 were 2.83 (95% CI 0.97–8.31) and 3.38 (1.14–9.98) as well as 1.54 (0.49–4.88) and 4.83 (1.64–14.23), respectively. Hazard ratios for the combination of ADMA and CRP for groups 2 and 3 compared with group 1 were 4.84 (1.36–17.22) and 8.43 (2.95–24.11).

CONCLUSIONS — Both, ADMA, and CRP predict the occurrence of cardio-

Table 2—*Median plasma concentrations (interquartile range) of ADMA and CRP grouped according to categorical baseline variables*

	ADMA	*P* value	CRP	*P* value
Sex				
Male ($n = 74$)	0.57 (0.49–0.64)		4.0 (2.0–6.8)	
Female ($n = 51$)	0.58 (0.51–0.71)	0.280	4.0 (1.7–7.0)	0.744
Smoking status				
Yes ($n = 26$)	0.56 (0.52–0.63)		5.0 (2.8–10.0)	
No ($n = 89$)	0.57 (0.49–0.68)	0.769	3.8 (1.5–6.9)	0.151
Hypertension				
Yes ($n = 118$)	0.57 (0.50–0.67)		4.0 (2.0–7.0)	
No ($n = 7$)	0.55 (0.47–0.64)	0.459	1.8 (0.6–5.3)	0.126
ACE/AT2*				
Yes ($n = 109$)	0.57 (0.50–0.67)		4.0 (2.0–7.0)	
No ($n = 16$)	0.59 (0.47–0.64)	0.564	2.9 (1.4–5.3)	0.268
Macrovascular disease				
Yes ($n = 51$)	0.62 (0.54 to −0.73)		5.0 (2.0–11.6)	
No ($n = 74$)	0.55 (0.48–0.63)	0.004	3.5 (1.7–6.0)	0.012
Hyperlipidaemia				
Yes ($n = 71$)	0.55 (0.47–0.65)		4.0 (2.0–6.1)	
No ($n = 54$)	0.57 (0.53–0.67)	0.215	3.7 (1.0–8.4)	0.744
Statin therapy				
Yes ($n = 67$)	0.55 (0.47–0.65)		4.0 (2.0–7.0)	
No ($n = 58$)	0.58 (0.53–0.67)	0.110	3.8 (1.2–7.0)	0.633
Insulin therapy				
Yes ($n = 70$)	0.58 (0.50–0.68)		4.1 (2.0–8.3)	
No ($n = 55$)	0.56 (0.49–0.63)	0.384	3.5 (1.7–6.0)	0.208

Mann Whitney *U* test was applied for comparisons between groups. *ACE/AT2, treatment with ACE inhibitors or angiotensin 2 receptor blockers.

vascular events in patients with type 2 diabetes. Combining both parameters showed that ADMA augments the predictive value of CRP as a cardiovascular risk marker. Patients with ADMA or CRP in the highest tertile, and none of these parameters in the lowest tertile, had a 4.5-times increased risk for the occurrence of cardiovascular events compared with those with neither ADMA nor CRP in the highest tertile. This imposes an important role of ADMA for cardiovascular risk stratification, adding to determination of CRP and traditional cardiovascular risk markers. A significant additional predictive value of ADMA to CRP for all-cause and cardiovascular mortality in patients with end-stage renal disease already has been shown in a previous study (19). Our study extends these findings to patients with type 2 diabetes. Risk stratification in patients with type 2 diabetes is of particular relevance. Patients with type 2 diabetes are prone to a dramatically high risk for cardiovascular events. This is reflected by the fact that subjects with diabetes without prior evidence of coronary heart disease have a similar risk to die from coronary heart as subjects with prior coronary heart disease without diabetes (20).

The predictive role of ADMA was independent of traditional cardiovascular risk markers and preexisting cardiovascular disease. Elevated ADMA has been found in patients with renal failure, indicating that ADMA is excreted by the kidneys (8). In subjects with normal renal function, circulating concentrations of ADMA mainly are determined by its degradation by dimethylamino dimethylarginine hydrolases (21). ADMA correlated with glomerular filtration rate, suggesting that renal excretion of this methylarginine plays a role for its plasma concentrations in our cohort of patients with type 2 diabetes.

