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INCREASED PLASMA CATHEPSIN S AT THE TIME OF PERCUTANEOUS TRANSLUMINAL ANGIOPLASTY IS ASSOCIATED WITH 6-MONTHS' RESTENOSIS OF THE FEMOROPOPLITEAL ARTERY

POVEĆAN KATEPSIN S U PLAZMI U VREME PERKUTANE TRANSLUMINALNE ANGIOPLASTIKE POVEZAN JE SA RESTENOZOM FEMOROPOPLITEALNE ARTERIJE 6 MESECI POSLE INTERVENCIJE

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Summary

Background: We tested the hypothesis that increased levels of cathepsin S and decreased levels of cystatin C in plasma at the time of percutaneous transluminal angioplasty (PTA) are associated with the occurrence of 6-months' restenosis of the femoropopliteal artery (FPA).
Methods: 20 patients with restenosis and 24 matched patients with patent FPA after a 6-months follow-up were included in this study. They all exhibited disabling claudication or critical limb ischemia and had undergone technically successful PTA. They were all receiving statins and ACE inhibitors (or angiotensin II receptor antagonist) before the PTA and the therapy did not change throughout the observational period. Plasma concentrations of C-reactive protein were < 10 mg/L and of creatinine within the reference range at the time of the PTA. Plasma concentration and activity of cathepsin S, together with its potent inhibitor cystatin C, were measured the day before and the day after the PTA.
Results: The increased plasma concentration and activity of cathepsin S at the time of PTA was associated with the occurrence of 6-months' restenosis of FPA, independently of established risk factors (lesion complexity, infrapopliteal run-off vessels, type of PTA, age, gender, smoking, diabetes, lipids) and of cystatin C. Plasma cystatin C concentration was not associated with restenosis and did not correlate with cathepsin S activity and concentration in the plasma.

Kratak sadržaj

Uvod: Testirali smo hipotezu da su povećani nivoi katepsina S i smanjeni nivoi cistatina C u plazmi u vreme izvođenja perkutane transluminalne angioplastika (PTA) povezani sa pojavom restenoze femoropopliteale arterije (FPA) 6 meseci posle intervencije.
Metode: Bolesnici sa restenozom (N=20) i bolesnici bez restenoze FPA (N=24) su uključeni u ovu studiju nakon 6 meseci praćenja. Svi bolesnici su imali intermitentnu klaudikaciju ili kritičnu ishemiju ekstremiteta i prošli su tehnički uspešnu proceduru PTA. Svi bolesnici su bili na terapiji statinima i ACE inhibitorima (ili antagonistima angiotenzin II receptora) pre PTA i terapije se nisu promenile tokom praćenja. Koncentracije C-reaktivnog proteina u plazmi bile su <10 mg/L i koncentracije kreatinina unutar referentnog opsega u vreme PTA. Koncentracije i aktivnosti katepsina S u plazmi, zajedno sa njenim endogenim inhibitorom cistatinom C, merene su dan pre i dan posle PTA.
Rezultati: Povećana koncentracija i aktivnost katepsina S u plazmi u trenutku PTA bile su povezane sa pojavom restenoze FPA 6 meseci nakon PTA, nezavisno od utvrđenih faktora rizika za pojavu ove komplikacije (kompleksnost lezija, izlivanje infrapoplitealnih sudova, tip PTA, starost, pol, pušenje, dijabetes, dislipidemija) i koncentracije cistatina C. Koncentracije cistatina C nisu bile povezane sa restenozom i nisu korelirale sa aktivnošću i koncentracijom katepsina S u plazmi.

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List of abbreviations: CTSS, cathepsin S; CysC, cystatin C; FPA, femoropopliteal artery; PTA, percutaneous transluminal angioplasty; CRP, C-reactive protein; TASC II, TransAtlantic Inter-Society Consensus-II classification

Conclusions: Increased level of plasma cathepsin S at the time of PTA is associated with 6-months' restenosis of PTA, independently of established risk factors.

