# Plasma asymmetric dimethylarginine predicts restenosis after coronary angioplasty

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## Abstract

**Introduction:** Asymmetric dimethylarginine (ADMA) is an endogenous competitive inhibitor of endothelial nitric oxide synthase. Asymmetric dimethylarginine may influence the process of restenosis after coronary angioplasty. The aim of the study was to determine if initial plasma ADMA level could predict restenosis after coronary angioplasty and stenting.

**Material and methods:** The study group consisted of 60 consecutive patients (10 women and 50 men, average age 58.9 ±10.4 years old), who underwent percutaneous coronary angioplasty with bare metal stenting for stable coronary artery disease. All patients underwent follow-up coronary angiography after a 6-month period. Patients were divided into two groups, one with restenosis ($n$ = 22), and the other one without restenosis ($n$ = 38). In addition to measuring acknowledged restenosis risk factors, plasma ADMA level was measured before initial angiography.

**Results:** Asymmetric dimethylarginine plasma level was significantly higher in the group with restenosis than in the group without restenosis (1.94 ±0.94 µmol/l vs. 0.96 ±0.67 µmol/l; $p < 0.05$). L-arginine/ADMA ratio was also decreased in the group with restenosis, when compared with the group without restenosis ($p < 0.05$). Multivariate logistic regression revealed that independent restenosis risk factors were characterised by an initially high ADMA level ($p < 0.01$), advanced age ($p < 0.05$) and low level of HDL cholesterol ($p < 0.05$).

**Conclusions:** Pre-procedural elevated plasma ADMA level increases the risk of restenosis in patients who underwent coronary angioplasty and stenting with bare metal stents.

**Key words:** asymmetric dimethylarginine, percutaneous coronary intervention, bare metal stent.

## Introduction

Percutaneous coronary intervention (PCI) is widely used in treatment of coronary artery disease (CAD). Unfortunately, the long-term efficacy of angioplasty is limited by the recurrent narrowing within the dilated part of the artery, which is known as restenosis. Based on experimental and clinical studies, understanding of the phenomenon of restenosis is still incomplete and needs further elucidation. Data obtained from several studies were controversial and did not help to determine all the factors

influencing the process. Injury of the endothelium within epicardial arteries occurring during angioplasty is believed to activate the process of restenosis [1]. Due to this fact it seems reasonable to search for other factors contributing to endothelial dysfunction, which may participate in development and progression of restenosis.

Asymmetric dimethylarginine (ADMA) is an endogenous competitive inhibitor of endothelial nitric oxide synthase (eNOS) [2]. Asymmetric dimethylarginine is regarded as a new cardiovascular risk factor and it may potentially influence the process of restenosis occurring after coronary angioplasty. A strong relationship was shown between ADMA and CAD, and plasma ADMA level was higher in patients with a documented CAD compared to a healthy control group [3]. Simultaneously, Valkonen *et al*. observed a significant increase in the risk of major cardiovascular events in middle-aged non-smoking men with higher plasma ADMA level [4].

The aim of the study was to determine if initial plasma ADMA level could predict restenosis after coronary angioplasty and stenting.

## Material and methods

The study group consisted of 60 consecutive patients (10 women and 50 men, average age 58.9 ±10.4 years) with stable coronary artery disease who underwent PCI. All subjects had chronic CCS (Canadian Cardiovascular Society) class II or III angina and were treated with simvastatin, in the dose of 20 mg or 40 mg, acetylsalicylic acid 75-150 mg, angiotensin-converting enzyme inhibitor and β-blocker, if possible. Short acting nitroglycerine was administered only in case of stenocardial pain. Clinical indications for coronary angiography were based on the stress treadmill test and symptoms. Subjects reported that angina was not controlled by the optimal medical therapy. Patients with a significantly positive stress treadmill with ST depressions more than 4 mm and with a low exercise tolerance (less than about 4 MET) were qualified for coronary angiography. Additionally, substantial objective evidence of extensive ischaemia in echocardiography was taken into account.

