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C-REACTIVE PROTEIN PREDICTS PROGRESSION OF PERIPHERAL ARTERIAL DISEASE IN PATIENTS WITH TYPE 2 DIABETES: A 5-YEAR FOLLOW-UP STUDY

C-REAKTIVNI PROTEIN PREDVIĐA PROGRESIJU BOLESTI PERIFERNIH ARTERIJA KOD PACIJENATA SA DIJABETESOM TIPA 2: REZULTATI PETOGODIŠNJEG PRAĆENJA

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Summary

Background: Previous studies have indicated that high sensitivity C-reactive protein (hs-CRP) is a risk factor for the peripheral arterial disease (PAD) in diabetes. This study aimed to evaluate the possible predictive significance of hs-CRP for the development and progression of PAD in patients with type 2 diabetes (T2D).
Methods: The study included 80 patients previously diagnosed with T2D, aged 45–70 years, divided into *group A* (T2D patients with PAD; n=38) and *group B* (T2D patients without PAD; n=42). After five years, all the patients were re-examined and divided into subgroups depending on *de novo* development of PAD or progression of previously diagnosed PAD. Ankle-Brachial Index (ABI) measurement was used for PAD diagnosis and hs-CRP was determined by nephelometry.
Results: We found significantly higher hs-CRP levels in group A compared to group B, but only at baseline. Among the patients in group A, those with later progression of PAD (subgroup A1) had the highest levels of hs-CRP at baseline, although not significantly different from those in subgroup A2 (non-progressors). In contrast, hs-CRP level was significantly higher in subgroup B1 (progressors) in comparison to subgroup B2 (non-progressors) at both the first and sec-

Kratak sadržaj

Uvod: Prethodne studije su istakle visokosenzitivni C-reaktivni protein (hs-CRP) kao faktor rizika za perifernu arterijsku bolest (PAB) u dijabetesu. Ova studija je imala za cilj da se proceni mogući prediktivni značaj hs-CRP u razvoju i progresiji PAD kod pacijenata sa tipom 2 dijabetesa (T2D).
Metode: U studiju je uključeno 80 pacijenata sa prethodnom dijagnozom T2D, starosti 45–70 godina, podeljenih u grupu A (T2D pacijenti sa PAB; n=38) i grupu B (T2D pacijenti bez PAB; n=42). Posle pet godina, pacijenti su podeljeni u podgrupe u zavisnosti od prisustva nove pojave PAB ili progresije prethodno postojeće PAB. Pedo-brahijalni indeks (PBI) korišćen je za dijagnozu PAB, dok je hs-CRP određen nefelometrijski.
Rezultati: Našli smo značajno više nivoe hs-CRP u grupi A u odnosu na grupu B, ali samo na početku studije. U okviru grupe A, pacijenti sa kasnijom progresijom PAD (podgrupa A1) imali su najviše nivoe hs-CRP na početku, mada bez značajne razlike u odnosu na podgrupu A2 (neprogresori). Suprotno tome, nivo hs-CRP bio je značajno viši u podgrupi B1 (progresori) u poređenju sa podgrupom B2 (neprogresori) i na početku i na kraju ispitivanja. Od svih ispitivanih metaboličkih parametara, hs-CRP je bio jedini nezavisni prediktor progresije PAD (OR=0,456, 95%

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ond exam. Of all the investigated metabolic parameters, hs-CRP was the only independent predictor of PAD progression (OR=0.456, 95% CI=0.267–0.7815, p=0.004). The cut-off point for hs-CRP was 2.5 mg/L (specificity 75% and sensitivity 73.3%) with the relative risk for PAD of 2.93 (95% CI=1.351–6.3629).
Conclusions: Our study implies that hs-CRP can be used as a reliable predictor for the progression of PAD in patients with T2D.

Keywords: Ankle-Brachial Index, C-reactive protein, peripheral arterial disease, type 2 diabetes

CI=0,267–0,7815, p=0,004). Nivo hs-CRP od 2,5 mg/L je utvrđen kao »cut-off« vrednost (specifičnost 75% i senzitivnost 73,3%) sa relativnim rizikom za PAD od 2,93 (95% CI=1,351–6,3629).
Zaključak: Naša studija je pokazala da se hs-CRP može koristiti kao pouzdan prediktor progresije PAB kod pacijenata sa T2D.