Keywords: cathepsin S; cystatin C; femoropopliteal artery; restenosis

Zaključak: Povećan nivo katepsina S u plazmi u vreme izvođenja PTA povezan je sa restenozom FPA u periodu od 6 meseci posle intervencije, nezavisno od utvrđenih faktora rizika.

Ključne reči: katepsin S, cistatin C, femoropoplitealna arterija, restenoza

Introduction

Percutaneous transluminal angioplasty (PTA) is a conventional method for treating peripheral artery disease in the lower limbs even though, in the first year after the treatment, restenosis and reocclusion occur in up to 50 % of patients (1). The understanding of the mechanisms of restenosis and reocclusion of the superficial femoral artery following PTA is incomplete. Several factors may affect the patency of the femoropopliteal arterial segment after successful PTA, including the clinical severity of peripheral artery disease, patient comorbidities, such as diabetes or renal failure, type and length of lesion, number of lesions, calcification of plaques (2, 3) and extent of vascular inflammation (4).

Cathepsin S (CTSS; EC 3.4.22.27) is a cysteine protease involved in autophagocytosis, clearance of damaged mitochondria, presentation of major histocompatibility complex class II antigen, and atherogenesis (5). In this latter, CTSS mRNA and protein levels are increased in human and animal atheroma but not in non-atherosclerotic arteries (6). CTSS is synthesized in activated macrophages, smooth muscle cells, and endothelial cells (7). When released, its elastolytic and collagenolytic activities cause elastic lamina degradation (8, 9), plaque rupture (10), and necrotic core development (9). Patients with cardiovascular disease have increased concentrations of CTSS in the plasma and therefore higher risk of developing atherosclerosis (11, 12). Our recent study revealed that increased plasma CTSS concentration and activity are associated with an atherogenic LDL subclass profile (decreased dominant LDL size and increased percentage of small, dense LDL particles) and that atorvastatin lowers plasma CTSS concentration and activity, concomitantly and interrelatedly with improvement of the LDL subclass profile (13).

Cystatin C (CysC) is an endogenous inhibitor of cysteine peptidases, being secreted from cells and present in high levels in various body fluids, including plasma (14). It has been proposed as a marker for atherosclerosis (14), even in patients without chronic kidney disease (15). Its increased plasma levels were shown to predict in-stent restenosis of coronary arteries (16). However, based on the fact that CysC is endogenous inhibitor of CTSS, we can rather propose a decreased plasma level to be associated with restenosis. Whatever, CysC role in femoropopliteal restenosis has not been studied yet.

The aim was to measure plasma concentrations and activities of CTSS and CysC in patients at the time of PTA of the femoropopliteal artery (FPA) and to relate the levels to the occurrence of restenosis. We hypothesized that increased CTSS and decreased CysC in plasma at the time of PTA are associated with the occurrence of 6-months' restenosis of the FPA.

Materials and Methods

Subjects

Among 88 consecutive patients treated with femoropopliteal PTA at the Department of Vascular Diseases of Ljubljana Medical Centre, ultrasound examination identified 24 patients with restenosis and 60 patients without restenosis; 4 patients were lost for follow-up (*Figure 1*). After matching for age, sex and other risk factors for atherosclerosis, 20 patients with restenosis were identified and 24 patients without restenosis, who were tested for levels of CTSS and CysC. They were all receiving statins and ACE inhibitors (or angiotensin II receptor antagonist) before the PTA, none started with this medication immediately prior to the undergoing PTA and the medication was not change throughout the observational period. The endocrine diseases, as well as acute inflammatory, thyroid, liver, neoplastic and renal diseases, were excluded by detailed history, clinical examination and laboratory analysis. Thus, they all had serum concentrations of C-reactive protein < 10 mg/L and of creatinine within the reference rage. All patients gave their written informed consent.

