

# Is there a Marker to Predict Restenosis After Carotid Stenting?

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**Abstract:** **Background:** Carotid atherosclerosis is the leading cause of ischemic stroke. Carotid Arteria Stenting (CAS) is a technique for carotid occlusive disease treatment. CAS procedures are performing in patients with carotid stenosis. **Methods:** We include 241 patients after stroke and 126 with high-graded asymptomatic stenosis in arteria carotis were included; eighteen of them had lesion type I and twenty one with lesion type IV (after stenting). Their results were compared to 91 age matched healthy controls. Included patients and controls were diagnosed by history of diseases, clinically examination, with ultrasound and MRI investigation. Included groups were evaluated by total cholesterol, LDL-cholesterol, homocysteine, hsCRP, D Dimer. **Results:** We found a correlation between serum ADMA and homocysteine levels in patients with asymptomatic stenosis ( $r = 0.612$ );  $P < 0.001$ . It seems to be the leading factor for the stroke. In after stroke patients an important correlations are ADMA to homocysteine ( $r = -0.751$ ), ADMA to LDL-cholesterol ( $r = 0.719$ ), and ADMA to hsCRP ( $r = 0.543$ );  $P < 0.001$ . In patients with in-stent restenosis (ISR) injury and restenosis we found a correlation ADMA to total cholesterol ( $r = 0.509$ ) and ADMA to RR systolic ( $r = -0.519$ );  $P < 0.001$ . **Conclusions:** Serum ADMA levels, homocysteine, LDL-cholesterol and hsCRP concentrations in combination are useful for early identification of highly risked asymptomatic and symptomatic patients for restenosis, as well as patients after stenting of arteria carotis. Serum ADMA might be a new marker to predict early diagnosis of restenosis after carotid stenting.

**Keywords:** ADMA, restenosis, stroke, stent, ISR lesion

## 1. Introduction

Stroke is leading cause of death worldwide. In stroke primary pathophysiological change is reduced regional blood flow. Carotid atherosclerosis is the leading cause of ischemic stroke. Overall, 20%-30% of ischemic strokes are related to extracranial carotid artery stenosis [1].

Traditional therapy for extracranial carotid artery occlusive disease, a significant risk factor for stroke, consists of optimal medical management and selective surgical treatment with carotid endarterectomy (CEA) for stroke risk reduction [2]. Carotid artery stenting (CAS) has become an accepted alternative to CEA over the past decade for patients at high surgical risk, and has progressively evolved into an elegant procedure over the past 3 decades, with dedicated equipment including proximal embolic occlusion devices that have minimized procedural strokes [3]. CAS is a valid technique for the treatment of carotid occlusive disease, with a very low rate of in-stent stenosis. Neurological complications were correlated with complex plaque morphology, which makes accurate pretreatment evaluation of the lesion mandatory if good CAS outcomes are to be achieved [2] three randomized-controlled trials and thirteen single centre studies The occurrence of ISR after CAS ranged from 2.7 to 33% and was detected within the first year in most of the studies. Because the clinical long-term

outcome is of crucial importance especially in younger patients, the occurrence of an in-stent restenosis (ISR) could be a factor endangering the long-term efficacy and safety of CAS [4].

Restenosis was most often asymptomatic and detected at routine ultrasound follow-up [5]. The incidence of in-stent restenosis (ISR) and periprocedural as well as long-term clinical complications were recorded. The combined stroke and death rate during long-term follow-up was significantly higher in the group with an ISR [6]. Predictors of stent restenosis are unknown yet. As such are considerable advanced ages, female gender, and implantation of multiple stents, prior revascularization treatment, and suboptimal result with residual stenosis, elevated postprocedural serum levels of acute-phase reactants, asymptomatic lesion, and use of balloon expandable stents [7].

Many studies have focused changes on plasma ADMA levels in patients with cardiovascular disease, hypertension, atherosclerosis and changes of IMT [8-12]. Asymmetric dimethylarginine (ADMA), an endogenous nitric oxide synthase (NOS) inhibitor, is known as mediator of endothelial cell dysfunction and atherosclerosis [11]. Clinical studies found evidence that increased ADMA levels are associated with a higher risk of cerebrovascular events - stroke and TIA [13-17].

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Changes in serum ADMA and homocysteine levels along with sCRP and LDL-cholesterol might be markers for ISR lesions, despite of lesion size. We evaluated changes of serum ADMA concentration in patients after stenting with different types of lesions.

## 2. Materials and Methods

We estimated 241 patients after stroke in the territory of the middle and anterior cerebral artery. They were stented 20 – 30 days after acute stroke. High graded stenosis (>70%) in arteria carotis in 126 asymptomatic patients was established. They have no symptoms (acute or chronic clinical symptoms) of carotid atherosclerosis. The decision for stent was taken after monitoring and progressing of stenosis, as well as evidence of irregular plaques. Eleven patients of these two groups went in-stent restenosis (ISR) type I (after stent) and twelve were with ISR lesions type IV [18].