Ključne reči: pedo-brahijalni indeks, C-reaktivni protein, periferna arterijska bolest, tip 2 dijabetesa

Introduction

Previous epidemiological studies have established a link between diabetes and increased prevalence of peripheral artery disease (PAD) (1, 2). It was also found that patients with diabetes and PAD are at greater risk for lower extremity amputation, cardiovascular and cerebrovascular events compared with patients without diabetes (1, 2). The Edinburgh Artery Study showed that after 5 years of follow-up both symptomatic and asymptomatic patients with PAD had increased risk for cardiovascular death (3). In addition, the German Epidemiological Trial on Ankle Brachial Index (ABI) pointed out a higher rate of cardiovascular events and death both in asymptomatic and symptomatic patients with ABI below 0.90 (4). On the other hand, the Framingham Heart Study revealed that approximately 20% of patients with symptomatic PAD have diabetes. It was also shown that the prevalence of PAD in patients with diabetes is greatly underestimated, given that more than 80% either have atypical symptoms or no symptoms at all (5).

Besides diabetes, the strongest risk factors for PAD are older age (6) and cigarette smoking (7). Other potential risk factors include hypertension (8) and dyslipidemia: elevations of total cholesterol (Ch), low-density lipoprotein cholesterol (LDL-Ch), triglyceride (TG) and lipoprotein (Lp) (a) levels (6). A follow-up study published in 2012 showed that insulin resistance analyzed by the homeostatic model of insulin resistance (HOMA-IR) was associated with a higher risk of PAD measured by ABI (9). Findings from the National Examination Survey, 1999 to 2004 demonstrated a strong and independent relationship between insulin resistance as assessed by HOMA-IR and PAD defined as ABI≤0.9 (10). Lately, high-sensitivity C-reactive protein (hs-CRP) has been indicated as a potential marker for the presence of PAD, but also as a factor that probably participates in the development and progression of the illness. CRP is produced primarily in the liver as a result of a non-specific acute response to tissue damage, infection, inflammation and malignant neoplasia (11). It binds with high affinity to phospholipids in apoptotic cells and damaged cell membranes, oxidized lipoproteins and cells attacked by microorganisms. Also, it was found that CRP can bind to lipoproteins and damaged cells in the atherosclerotic plaque to induce complement activation, thereby promoting inflammation and disease progression (11). The data, primarily from *in vitro* studies, suggest the possibility that CRP itself contributes to the development and progression of atherosclerosis, and that it can be considered not only as a marker but also as a genuine risk factor. CRP levels, thus, could reflect the severity of atherosclerosis and risk of future cardiovascular events. However, the detailed mechanisms of the role of CRP in the development of PAD in patients with type 2 diabetes have not yet been elucidated. For that reason, this follow-up study aimed to evaluate the possible predictive significance of hs-CRP for PAD development and progression in patients with type 2 diabetes.

Methods

In this prospective, cohort observational study, we assessed the relationship between hs-CRP and the occurrence and progression of PAD in patients with type 2 diabetes. The study was carried out according to Good Clinical Practice and was approved by the local medical Ethics Committee and conformed to the principles outlined in the Declaration of Helsinki (12). All patients included in the study gave their written Informed Consent.

Study population

From a larger group of patients, 80 patients previously diagnosed with type 2 diabetes, aged 45–70 years, were randomly chosen for the study from our outpatient clinic. After a thorough basal examination, patients were divided according to the presence of PAD (based on the value of ABI) into two groups: *group A* consisted of patients with PAD (n=38) and *group B* consisted of patients without PAD (n=42). After five years, all the patients were re-examined and divided into subgroups depending on *de novo* development of PAD or progression of previously diagnosed PAD. During this period, 8 patients (21.1%) from group A showed a decrease in ABI and progression of PAD (*subgroup A1; progressors*) and 30

patients (79.9%) had no change in ABI (*subgroup A2; non-progressors*). At the same time, in group B 22 patients (52.4%) had a decrease in ABI (*subgroup B1; progressors*) and *de novo* development of PAD, while 20 patients (47.6%) had neither ABI changes nor development of PAD (*subgroup B2; non-progressors*).