The technical success of PTA, performed by simple balloon angioplasty or bailout stenting, was assessed by peri-procedural angiography, and judged satisfactory with a residual stenosis after ≤ 30 %. Infrapopliteal run-off, which was assessed after endovascular intervention, was scored by a modification of the Society for Vascular Surgery criteria (17). With this scoring system, we divided patients' limbs into two categories: good run-off and compromised run-off. Patients were followed up by vascular ultrasound imaging 6-months after PTA. Adverse outcome of PTA was defined by a restenosis of $\geq 50\%$ that had been confirmed by at least doubling of the maximal systolic velocity in comparison to that of a proximal, non-obstructed arterial segment. As we followed the guidelines for management of patients with peripheral arterial disease (18), there were no changes in the prescribed treatment regimen in the observational

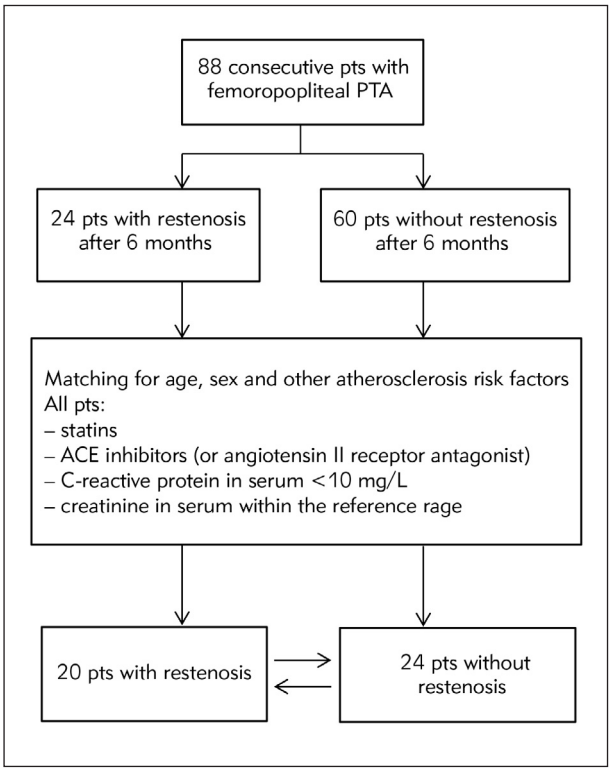

Figure 1 Selection of patients. Among 88 consecutive patients treated with femoropopliteal percutaneous transluminal angioplasty (PTA), ultrasound examination identified 24 patients with restenosis and 60 patients without restenosis; 4 patients were lost for follow-up. After matching for age, sex and other risk factors for atherosclerosis, 20 patients with restenosis were identified and 24 patients without restenosis, who were tested for levels of cathepsin S and cystatin C.

period, except for dual antiplatelet treatment, which was prescribed for a maximum of three months after bailout stenting and thereafter continued as antiplatelet monotherapy, usually with acetylsalicylic acid.

The study was performed according to the Declaration of Helsinki guidelines and was approved by the National ethics committee.

Laboratory measurements

Blood was collected from the antecubital vein into vacuum tubes containing 0.11 mol/L sodium citrate (9:1 v/v) the day before and the day after the PTA. Plasma was prepared by 30-minute centrifugation at 4 °C and transferred to small plastic vials, snap frozen in liquid nitrogen and stored at -70 °C until analyzed.

Quantitative CTSS, ELISA with a combination of 1E3 MAb and 2B4 Mabs (Krka, d.d., Ljubljana, Slovenia), was used as described (19). CTSS activity was determined using fluorogenic substrate Z-VVR-AMC (Biomol International, Hamburg, Germany) as described (13). Samples from the same patient (before and after the PTA) were processed in the same run.

CysC was determined as a protein concentration in plasma by Cystatin C Immunoparticles assay (Dako, Glostrup, Denmark).

Total, LDL and HDL cholesterol, triglycerides, creatinine and C-reactive protein (CRP) were measured using standard procedures based on dry-chemistry on Fusion 5.1 (Ortho Clinical Diagnostics, Raritan, USA). In CRP, the lowest concentration measured was 5 mg/L.