All patients were diagnosed by history of diseases, clinically examination, with ultrasound and MRI investigation. All stents were with cerebral protection. Stenting was successful in all cases. In only 3 (0.68%) patients were observed complications after stenting TIA. Patients went under standard therapy after this procedure. After stenting of arteria carotis patients were monitored with clinical examination, ultrasound and MRI arteriography in patients. The monitoring was in continuation for three years (at 1<sup>st</sup>, 6<sup>th</sup>, 12<sup>th</sup>, 24<sup>th</sup> and 36<sup>th</sup> month).

All results were compared to 91 age matched healthy controls. Healthy controls showed no clinical symptoms, and no history of disease. Ultrasound showed no evidence of stenosis and thrombosis. Demographic and clinical evaluation of included patients is showed in Table 1.

**Table 1: Demographic and clinical evaluation of included patients**

Patients	After stroke		Asympt. stenosis		ISR type IV		ISR type I	
	n		n		n		n	
	99		82		16		15	
	m	f	m	f	m	f	m	f
Age	62.9± 4.5	63.5± 4.4	64.1± 3.9	64.0 ± 4.1	65.8± 3.9	64.6 ± 2.9	65.9± 3.8	64.5 ± 2.6
Gender (%)	61 (61.6%)	38 (38.4%)	53 (64.6%)	29 (35.4%)	3 (25%)	9 (75%)	5 (45.5%)	6 (54.5%)
ACC	32 (32.3%)		23 (28%)		4 (25%)		4 (26.7%)	
ACI	67 (67.7%)		59 (82%)		12 (75%)		11 (73.3%)	
Time to stent (after diagnosis)	20 – 30 days		5 – 8 days		n.a.		n.a.	
Stroke/severity/								
Mild/	37 (37.4%)							
Moderate/-	57 (57.6%)							
Severe	5 (5.1%)							

From the group of patients in different time of observation dropped out 143: with diabetes (type 2) 32 patients (22.4%), liver diseases 9 (6.3%), thyroid diseases 6 (4.2%), cranial trauma 2 (1.4%), kidney injury 7 (4.9%), rheumatology diseases 7 (4.9%), smokers 67 (46.9%), tumor diseases 2 (1.4%) and patients with cognitive deficiency 11 (7.7%).

Ultrasound investigation (Philips, iU22) were performed on extracranial arteries (ACC, ACI, vertebral) and intracranial (ACM, ACA, ACP) both sides according to certain criteria for assessment of acute stroke and stenosis [19-21].

MRI investigation was made using Siemens Essex a 1.5T. Tracing changes in carotid stenosis before and after stenting we use Magnetic Resonance Imaging (MRI). We perform ToF 3D natively technology. The source images are transverse plane. According to correlations between Doppler ultrasound findings and after analysis of MIP (maximum intensity projection) and VRT (volume rendering images) in registered focal reductions in carotide blood flow we perform targeted and consistent: t1\_db\_fs, t2\_db\_fs pd\_db\_fs and to assess the extent of luminal narrowing in an axial run and the state of the wall of the carotid arteries. Reference is analysis of the condition for the presence of calcification and/or volume of hematoma in atheromatous plaque.

All included groups were analyzed for laboratory parameters: RBC, WBC, Platelets, AST, ALT, γ-GT, serum creatinine, urea, K, Na, Ca. Main risk factors were

evaluated: total cholesterol, LDL and HDL-cholesterol, triglycerides, homocysteine, glucose, hsCRP, D Dimer. Blood samples from controls were collected at enrolment. Biochemical analyses were performed on Cobas Integra (Roche Diagnostics). BN ProSpec (Siemens Healthcare) nephelometry was used for evaluation of hsCRP. Homocysteine and ADMA were evaluated using ELISA methods on Anthos Zenyth 3100 Multimode Detector.

Informed consent was obtained from all included patients and controls according to the Declaration of Helsinki (Directive 2001/20/EO).

## 3. Statistics

Data were analyzed using SPSS 13.0 (IBM). The results are showed as mean value ± SD. Correlation between parameters was evaluated by Pearson's correlation. Statistical significance was evaluated by paired Student's t-test. We verified the hypothesis that ADMA as an early marker for restenosis in patients after carotid stenting.

## 4. Results

There were no significant differences in baseline characteristics of the patients (age, gender, P > 0.5). Established correlations in serum ADMA levels to other parameters in all included groups are showed in Table 2.