All patients included in the study were treated with diet and/or oral antidiabetic drugs only. The patients treated with insulin as well as smokers (former or current) were excluded. Subjects with ABI less than 0.40 and/or ABI greater than 1.30, subjects with CRP>10 g/L, and subjects who refused to be followed and investigated were excluded also. We excluded patients with acute or chronic infectious disease, renal disease, hepatic disease, malignancies, autoimmune disorders and chronic inflammatory diseases. Medications that might affect lipoprotein levels were discontinued 24–48 h before testing. Participants did not receive medications and/or foods that might affect glucose levels at least 12 hours before providing blood samples for analysis.

Degree of obesity was determined by using the body mass index (BMI) which was calculated according to the formula: BMI (kg/m^2) = body weight (kg) / body height (m^2) (13). The type of obesity was determined by the value of waist circumference and waist to hip ratio (WHR) (14, 15). Coronary and cerebrovascular diseases were diagnosed on the basis of medical history and data from relevant medical records. Hypertension was defined as systolic/diastolic blood pressure (≥140/≥90 mmHg) according to the previous description as recommended by the World Health Organization (WHO) and ESH/ESC Guidelines for management of arterial hypertension (16), or by a patient's history of antihypertensive therapy.

Vascular assessment

Diagnosis of PAD was based on the ABI on either leg, as previously described (17). Briefly, Doppler ultrasound (The Huntleigh Multi Doppler II Bi-directional Doppler with an 8 MHz vascular probe) was used to measure systolic pressure in the bilateral brachial, posterior tibial and dorsal pedal arteries. ABI was calculated by dividing the higher pressure in the dorsal pedal and posterior tibial arteries on the right and left sides respectively by the higher brachial pressure on either side (at least two measurements were done).

PAD diagnosis and categorization based on the determination of ABI were interpreted as follows: *without PAD:* ABI of 0.91–1.30; *mild obstruction:* ABI of 0.70–0.90; *moderate obstruction:* ABI of 0.41–0.69; *severe obstruction:* ABI less than 0.40; and non-compressible blood vessels when ABI is greater than 1.30 (due to medial arterial calcification) (17). After 5 years of follow-up, ABI measurements were repeated in all patients and we determined an ABI decline from one category to another (from mild to moderate and from moderate to severe) as a marker of PAD progression, and an ABI decline from normal to values below 0.90 as a marker of *de novo* PAD development in these patients.

Laboratory analyses

From each patient included in the study blood samples for laboratory analyses were drawn after a 12–14 hours overnight fast, twice: at the beginning of the study and after 5 years.

Venepunction (two tubes BD Vacutainer – one SST® II Advance and one with K_2EDTA) was performed after overnight fasting. Serum was separated by centrifugation for 15 minutes at 1500 g, and used for the measurement of glucose, total, HDL- and LDL-cholesterol, triglyceride and CRP concentrations, while the HbA1C concentration was measured in the whole blood samples collected with K_2EDTA. All biochemical parameters were measured immediately after collection. Commercial tests produced by Beckman Coulter® (USA) were used to measure the concentration of glucose (hexokinase/glucose-6-phosphate dehydrogenase (G-6-PDH) method), HbA1c (immuno-inhibition method), cholesterol (method based on the coupled action of cholesterol oxidase (CHO) and peroxidase (POD)), HDL- and LDL-cholesterol (direct homogenous assays) and triglycerides (method utilizing a series of reactions catalyzed by lipase, glycerol kinase (GK), glycerol phosphate oxidase (GPO) and peroxidase (POD)). All the mentioned tests were performed on the analyzer Beckman Coulter AU 400®. For hs-CRP concentrations, commercial immunoassay reagent kits manufactured by Siemens Healthcare® were used and tests were performed on the Behring Nephelometer Analyzer II®.

The levels of analyzed high-sensitivity CRP (hs-CRP) were classified as low (<1 mg/L), intermediate (1 to 3 mg/L) and elevated (>3 mg/L) according to the American Heart Association and Centers for Disease Control (18).