Statistical analysis

All calculations were performed using SPSS v.23.0 (SPSS Inc.Chicago, IL, USA). Numerical data are shown as medians and interquartile range, and categorical variables as the number of cases. Mann-Whitney U-test, Fisher-Exact tests and ROC analysis were used to compare data between groups. Relations between variables were determined using the Spearman rank-order correlation test. Multivariable logistic regression analysis was used to access independent associations with 6-months' restenosis of FPA.

Results

Clinical characteristics of the patients are summarized in the *Table I*. The groups with and without restenosis did not differ in demographic data and atherosclerosis risk factors (age, gender, hypertension, smoking habits, presence of diabetes), clinical severity of peripheral artery disease, lesion complexity (as evaluated by TransAtlantic Inter-Society Consensus-II (TASC-II) classification) (20), type of PTA and therapy (statins, ACE inhibitors or angiotensin II receptor antagonist, beta blockers). However, infrapopliteal run-off was more frequently compromised in the restenosis group than in the group without restenosis ($p = 0.036$).

Laboratory findings are summarized, separately for the venepuncture the day before the PTA and for that the day after PTA (*Table II*). Before the PTA, increased CTSS concentration and CTSS activity were the only parameters discriminating the restenosis group from the group without restenosis. After the PTA, increased CTSS activity was the only parameter discriminating the restenosis group from that without restenosis. ROC analysis yielded exactly the same conclusions (results not shown). Diabetic patients exhibited higher CTSS activity in plasma (before PTA: $p = 0.045$; after PTA: $p = 0.017$), but were equally distributed in both groups (*Table I*). Plasma CTSS and CysC levels did not differ between patients with advanced lesion complexity (D or C in TASC II) and

Table I Clinical characteristics of patients.

Parameter	Restenosis (n = 20)	Without restenosis (n = 24)	p
Age (years)	68.5 (62.0/72.0)	63.0 (58.3/71.8)	NS
Gender (male/female)	13/7	19/5	NS
Hypertension (yes/no)	20/0	24/0	NS
Smoking (yes/quit/no)	2/10/8	5/14/5	NS
Type 2 diabetes (yes/no)	10/10	15/9	NS
Clinical severity (critical ischemia/disabling claudication)	1/19	0/24	NS
Lesion complexity (TASC II; D/C/B/A)	2/9/9/0	0/12/12/0	NS
Run-off vessels (compromised/good)	4/16	0/24	0.036
PTA (bailout stenting/balloon angioplasty)	4/16	6/18	NS
Statin (yes/no)	20/0	24/0	NS
ACE inhibitor (yes/no)	20/0	24/0	NS
Beta blocker (yes/no)	11/9	14/10	NS

The values are median (25th percentile/75th percentile) or number of patients.
Abbreviations: TASC II, TransAtlantic Inter-Society Consensus-II classification (20); p, level of significance; NS, p > 0.100.

Table II Laboratory findings.

Parameter	Venepuncture one day after PTA			Venepuncture one day after PTA		
	Restenosis	Without restenosis	p	Restenosis	Without restenosis	p
Total cholesterol (mmol/L)	3.1 (2.8/3.4)	2.8 (2.6/3.3)	NS	2.9 (2.5/3.1)	2.7 (2.4/2.9)	NS
LDL cholesterol (mmol/L)	1.6 (1.2/1.9)	1.4 (1.2/1.9)	NS	1.4 (1.1/1.6)	1.3 (1.1/1.7)	NS
HDL cholesterol (mmol/L)	0.87 (0.69/1.03)	0.81 (0.70/0.93)	NS	0.76 (0.65/0.92)	0.71 (0.61/0.83)	NS
Triglycerides (mmol/L)	1.5 (1.0/1.9)	1.2 (1.0/2.2)	NS	1.4 (1.0/1.9)	1.4 (1.1/2.1)	NS
Total/HDL cholesterol	3.68 (2.94/4.39)	3.97 (3.14/4.60)	NS	3.58 (3.06/4.19)	4.11 (3.44/4.52)	NS
Triglycerides/HDL cholesterol	1.73 (1.12/2.55)	1.67 (1.11/2.54)	NS	2.09 (1.14/2.47)	2.12 (1.30/2.69)	NS
CTSS concentration (μg/L)	20.5 (18.6/23.3)	18.0 (14.0/20.7)	**0.015***	19.6 (17.3/23.9)	17.0 (14.3/20.9)	NS
CTSS activity (RFU/s)	5.79 (5.51/7.20)	5.16 (4.40/6.24)	**0.038**	6.76 (5.62/7.53)	5.14 (4.57/6.20)	**0.003***
CysC (mg/L)	0.91 (0.75/0.99)	0.84 (0.74/0.94)	NS	0.79 (0.69/0.94)	0.82 (0.73/0.94)	NS