Coronary angiography was performed through the femoral artery with the help of Judkins' technique, using a 6 F haemostatic sheath with Advantax equipment (General Electric, USA). Non-ionic contrast agents were used only to visualize the coronary circulation. Epicardial coronary arteries were visualized with selective coronary angiography in standard projections. In selected cases standard projections were supported by individually matched projections. Coronary angioplasty was performed when the stenosis of the coronary artery exceeded 70%. Bare metal stents (BMS) were used in all cases. Patients with drug-eluting stents (DES), and patients who underwent solely balloon angioplasty were not included in the study. Only patients after the implantation of one stent were examined. Stent implantation was performed after balloon predilatation with inflation pressure of 12 atm to 18 atm or with direct stenting. Balloon inflation lasted between 30 and 40 s in case of balloon predilatation, and about 20-30 s in case of direct stent implantation. Maximally three inflations were accepted. Time and number of inflations, as well as balloon diameter, were individually adjusted. All patients who underwent a successful PCI with residual narrowing not exceeding 20% followed by BMS in a single coronary artery were qualified for the study.

Exclusion criteria for this study included atherosclerotic lesions which were over 30 mm long, lesions of the left main coronary artery, ostial lesions or lesions located in bifurcations, and lesions located within native coronary arteries except those of diameter between 2.5 mm and 3.5 mm. Furthermore, patients with any acute or chronic inflammatory diseases, as well as patients with diabetes, malignancies, heart failure, kidney or liver insufficiency, with massive haemorrhages, acute myocardial infarctions and with indications for acute revascularisation or with fatal complications, were excluded from the study.

In order to assess the final result of the procedure, angiographic evaluation was performed immediately after coronary angioplasty. The long-term result was assessed 6 months after the procedure, by means of control coronary angiography. Restenosis was defined as narrowing within the vessel lumen at the location of stent implantation or within 1 cm from the stent margin, exceeding 50%. Objective evaluation of the vessel lumen was performed with digital quantitative coronary angiography (QCA) analysis. Patients with recurrent vessel narrowing ≥ 50% were included in the group with restenosis (group I; $n = 22$). Patients without stenosis or with narrowing < 50% were included in the group without restenosis (group II; $n = 38$). The recruitment of patients to the specific group according to the definition of restenosis was made blindly to ADMA levels.

Venous blood was collected directly before the procedure. The ethylenediaminetetra-acetate (EDTA) blood was centrifuged (at speed of 10 000/per minute); plasma was collected and frozen at the temperature of –70°C, in order to perform ADMA measurement by high performance liquid chromatography (HPLC). Total cholesterol, LDL cholesterol, HDL cholesterol and triglyceride serum levels were determined by using commercial tests (Boehringer Mannheim, Germany).

Informed consent was obtained from each patient within both study groups. The Bioethical Committee of Wroclaw Medical University approved the study.

Statistical analysis was performed with the Statistica PL 6.0 package (StatSoft, Poland). Average ($x$) and standard deviations (SD) concerning quantitative variables and percentage values for qualitative variables were measured in the studied groups. The distribution was verified with the Shapiro-Wilk $W$-test. In the case of quantitative variables with abnormal distribution, a non-parametric Mann-Whitney $U$-test was used. To determine the independent risk factors of restenosis, multivariate logistic regression analysis was performed. The analysis involved total, LDL and HDL cholesterol, triglycerides, ADMA, and the age of patients. Values of $p < 0.05$ were accepted as statistically significant.

## Results

The study subgroups with and without restenosis did not differ as far as demographics, medications, laboratory parameters and clinical factors are concerned. Demographics and laboratory parameters of both groups are presented in Table I. No significant differences in basic angiographic parameters reported before and after coronary angioplasty were observed between group I and group II (Table II).