Plasma insulin levels were determined by the radioimmunoassay method (PEG, INEP Insulin RIA kit) according to the standardized WHO reference preparation. Insulin resistance (IR) was assessed by HOMA-IR, which was calculated by the formula: HOMA-IR = insulin (mIU/L) × fasting glucose (mmol/L) / 22.5 (19–22).

Statistical analyses

SPSS 10.0 for Windows v18 (SPSS Inc, Chicago Ill) was used for statistical analysis. Values and the results are expressed as mean ± standard deviation

(SD) and descriptive statistics were calculated to summarize the clinical features of the patients. Normality of variable distribution was assessed using the Kolmogorov-Smirnov test. For testing the difference between groups we used the Student t-test for variables with normal distribution, and for data without normal distribution we used the Mann-Whitney U test. Receiver operating characteristic curves (ROC) were used to determine a cut-off value for hs-CRP that would discriminate patients at risk for PAD development/progression. A forward stepwise multivariate binary logistic regression analysis was conducted to identify independent predictors of PAD progression, and odds ratio (OR) with the 95% confidence interval (CI) was determined. All the tests used were two-tailed and p-values less than 0.05 were considered significant.

Results

The clinical and anthropometric characteristics of type 2 diabetes patients involved in the study are shown in *Table I*. No significant differences were seen among the groups with respect to mean age, duration of diabetes, BMI and presence of diabetic complications (retinopathy, nephropathy and polyneuropathy and coronary artery disease). However, claudication was more prevalent in group A in comparison to group B, as well as in subgroups A1 and A2 in comparison to subgroups B1 and B2 ($p<0.05$). Also, waist circumference was significantly higher in group A compared to group B at the beginning of study (first exam, $p<0.05$), but we failed to find such a difference at the 5-year follow-up (second exam) (data not shown).

As for fasting glucose, HbA1c, lipid parameters, plasma insulin and HOMA IR, their levels were similar in both groups (A and B) at the first, as well as at the second exam after 5 years. Additionally, when we compared these parameters in patients from subgroups A1 (progressors) and A2 (non-progressors), as well as from subgroups B1 (progressors) and B2 (non-progressors), at the first and second exam, we also failed to find any significant differences (data are shown in *Table II*).

Simultaneously, we found significantly higher hs-CRP levels in group A (with PAD) compared to group B (without PAD), but only at the first exam (*Table II*). Among the patients in group A, those with later progression of PAD (subgroup A1) had the high-

Table I Demographic, anthropometric and clinical characteristics of examined patients in Group A (patients with PAD), Group B (patients without PAD), Subgroup A1 (patients with PAD progression), Subgroup A2 (patients without PAD progression), Subgroup B1 (patients who developed PAD), and Subgroup B2 (patients without development of PAD).

Characteristics	Group A	Group B	p	Subgroup A1	Subgroup A2	p	Subgroup B1	Subgroup B2	p
Number	38	42		8	30		22	20	
Age (years)*	64.6±6.2	62.3±6.8	0.359	62.9±4.3	63.8±6.7	0.704	62.2±8.2	62.4±5.0	0.918
Gender (%) (m/f)**	36.8/63.2	42.9/57.1	0.583	62.5/37.5	30.0/70.0	0.095	18.2/81.8	70.0/30.0	0.001
Diabetes duration (years)*	7.8±2.1	7.8±1.8	0.989	8.0±2.3	8.8±2.1	0.787	8.0±1.7	7.6±1.9	0.479
BMI (kg/m^2)*	30.1±4.4	29.1±3.9	0.272	29.6±4.3	30.2±4.4	0.727	29.2±4.2	28.9±3.6	0.835
Waist (cm)*	102.2±12.5	96.6±9.7	0.028	100.9±13.1	102.5±12.5	0.753	94.6±8.9	98.6±10.4	0.184
WHR*	0.95±0.07	0.92±0.08	0.110	0.95±0.07	0.95±0.07	0.956	0.91±0.07	0.94±0.09	0.182
Claudication (%)*	60.5	28.6	0.004	50.0	63.3	0.496	22.7	35.0	0.379
Complications**									
Retinopathy (%)	15.8	4.8	0.945	25.0	13.3	0.442	4.5	5.0	0.095
Nephropathy (%)	10.5	10.5	0.251	12.5	10.0	0.841	4.5	0.0	0.123
Polyneuropathy (%)	21.2	11.9	0.113	12.5	23.3	0.484	4.5	20.0	0.268
CAD (%)**	36.8	38.1	0.908	37.5	36.7	0.965	40.9	35.0	0.694
Hypertension (%)**	78.9	69.6	0.315	87.5	76.7	0.504	68.2	70.1	0.899