It was previously reported that LDL cholesterol suppressed dimethylamino dimethylarginine activity and thereby increased ADMA in an vitro experiment

Table 3—*Cox regression analysis assessing the univariate and multivariate hazard ratios for tertiles of ADMA*

	ADMA		CRP	
	Hazard ratio (95% CI)	*P* value	Hazard ratio (95% CI)	*P* value
Univariate model				
Tertile 1	1.00		1.00	
Tertile 2	1.62 (0.74–3.56)	0.226	1.76 (0.80–3.88)	0.164
Tertile 3	2.28 (1.08–4.85)	0.032	3.12 (1.46–6.68)	0.003
Multivariate model				
Tertile 1	1.00		1.00	
Tertile 2	1.73 (0.76–3.93)	0.194	1.79 (0.79–4.06)	0.164
Tertile 3	2.37 (1.05–5.35)	0.038	3.63 (1.59–8.28)	0.002

ADMA: tertile 1: ≤0.53 μmol/l, tertile 2: >0.53 and ≤0.63 μmol/l, and tertile 3: >0.63 μmol/l; CRP: tertile 1: ≤2.0 mg/l, tertile 2: >2.0 and ≤6.0 mg/l, and tertile 3: >6.0 mg/l. Multivariate model for ADMA: adjustment for age, sex, history of macrovascular disease, and glomerular filtration rate; multivariate model for CRP: adjustment for age, sex, history of macrovascular disease, and HDL cholesterol.

Table 4—*Cox regression analysis assessing the univariate and multivariate hazard ratios for the joint effect of ADMA and CRP*

	Hazard ratio (95% CI)	*P* value
Univariate model		
Group 1	1.00	
Group 2	2.37 (1.00–5.60)	0.050
Group 3	3.48 (1.78–6.83)	<0.001
Multivariate model		
Group 1	1.00	
Group 2	3.39 (1.32–8.68)	0.011
Group 3	4.59 (2.07–10.15)	<0.001

Group 1: neither ADMA nor CRP in the highest tertile. Group 2: ADMA or CRP in the highest tertile and the other parameter in the lowest tertile. Group 3: ADMA and CRP or one of them in the highest tertile and none in the lowest. Multivariate model: adjustment for age, sex, history of macrovascular disease, glomerular filtration rate, and HDL cholesterol.

(22). Further, it was shown that ADMA is elevated in patients with hyperlipidemia (6,23) and essential hypertension (24). But ADMA was not associated with hyperlipidemia or elevated blood pressure in our study, which also was found in other clinical trials (11,12). The influence of concomitant medication in our patients could have obscured a possible relationship between ADMA and these risk factors. However, ADMA concentrations were not different in patients on statin therapy in the present cohort. Most studies (25–27) investigating ADMA and statin therapy provided similar results with the exception of a study (28) describing an ADMA-lowering effect of rosuvastatin. Therefore, an influence of statin therapy on the relationship between ADMA and hyperlipidemia seems unlikely.

CRP was independently associated with future cardiovascular events in the present study, which was previously found in patients with type 2 diabetes (6,29,30). This confirms the importance of inflammation for incident cardiovascular disease. However, CRP and other traditional cardiovascular risk markers may not sufficiently predict the cardiovascular risk of patients with type 2 diabetes. As shown in the present study, the addition of ADMA to CRP enhances cardiovascular risk prediction in patients with type 2 diabetes. More accurate risk assessment by additional ADMA determination could lead to more stringent management of cardiovascular risk factors and might therefore be of major clinical relevance. Future prospective studies will be needed to examine if risk marker–guided therapy will lead to improvement of cardiovascular morbidity and mortality in patients with type 2 diabetes. As a limitation of the study, only patients attending a specialized outpatient clinic who might have a more pronounced cardiovascular risk profile than the general diabetic population were included in this analysis. This could explain the rather high incidence of cardiovascular events found in our study. Larger prospective studies with longer follow-up will be necessary to strengthen our findings.

Piatti et al. (15) reported that L-arginine administration has beneficial effects on endothelial function and the cardiovascular risk profile in patients with type 2 diabetes. L-arginine was not associated with future cardiovascular events in the present cohort. Therefore, our results do not support a protective role of endogenous circulating L-arginine.

In conclusion, this study identified ADMA as new cardiovascular risk marker in patients with type 2 diabetes and high cardiovascular risk. ADMA significantly enhanced the predictive role of CRP for incident cardiovascular disease and was independent of traditional risk predictors. In patients with type 2 diabetes the consideration of ADMA for risk stratification in addition to other markers may be important. Large prospective studies are necessary to evaluate if ADMA also predicts cardiovascular events in patients with type 2 diabetes and minor cardiovascular risk.

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