In the group with restenosis ADMA plasma concentration was significantly higher than in the group without restenosis. L-arginine/ADMA ratio was also lower in the group with restenosis, compared to the group without restenosis (Table III).

Multivariate logistic regression revealed that independent risk factors of restenosis were an initial high ADMA level [OR = 5.96 (2.45, 11.26); $p < 0.01$], advanced age [OR = 1.02 (1.00, 1.17); $p < 0.05$] and low level of HDL cholesterol [OR = 0.96 (0.76, 0.99); $p < 0.05$], [OR – odds ratio for unit change (confidence interval –95%, 95%)]. Plasma glucose levels were normal and this parameter was not included in the regression model.

## Discussion

The role of biochemical factors in the pathogenesis of coronary artery restenosis after PCI continues to be the subject of multiple investigations. The analysis is quite difficult due to the complex character of the phenomenon. Findings resulting from the experimental environment frequently have no confirmation in clinical studies. Earlier studies of this phenomenon were based on angiographic evaluation of atherosclerotic lesions. It was proved that long lesions with calcifications, located within arteries of small diameter and of type C stenosis are more prone to restenosis. Other risk factors for restenosis included diabetes mellitus, unstable coronary heart disease, signi-

**Table I.** Study group demographics

| Parameters | Whole group ($n$ = 60) | Group I ($n$ = 22) | Group II ($n$ = 38) | Value of $p$ |
|---|---|---|---|---|
| Age [years] | 58.9 ±10.4 | 58.6 ±9.6 | 592 ±10.9 | NS |
| Men [%] | 83.7 | 81.3 | 84.8 | NS |
| Total cholesterol [mg/dl] | 198.9 ±45.5 | 200.1 ±56.4 | 198.3 ±39.9 | NS |
| LDL cholesterol [mg/dl] | 123.5 ±36.8 | 119.3 ±44.1 | 125.2 ±34.2 | NS |
| HDL cholesterol [mg/dl] | 45.1 ±9.6 | 45.2 ±9.9 | 45.1 ±9.6 | NS |
| Triglycerides [mg/dl] | 155.6 ±90.2 | 184.9 ±114.2 | 140.9 ±73.2 | NS |
| Previous myocardial infarction [%] | 51.7 | 52.5 | 50.5 | NS |
| Active smoking [%] | 50.0 | 53.1 | 48.3 | NS |
| Arterial hypertension [%] | 46.7 | 48.6 | 43.6 | NS |

**Table II.** Basic angiographic parameters before and after coronary angioplasty

| | Parameter | Group I ($n$ = 22) | Group II ($n$ = 38) | Value of $p$ |
|---|---|---|---|---|
| Before angioplasty | Reference diameter [mm] | 3.0 ±0.2 | 3.1 ±0.1 | NS |
| | Percent stenosis | 74.2 ±12.2 | 74.1 ±13.7 | NS |
| | Lesion length [mm] | 16.4 ±2.4 | 16.9 ±1.1 | NS |
| | Stent length [mm] | 18.2 ±3.9 | 19.1 ±5.3 | NS |
| | Maximal inflation pressure [atm] | 15.9 ±3.7 | 15.4 ±3.1 | NS |
| After angioplasty | Percent residual stenosis | 12.8 ±10.9 | 14.7 ±11.8 | NS |

ficant length of the implanted stent and suboptimal result of coronary angioplasty with large residual stenosis [5].

Patients with diabetes mellitus, acute or chronic inflammatory disease and with acute coronary syndrome were excluded from our study. Moreover, taking the characteristics of anatomy, anthropometrics and angioplasty procedure into account, as well as pharmacotherapy, special exclusion criteria were applied to make the study group homogeneous. Such restrictive inclusion criteria limited the influence of various anatomical and anthropometric factors, as well as the influence of factors connected with the procedure itself, on the recurrence of restenosis and on ADMA concentrations. Only bare metal stents were used in our study, as their influence on the function of the artery wall is weak in comparison to drug-eluting stents.