m, male; f, female; WHR, Waist to Hip Ratio; CAD, coronary artery disease; *results are shown as mean ± standard deviation (X±SD), for testing we used student t test, data have normal distribution; ** for categorical data we used Pearson chi square test Likelihood ratio for measuring association between parameters and groups.

Table II Comparison of examined biochemical parameters between group A (with PAD) versus B (without PAD), Subgroup A1 (with PAD progression) versus A2 (without PAD progression) and Subgroups B1 (with PAD development) versus B2 (without PAD development) during the first exam and five years later, during the second exam.

Parameters		Fasting glucose (mmol/L)	HbA1c (%)	Total cholesterol (mmol/L)	Triglycerides (mmol/L)	HDL-Ch (mmol/L)	LDL-Ch (mmol/L)	Plasma insulin (mIU/L)	HOMA-IR	hs-CRP (mg/L)
First exam	Group A	8.82±2.99	8.06±1.88	6.40±1.52	2.56±1.48	1.20±0.25	4.05±1.04	21.48±6.91	8.40±3.75	4.09±2.36
	Group B	8.59±2.58	7.58±1.63	6.23±1.51	2.82±2.75	1.20±0.30	4.02±1.21	22.26±10.64	8.74±5.27	3.09±2.21
	p	0.806*	0.229**	0.616**	0.658*	0.900*	0.9651*	0.825*	0.893*	0.034*
Second exam	Group A	9.42±9.20	7.50±1.14	6.20±1.10	2.42±1.16	1.25±0.27	4.06±0.91	20.96±5.72	7.35±2.41	4.51±1.91
	Group B	8.77±2.32	7.72±1.54	6.27±1.40	3.01±2.81	1.27±0.32	4.09±1.25	21.64±8.98	8.69±4.64	3.86±2.27
	p	0.421*	0.473**	0.814**	0.927*	0.927*	0.900*	0.761*	0.236*	0.047*
First exam	Subgroup A1	9.04±2.65	8.34±1.68	6.04±1.12	2.04±0.76	1.19±0.31	3.92±0.85	19.98±6.23	7.71±3.40	4.69±2.69
	Subgroup A2	8.77±3.11	7.98±1.95	6.50±1.62	2.71±0.16	1.20±0.24	4.09±1.09	22.15±7.03	8.58±3.87	3.94±2.28
	p	0.686*	0.462**	0.460**	0.250*	0.901**	0.902*	0.254**	0.802*	0.432**
Second exam	Subgroup A1	9.42±1.28	7.71±1.19	5.94±0.99	2.10±0.72	1.27±0.30	3.98±0.65	19.60±5.03	6.70±2.77	5.04±1.79
	Subgroup A2	9.87±1.33	7.44±1.14	6.27±1.14	2.50±1.25	1.25±0.26	4.08±0.98	21.33±5.2	7.52±2.33	4.37±1.95
	p	0.221*	0.558**	0.464**	0.449*	0.847*	0.787**	0.456**	0.398**	0.374*
First exam	Subgroup B1	8.01±2.49 *	7.55±1.76	6.41±1.66	2.34±1.31	1.27±0.26	4.12±1.36	20.68±8.10	7.77±4.85	4.04±2.56
	Subgroup B2	9.22±2.59	7.61±1.53	6.04±1.35	3.34±3.73	1.13±0.32	3.91±1.05	24.01±12.88	9.80±5.63	2.05±1.06
	p	0.131**	0.922**	0.430**	0.580*	0.091**	0.580**	0.399*	0.215**	0.003**
Second exam	Subgroup B1	8.58±2.67	7.70±1.39	6.32±1.50	2.72±2.21	1.36±0.34	4.07±1.36	21.41±9.26	8.66±5.72	5.16±2.39
	Subgroup B2	8.98±1.91	7.74±1.72	6.21±1.33	3.32±3.39	1.18±0.29	4.10±1.17	21.88±8.89	8.72±3.21	2.43±0.85
	p	0.262*	0.930*	0.798**	0.950*	0.079**	0.953**	0.920*	0.473*	0.000**