**Table 2:** Correlations in serum ADMA levels to other parameters in all included groups

Correlations	After stroke	Asympt. stenosis	ISR type I	ISR type IV
ADMA to homocysteine	-0.751	0.612	-0.649	-0.719
ADMA to LDL-cholesterol	0.729	0.412	-0.709	-0.741
ADMA to hsCRP	0.543	-0.084	-0.724	-0.794
ADMA to RR systolic	-0.134	0.751	-0.491	-0.519
ADMA to total cholesterol	-0.121	-0.091	0.484	0.509
ADMA to D Dimer	0.053	0.089	0.067	0.081
ADMA to glucose	n.a.	0.029	0.051	0.031

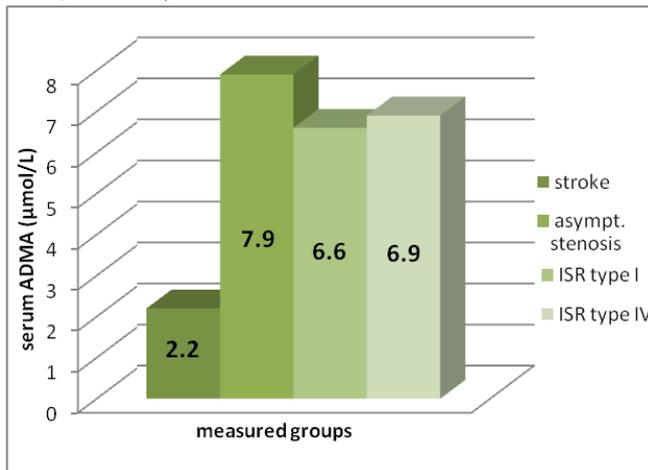
P < 0.001

In ISR type IV restenosis was in twelve ACI patients and four ACC case after twelve months.

In ISR type I restenosis was in eleven ACI patients and four ACC cases after eighteen months.

Laboratory parameters and serum ADMA levels were in reference ranges in the control group.

The comparison of serum ADMA levels between groups showed high correlation between stroke and asymptom, ISR type IV and ISR type I ( $r = -0.598$ ;  $P < 0.001$ ); correlation between stroke and restenosis ( $r = 0.509$ ;  $P < 0.001$ ); and no correlation asymptom to ISR type IV and ISR type I ( $r = -0.019$ ;  $P > 0.05$ ).



**Figure 1:** Serum ADMA levels in measured groups.

## 5. Discussion

Elevated serum hsCRP concentration correlates with increased risk of cerebro- and cardiovascular events [8]. In our patients we found higher hsCRP levels in patients with ISR type IV and ISR type I, compared to stroke cases. Probably it is due to fact that in patients with stroke before stenting the risk of new events was lower, and in ISR type IV and ISR type I this process is still active. Elevated levels of pre-procedural hsCRP may be predictive of the development of neointimal hyperplasia in patients treated with extra- or intracranial stenting procedures [22], as a separate marker.

Several animal and clinical studies have demonstrated a strong association between plasma total homocysteine, plasma ADMA, and endothelial dysfunction [23]. Increasing ADMA accumulation and, subsequently, reducing NO

elaboration by endothelial cells and aortic segments. These observations may explain the observation that endothelial vasodilator function is impaired in individuals with hyperhomocysteinemia [23]. High serum homocysteine levels in our study in asymptomatic restenosis, ISR type IV and ISR type I proves this. This might partly explain the acceleration of vascular disease by hyperhomocysteinemia [24], because of chronic suppression of endothelial NOS activity accelerates atherosclerosis [25], which leads to restenosis.

A study indicates that serum ADMA level is a strong and independent determinant of IMT of the carotid artery in the large number of subjects without overt cerebro-cardiovascular diseases [26]. Serum ADMA was a predictor of carotid IMT progression [27]. In our study a progression of carotid atherosclerosis (intimal hyperplasia) is proved by changes in patients with ISR injury. There were no changes in ADMA levels between asymptomatic patients, ISR type IV and ISR type I ( $P > 0.5$ ), while ADMA concentrations after stroke are lower. This means that ADMA is useful as a predictor for ongoing atherosclerosis. In cases with asymptomatic restenosis there is a leading role of connection between ADMA, homocysteine and hypertension. Total cholesterol, LDL-cholesterol, triglycerides and hsCRP in serum are significantly associated with asymptomatic carotid atherosclerosis [28]. Probably it is because of lack of other atherogenic factors, and because of ongoing therapy. In cases after stroke there is a connection between ADMA and homocysteine, ADMA to LDL-cholesterol and ADMA to hsCRP. This partly shows patophysiological mechanisms of changes, and probably ongoing therapy. In patients with ISR type I and type IV a moderate correlation between ADMA and total cholesterol, and ADMA to blood systolic pressure shows the meaning of these factors in pathogenesis of ongoing atherosclerosis. There is a high correlation between ADMA and homocysteine, ADMA and LDL-cholesterol, ADMA and hsCRP, which means that these parameters might be useful as markers for development of ISR injury and as a predictors of ongoing atherosclerosis, quite similar to patients with carotid endarterectomy cases after coronary stenting [29,30]. Serum HDL-cholesterol levels after procedures predict patients with carotid stent after one year as a single marker [31]. But results of HDL-cholesterol as only marker might be affected easiest compared to other markers.

## 6. Conclusion

Serum ADMA, homocysteine, LDL-cholesterol, hsCRP quantification will facilitate identification of high-risked patients. The combination of four parameters is useful in monitoring and therapy in both asymptomatic and symptomatic patients with carotid stenting. ADMA is a new predictor for early diagnosis of restenosis after carotid stenting.

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