The values are median (25th percentile/75th percentile) or number of patients.

Abbreviations: PTA, percutaneous transluminal angioplasty; CTSS, cathepsin S; CysC, cystatin C; p, level of significance; NS, p > 0.100; *, power of the difference >78% (one-tail test).

patients with lower lesion complexity (B or A in TASC II) (results not shown). Reasonably, plasma CTSS and CysC levels highly correlated between the day before the PTA and the day after PTA (results not shown). Importantly, neither CTSS activity (p = 0.417) nor concentration (p = 0.164) correlated with CysC concentration in the plasma.

Before the PTA, CTSS concentration and CTSS activity were associated, by multivariate analysis, with occurrence of restenosis, independently of established risk factors for restenosis and of CysC, a potent endogenous CTSS inhibitor (*Table III*; column Venipuncture one day before PTA). Large number of established risk factors is divided into the disease/intervention group (lesion complexity, run-off vessels, type of PTA) and the atherosclerosis group (age, gender, smoking, diabetes, lipids). Furthermore, after the PTA, CTSS concentration and CTSS activity remained associated with restenosis, again independently of the above mentioned established dis-

Table III Cathepsin S association with restenosis in multivariable analysis.

Model	Parameters in the model	Venepuncture one day before PTA Relative risk (95 % interval)		Venepuncture one day after PTA Relative risk (95 % interval)	
Disease/ intervention parameters	Lesion complexity (TASC II; D+C/B+A)	1.25 (0.26–6.07)	p = 0.780	1.19 (0.29–4.91)	p = 0.808
	Run-off vessels (compromised/good)	>99 (0.00–>999)	p = 0.999	>99 (0.00–>999)	p = 0.999
	PTA (bailout stenting/balloon angioplasty)	0.90 (0.18–4.66)	p = 0.904	0.66 (0.12–3.61)	p = 0.634
	Constant	0.01 (0.00–1.77)	p = 0.083	0.17 (0.00–9.97)	p = 0.395
	CTSS concentration (μg/L)	**1.20 (1.02–1.42)**	**p = 0.031**	**1.13 (1.01–1.26)**	**p = 0.040**
	CysC (mg/L)	0.83 (0.01–56.6)	p = 0.929	0.23 (0.00–16.3)	p = 0.496
Atherosclerosis risk factors	Age (years)	1.02 (0.91–1.13)	p = 0.775	1.02 (0.92–1.13)	p = 0.722
	Gender (male/female)	1.07 (0.20–5.79)	p = 0.940	1.62 (0.34–7.67)	p = 0.547
	Smoking (yes/quit+no)	0.37 (0.02–6.42)	p = 0.493	1.19 (0.14–9.91)	p = 0.875
	Type 2 diabetes (yes/no)	2.22 (0.49 –10.1)	p = 0.302	1.81 (0.43 –7.68)	p = 0.422
	Total/HDL cholesterol	0.91 (0.26–3.25)	p = 0.885	0.60 (0.18–1.97)	p = 0.399
	Triglycerides/HDL cholesterol	0.50 (0.15–1.60)	p = 0.241	0.89 (0.34–2.34)	p = 0.808
	Constant	0.02 (0.00–458)	p = 0.441	2.41 (0.00–14.1)	p = 0.843
	CTSS concentration (μg/L)	**1.26 (1.03–1.53)**	**p = 0.025**	1.10 (0.99–1.22)	p = 0.093
	CysC (mg/L)	0.59 (0.01–57.9)	p = 0.822	0.06 (0.00–7.42)	p = 0.251
Disease/ intervention parameters	Lesion complexity (TASC II; D+C/B+A)	0.89 (0.19–4.11)	p = 0.882	0.76 (0.18–3.26)	p = 0.711
	Run-off vessels (compromised/good)	>99 (0.00–>999)	p = 0.999	>99 (0.00–>999)	p = 0.999
	PTA (bailout stenting/balloon angioplasty)	1.05 (0.21–5.33)	p = 0.949	1.01 (0.21–4.99)	p = 0.989
	Constant	0.03 (0.00 –2.27)	p = 0.109	0.04 (0.00 –4.53)	p = 0.179
	CTSS activity (RFU/s)	**1.89 (1.07–3.33)**	**p = 0.028**	**1.69 (1.06–2.68)**	**p = 0.027**
	CysC (mg/L)	0.66 (0.01–60.8)	p = 0.856	1.23 (0.01–116)	p = 0.930
Atherosclerosis risk factors	Age (years)	0.99 (0.89–1.11)	p = 0.863	1.01 (0.90–1.12)	p = 0.897