HOMA-IR–HOMA insulin resistance index; hs-CRP–high-sensitivity C-reactive protein; Ch–cholesterol, results are shown as mean ± standard deviation (X±SD); for testing the difference between groups and subgroups we used Mann-Whitney U test for data without normal distribution *, and student t test** if data had normal distribution.

est level of hs-CRP at the first exam, although this value was not significantly different from that in patients from subgroup A2 (non-progressors), neither at the first nor at the second exam (data are shown in *Table III*). By contrast, the hs-CRP level was significantly higher in subgroup B1 (progressors) in comparison to subgroup B2 (non-progressors) at both the first and the second exam after 5 years (data are shown in *Table IV*). Also, the majority of patients in subgroups A1, A2, and B1 had elevated hs-CRP levels (>3 mg/L) according to AHA and CDC (20), but the majority of patients in subgroup B2 had intermediate levels (1–3 mg/L) (*Table II*).

In order to establish whether the determination of hs-CRP might be useful for prediction of the existence, development and progression of PAD, patients with PAD at the beginning of the study and those in whom PAD developed or progressed during 5 years (subgroups A1, A2 and B1, n=60) were compared to patients who remained PAD-free during the whole follow-up period (subgroup B2, n=20). The level of hs-CRP was significantly higher in all patients with PAD at the beginning of the study (first exam, $p<0.01$) and also five years later (second exam, $p<0.001$) when compared to those who remained PAD-free (*Figure 1*).

Forward stepwise multivariate logistic regression analysis showed that the only potentially significant predictor of PAD was hs-CRP (HR=1.961, 95% CI=1.295–2.976, p=0.001). In addition, of all the investigated metabolic parameters, hs-CRP was the only independent predictor of PAD development and

Table III Area under the curve of examined parameters in patients with PAD (subgroups A1, A2 and B1, n=60) versus patients without PAD (subgroup B2, n=20).

Variables		AUC		
		AUC	(95% CI)	*p-value*
Fasting glycemia (mmol/L)	First exam	0.401	0.254–0.549	0.188
	Second exam	0.382	0.238–0.525	0.115
HbA1c (%)	First exam	0.530	0.389–0.672	0.685
	Second exam	0.498	0.323–0.672	0.973
Total cholesterol (mmol/L)	First exam	0.546	0.402–0.672	0.537
	Second exam	0.510	0.362–0.658	0.894
Triglycerides (mmol/L)	First exam	0.475	0.313–0.637	0.739
	Second exam	0.509	0.348–0.670	0.903
HDL cholesterol (mmol/L)	First exam	0.643	0.498–0.788	0.057
	Second exam	0.620	0.473–0.767	0.110
LDL cholesterol (mmol/L)	First exam	0.548	0.403–0.694	0.519
	Second exam	0.507	0.358–0.656	0.925
Fasting plasma insulin (mIU/L)	First exam	0.455	0.296–0.613	0.545
	Second exam	0.525	0.370–0.679	0.743
HOMA IR	First exam	0.406	0.260–0.552	0.209
	Second exam	0.396	0.253–0.539	0.167
hs-CRP (mg/L)	First exam	0.780	0.678–0.882	0.000
	Second exam	0.866	0.787–0.945	0.000

AUC – area under the receiver-operating curve; dCI – confidence interval; hs-CRP – high sensitivity C-reactive protein; HOMA-IR – HOMA insulin resistance index.