Our study is one of the first studies to report that the initial asymmetric dimethylarginine plasma level preceding the performance of coronary angioplasty with stenting increases risk of restenosis after 6 months. A recent study found similar results in a group of 105 patients. The levels of ADMA obtained before the procedure predicted the development of restenosis and major adverse cardiac events in patients who underwent elective PTCA and bare metal stent procedures [6].

In the group of patients with restenosis the initial plasma level of ADMA was significantly higher ($p < 0.01$) and the ratio of L-arginine/ADMA was significantly lower in comparison to patients without restenosis ($p < 0.01$). Additionally, logistic regression analysis revealed that the independent risk factors of restenosis include ADMA, age and HDL cholesterol level.

An earlier study by Lu *et al*. showed an increased number of cardiovascular events, including restenosis, after PCI in patients with increased plasma ADMA levels [7]. The study revealed the negative influence of age and the protective role of HDL cholesterol level on the recurrent narrowing in the coronary artery after coronary angioplasty [8].

Elevated plasma ADMA level was reported in patients with traditional cardiovascular risk factors: endothelial dysfunction, elevated total cholesterol level, arterial hypertension, impaired glucose tolerance, and increased intima-media thickness [9–14]. Asymmetric dimethylarginine – an endogenous inhibitor of endothelial nitric oxide (NO) synthase – impairs endothelial NO production, leading to NO deficiency and increased production of oxygen free radicals [15, 16]. Endogenous nitric oxide has anti-inflammatory capabilities, as it inhibits the interaction between endothelium and circulating monocytes [17]. Nitric oxide also inhibits platelet aggregation and their interactions with endothelium, simultaneously decreasing *in vitro* proliferation of vascular smooth muscle cells [18, 19]. Significant damage of the endothelium resulting from coronary angioplasty stimulates the smooth vascular muscle [20]. Furthermore, it was also found in an animal model that the nitric oxide produced in the endothelium decreases the *in vivo* proliferation of smooth muscles in vessels, as well as the growth of neointima [21]. Decreased endothelial production of NO in patients with elevated ADMA levels may accelerate the process of restenosis. Increased ADMA plasma concentration is associated with impaired flow-mediated vasodilation and the progression of atherosclerosis [14]. Several studies have reported an increased rate of coronary restenosis in patients with impaired flow-mediated vasodilatation [22-24]. Increased plasma ADMA concentrations may also cause the upregulation of angiotensin-converting enzyme and increased oxidative stress by means of angiotensin I receptor [25, 26]. Concentrations of ADMA obtained before PCI predict the development of restenosis and major adverse cardiac events. However, there have been reports not confirming the predictive value of ADMA levels in acute coronary syndrome [6, 27].

**Table III.** Asymmetric dimethylarginine and L-arginine in studied subjects

| Parameter | Group I ($n$ = 22) | Group II ($n$ = 38) | Value of $p$ |
|---|---|---|---|
| L-arginine [µmol/l] | 100.5 ±34.5 | 74.6 ±23.9 | < 0.05 |
| ADMA [µmol/l] | 1.94 ±0.94 | 0.96 ±0.67 | < 0.05 |
| L-arginine/ADMA | 49.6 ±23.8 | 121.4 ±100.0 | < 0.05 |

Further clinical implications for the use of ADMA levels could be based on the observation that levels of ADMA obtained before the procedure predict the development of restenosis and major adverse cardiac events in patients who underwent PCI and bare metal stent procedures and thus it may be applied in such a group of patients.

A limitation of the study is the small number of participants. The study should be treated as a preliminary report.

In conclusion, there are several known mechanisms that may explain acceleration of restenosis in patients with elevated ADMA levels. Pre-procedural elevated plasma ADMA level increases the risk of restenosis in patients who underwent coronary angioplasty and stenting with bare metal stents.

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