	Gender (male/female)	1.31 (0.25–6.91)	p = 0.750	2.31 (0.45–11.7)	p = 0.314
	Smoking (yes/quit+no)	0.28 (0.01–5.57)	p = 0.407	1.50 (0.13–17.7)	p = 0.748
	Type 2 diabetes (yes/no)	1.43 (0.33–6.27)	p = 0.632	1.38 (0.30–6.31)	p = 0.675
	Total/HDL cholesterol	0.90 (0.29–2.81)	p = 0.862	0.77 (0.22–2.74)	p = 0.683
	Triglycerides/HDL cholesterol	0.72 (0.28–1.85)	p = 0.493	0.91 (0.33–2.44)	p = 0.844
	Constant	0.12 (0.00–423)	p = 0.609	0.09 (0.00–1035)	p = 0.609
	CTSS activity (RFU/s)	**1.88 (1.02–3.45)**	**p = 0.042**	**1.78 (1.04–3.02)**	**p = 0.034**
	CysC (mg/L)	0.73 (0.01–49.0)	p = 0.885	0.34 (0.00–47.4)	p = 0.665

Abbreviations: PTA, percutaneous transluminal angioplasty; TASC-II, TransAtlantic Inter-Society Consensus-II classification (20); CTSS, cathepsin S; CysC, cystatin C; p, level of significance.

ease/intervention and atherosclerosis risk factors and of CysC (*Table III*; column Venipuncture one day after PTA). In some cases, the number of variables in the model is high compared to the total number of subjects included in our study, but the exclusion of a particular risk factor from the model (age, gender, smoking) did not change the strength of association (results not shown).

Discussion

We have shown, for the first time, that increased plasma concentration and activity of CTSS at the time of PTA are associated with the occurrence of restenosis of FPA within 6-months of follow-up, independently of established disease/intervention and atherosclerosis risk factors and of CysC concentration. Contrary to our expectation, plasma CysC concentration was not associated with the restenosis and did not correlate with activity or concentration of CTSS in the plasma.

Our understanding of the mechanisms of restenosis and reocclusion of the superficial femoral artery after PTA enhanced tremendously, but some stimuli and some pathways still need to be elucidated. Moreover, the best treatment and ideal predictor of its failure are lacking. For instance, clinical severity of peripheral artery disease, lesion complexity, diabetes and renal failure are established predictors of restenosis and reocclusion of the femoropopliteal arterial segment following technically successful PTA (2, 3). On other hand, data associating inflammatory biomarkers with the rate of restenosis and reocclusion are ambiguous (22, 23). Notably, conventional therapy, such as statins or ACE inhibitors (or angiotensin II receptor antagonist), may influence biomarkers, thus raising further difficulties in interpreting their predictive ability. In our study the groups of patients with and without restenosis did not differ with regard to established disease/intervention and atherosclerosis risk factors (except for infrapopliteal run-off vessels, which was appraised in multivariable analysis) and with regard to conventional therapy (all patients were

receiving statins and ACE inhibitors or angiotensin II receptor antagonist before the PTA and the medication was not change throughout the observational period). In this respect, the association of CTSS with the occurrence of restenosis of FPA after successful PTA is of fundamental interest. Atherogenesis is a multifactorial process and the role of CTSS-mediated pathway is well documented (6–10). Presumably, this pathway performs a vivid role in the complex process of restenosis and reocclusion of the superficial femoral artery after successful PTA, which merits further consideration.