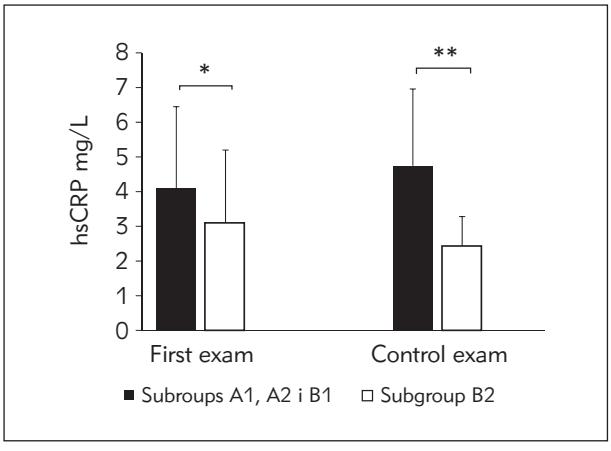

Figure 1 Mean values of hs-CRP at baseline (first exam) and after 5 years of follow-up (second exam) in patients with PAD (subgroups A1, A2 and B1, n=60) and in patients without PAD (subgroup B2, n=20), *p=0.000, ** p=0.000.

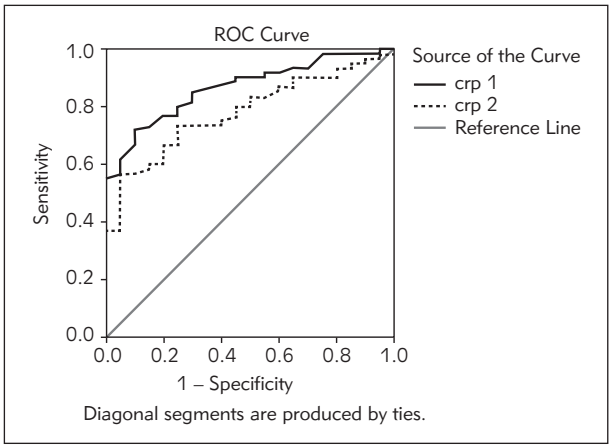

Figure 2 ROC curve for hs-CRP at first exam (CRP 1), AUC=.780, 95% CI .678–.882, p=0.000, and second exam (CRP 2) between all examined patients with PAD (Subgroups A1, A2, B1, n=60) versus patients without PAD (subgroup B2, n=20), AUC=.866, 95% CI .787–.945, p=0.000.

progression in the ROC analysis (AUC=0.780, SE=0.052, 95% CI=0.678–0.882, p=0.000) (*Table III*, *Figure 2*). Moreover, the cut-off point for hs-CRP was 2.5 mg/L, with 75% specificity and 73.3% sensitivity (χ^2=14.76, p<0.001). The cut-off for hs-CRP tested by the Cochran Mantel-Haenszel method (24, 25) was also used for differentiating patients with PAD (subgroups A1, A2, B1) and those without PAD (subgroup B2). Odds Ratio for the obtained cut-off point for hs-CRP was 8.25 (95% CI=2.58–26.39, p<0.000) which indicates that the relative risk for PAD was almost three times higher if the value of hs-CRP was greater than or equal to 2.5 mg/L (RR=2.933, 95% CI 1.35–6.36).

Discussion

Data presented in this 5-year follow-up study showed that among the traditional and non-traditional risk factors investigated (age, BMI, waist circumference, HbA1c, lipids, IR) hs-CRP represents the most important predictive biomarker not only for the presence of PAD, but also for new development and/or progression of existing PAD in patients with type 2 diabetes. Furthermore, our results indicated that the hs-CRP level of 2.5 mg/L might be the discriminative value above which the risk for development and/or progression of PAD in patients with type 2 diabetes increases significantly (approximately three times). Our results are in agreement with the results of several published studies that have pointed out CRP as an independent risk factor for PAD and suggested that patients with increased levels of CRP also have two-fold increased risk for PAD development (25).

It has been previously shown that PAD, as a manifestation of atherosclerotic vascular disease, is more prevalent and tends to be much more severe in patients with type 2 diabetes, but presence of PAD in these patients also significantly increases the risk for ulcers, gangrene and amputation (26). Given that the traditional risk factors such as hyperglycemia, hyperlipidemia and smoking do not fully explain why PAD is more common and has serious clinical consequences in patients with diabetes, a number of studies have investigated the association of novel risk factors and biomarkers with PAD. In recent years, an increasing body of evidence has supported the finding that inflammation could play an important role in the development and progression of atherosclerosis (27), but the association of inflammation and various acute phase proteins, particularly CRP, with PAD has not yet been clarified. Recent findings suggest that CRP, as an inflammatory marker, could be used to assess cardiovascular risk (28). As demonstrated in a comprehensive meta-analysis of 54 prospective cohort studies, hs-CRP was an independent risk factor for future cardiovascular events characterized by a magnitude of effect similar to or larger than that of blood pressure or cholesterol and long-term stability and reproducibility, at least as good as these widely-accepted risk factors (29).

Ridker et al. (30) were the first to indicate a link between CRP and PAD, independent of other risk factors (31, 32). The National Academy of Clinical Biochemistry clearly pointed out that hs-CRP is the only emerging risk marker for clinical use, considering its characteristics and the results of some prominent studies such as JUPITER, PROVE-IT and A to Z (33).

In some of the previously published prospective studies, a major novel finding was that the basal levels of CRP were significantly higher in patients who later, during the follow-up, developed PAD and that CRP was also the strongest predictor for PAD (34). The Edinburgh Artery Study identifies CRP as a predictor of the development and progression of PAD (35) and Van der Meer and his colleagues confirm the importance of hs-CRP in the assessment of PAD progression (36).

Our results have confirmed these findings regarding the presence of increased hs-CRP not only in type 2 diabetes patients with established PAD, but also in the patients who went on to develop PAD. In our study we used ABI as a marker of PAD, and also as a measure of PAD progression in patients with type 2 diabetes. So, the diagnosis of PAD was not based on imaging methods (like arteriography and other visualization techniques) which could influence the results, especially given that almost one third of patients without PAD according to the ABI method (group B) had claudication. However, nowadays, the ABI is widely used in clinical practice to establish the presence of PAD. Also, as a noninvasive measure of systemic atherosclerotic disease, the ABI is a valuable tool for investigating the relationship between novel biomarkers and atherosclerosis, particularly PAD, in a large number of subjects, which was the reason for using this method in our study. A number of investigations used ABI and found a significant inverse relationship between ABI and CRP levels (37, 38), or even a link between CRP, PAD severity (assessed by using the ABI) and future cardiovascular events (26). The added value of our investigation might be the set up of a cut-off point for hs-CRP (2.5 mg/L) as a predictive value for future PAD, with satisfactory sensitivity (73.3%) and specificity (75%). This may be useful in clinical practice, at least in our clinical settings, to identify patients with type 2 diabetes at higher risk for development or progression of PAD.

A possible shortcoming of our study primarily relates to the relatively small number of patients analyzed and the relatively short length of follow-up (5 years). However, patients engaged in our research were carefully chosen from the large number of type 2 diabetes patients treated in our outpatient service who met the strict inclusion criteria. We excluded those with acute inflammatory illness, other acute and chronic diseases and malignancy, smokers, etc,

to avoid possible confounding factors. Moreover, when comparing patients with and without PAD, we failed to find any difference in the level of metabolic control of diabetes, degree of obesity, IR and lipid parameters at the beginning of the study and after 5 years which further emphasizes the importance of hs-CRP as an independent predictive biomarker for future PAD. It has to be noted that future studies should include examinees followed from the time of diagnosis of diabetes in order to determine whether the increased levels of CRP precede the occurrence of PAD or are simply a side effect of the already established PAD. In this regard, we have planned to continue the follow-up of these groups of patients with re-evaluation after a ten-year interval.

In conclusion, our prospective study indicates that patients with type 2 diabetes and elevated levels of hs-CRP have almost 3-fold higher risk for PAD irrespective of the present moderate degree of obesity, hyperglycemia and dyslipidemia. The data presented also suggest that hs-CRP can be used as a reliable predictor for PAD progression in patients with type 2 diabetes.

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Conflict of interest statement

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

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