There is accumulating evidence that conventional therapy (statins and ACE inhibitors or angiotensin II receptor antagonist) is only partially successful in preventing CTSS-mediated pathway of atherogenesis. A study in humans indicated that statin treatment did not change CTSS activity in the wall of abdominal aortic aneurysm (23). Furthermore, patients with coronary atherosclerosis were shown, by plasma mRNA analysis, to exhibit much higher CTSS expression than control subjects, despite treatment with statin (24). However, in an animal model the levels of CTSS mRNA and protein were increased significantly in myocardium of apoE$^{-/-}$ mice fed with western-style diet and, further, simvastatin decreased both levels 32 weeks after treatment (25). ACE inhibitors or angiotensin II receptor antagonist also decrease CTSS mRNA and/or activity (26, 27). In our study all patients had the same therapy regiment (statins and ACE inhibitors or angiotensin II receptor antagonist) at the entrance, which did not change throughout the observational period. Despite that, we identified a subgroup of patients with increased CTSS plasma levels at the time of PTA and this subgroup experienced restenosis within the short period of 6-months, presumably due to the persisting vivid CTSS-mediated pathway of atherogenesis that was not successfully treated. In this respect it is worth to mention that atorvastatin reduce plasma CTSS levels in LDL phenotype B patients only (patients with small, dense LDL particles, with a dominant LDL diameter of ≤ 25.5 nm before statin intervention) (13). Future studies should discretely evaluate whether the prescribed therapy prevents CTSS-mediated pathway of atherogenesis.

Contrary to our expectation and to published data, plasma CysC concentration at the time of PTA was not associated with the occurrence of 6-months' restenosis of FPA. The frequently described background mechanism linking CysC to atherogenesis is that it is a potent endogenous inhibitor of CTSS (14, 15), thus preventing CTSS-mediated pathway of atherogenesis. In this respect we expected the association of decreased CysC concentrations with the restenosis. The hypothesis was not proved. Furthermore, CysC concentration did not correlate with CTSS activity (and concentration) in the plasma. Altogether, this rather points to CysC not being the main regulator of CTSS activity and CTSS-mediated effects of atherogenesis. On the other hand, some recent publications indicate that increased plasma CysC concentration predicts coronary artery disease (15, 28) and in-stent restenosis of coronary arteries (16) in patients without chronic kidney disease, leading to the assumption that increased CysC concentration is proatherogenic. This too was not confirmed in our study. However, previously published studies included patients with serum CRP concentrations well above 10 mg/L (15, 16), which was the upper limiting value for entering the patient to our study. Thus, it is reasonable to suspect that, in previous studies, patients had etiologies of inflammation other than atherosclerosis, thus yielding additional increase of plasma concentrations of CRP and CysC, beside. Last, but not least, the role of inflammation in coronary atherosclerosis cannot easily be extrapolated to peripheral artery disease, in which inflammatory biomarkers may play a different role (29). Thus, our results are incomparable to the data of previous publications in this field. Whatever the background mechanism is, in our study CysC concentration in plasma at the time of PTA did not associate with the occurrence of 6-months' restenosis of FPA.

In conclusion, the increased plasma concentration and activity of CTSS at the time of PTA was associated with the occurrence of 6-months' restenosis of FPA, independently of established risk factors. Plasma CysC concentration was not associated with the occurrence of 6-months' restenosis of FPA and did not correlate with CTSS activity and concentration in the plasma.

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Conflict of interest statement

The authors stated that they have no conflict of interest regarding the publication of this article.

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