

Altered fibrin clot properties in patients with premature peripheral artery disease

Agnieszka Okraska-Bylica¹, Tadeusz Wilkosz¹, Lidia Słowik³,
Marta Bazanek¹, Małgorzata Koniecznyńska¹, Anetta Undas²

¹ Department of Diagnostics, John Paul II Hospital, Kraków, Poland

² Institute of Cardiology, Jagiellonian University Medical College, Kraków, Poland

³ Outpatient Clinic of Angiology, Jagiellonian University Medical College, Kraków, Poland

KEY WORDS

ankle brachial index,
D-dimer, fibrin clot,
peripheral artery
disease

ABSTRACT

INTRODUCTION It has been shown that formation of denser and poorly lysable fibrin clots is observed in elderly patients with peripheral artery disease (PAD).

OBJECTIVES The aim of the study was to test the hypothesis that premature PAD is associated with more prothrombotic fibrin clot phenotype.

PATIENTS AND METHODS Ex-vivo plasma fibrin clot permeability, turbidity, and susceptibility to lysis were evaluated in 31 premature PAD patients (median ankle brachial index [ABI], 0.75; interquartile range, 0.5–0.8) aged 55 or less and 32 PAD patients (ABI, 0.66; 0.56–0.76) aged over 55 years. Subjects without PAD matched for age and sex ($n = 40$) served as controls.

RESULTS Premature PAD patients were characterized by 32% lower clot permeability (K_s) ($P < 0.001$), 7% longer clot lysis time ($t_{50\%}$) ($P = 0.004$), and 31% higher maximum D-dimer levels released from fibrin clots ($D-D_{max}$) ($P < 0.001$) compared with controls. These differences remained significant after adjustment for risk factors and medications. None of the fibrin clot parameters differed between premature and older PAD patients. There were correlations between fibrin clot parameters and CRP in premature PAD patients and with ABI in older PAD patients. In a multiple regression model, premature PAD and ABI were independent predictors of K_s , and premature PAD and plasma fibrinogen of the maximum absorbance of a fibrin gel.

CONCLUSIONS Plasma fibrin clots show similarly abnormal prothrombotic phenotype in premature and older PAD patients. However, different factors influence fibrin clot parameters in these patient groups. Premature PAD was an independent predictor of clot permeability and maximum absorbance of a fibrin gel.

Correspondence to:

Agnieszka Okraska-Bylica, MD, PhD,
Krakowski Szpital Specjalistyczny
im. Jana Pawła II, ul. Pędziczna 80,
31-202 Kraków, Poland, phone/
fax: +48-12-614-25-56, e-mail:
aokraska@szpitalip2.krakow.pl

Received: November 13, 2012.

Revision accepted: December 11,
2012.

Published online: December 11, 2012.

Conflict of interest: none declared.

Pol Arch Med Wewn. 2012;

122 (12): 608-615

Copyright by Medycyna Praktyczna,
Kraków 2012

INTRODUCTION Peripheral artery disease (PAD) has a prevalence between 3% and 10% in the general population and is associated with a 6-fold increase in cardiovascular mortality.¹ PAD is strongly associated with advanced age. Patients with early onset of the disease represent only 1% of the PAD population but have a particularly progressive disease with early involvement of carotid and coronary arteries.² Higher risk of failed bypass surgery leading to amputations is reported in patients with premature PAD compared with their older counterparts.³

Despite the fact that traditional atherosclerosis risk factors explain 75% to 95% of PAD cases, they are less common among younger patients.^{4,5} Enhanced inflammation has been implicated in premature PAD. In a cross-sectional community-based study, the odds ratios of PAD in the highest C-reactive protein (CRP) quartile vs. the 3 lower quartiles were larger for patients aged less than 55 years compared with older subjects.⁶ Co-existing coronary artery disease (CAD) and elevated plasma fibrinogen were associated with premature peripheral vascular disease.⁷ It has

been shown that fibrinogen, CRP, serum amyloid A, and D-dimer are higher in PAD patients, and CRP is associated with severity of the disease.⁸ Higher plasma levels of D-dimer, plasminogen, prothrombin fragment 1 + 2, and plasminogen activator inhibitor type 1 (PAI-1) were observed in a small sample of patients with PAD compared with healthy controls.⁹ CRP, fibrinogen, and D-dimer have also been associated with progression of PAD symptoms.¹⁰

Thrombophilia is observed in 15% to 30% of patients with PAD presenting at a young age.^{11,12} High thrombin activatable fibrinolysis inhibitor (TAFI) levels have been reported in this patient group.¹³ The role of these factors in the pathogenesis of PAD remains to be elucidated.

The final step of the blood coagulation cascade is thrombin-mediated conversion of fibrinogen into fibrin and the formation of clots relatively resistant to lysis. Fibrin has been demonstrated to be a consistent component of atherosclerotic plaques involved in their growth.¹⁴ The structure and function of a fibrin clot is influenced by environmental and genetic factors.¹⁵ Fibrin clots composed of tightly packed thin fibers with small pores are relatively resistant to lysis and have been shown in patients with acute or previous myocardial infarction (MI),^{16,17} cryptogenic ischemic stroke,¹⁸ and those with venous thromboembolism (VTE).¹⁹ Moreover, prolonged clot lysis time has been documented in young survivors of a first arterial thrombosis compared with healthy individuals.²⁰

Recently, unfavorably altered fibrin clot properties have been demonstrated in middle-aged and elderly PAD patients.²¹ Lower clot permeability and prolonged clot lysis were associated with progression of the disease during follow-up.²¹ First-degree relatives of PAD patients had similarly altered clot characteristics as the patients.²²

We hypothesized that premature PAD is associated with particularly unfavorable fibrin clot characteristics compared with elderly patients with PAD.

PATIENTS AND METHODS We recruited 31 consecutive patients with premature PAD, defined as age at symptom onset of 55 years or less and 32 patients with PAD aged 56 years or more. PAD was defined as intermittent claudication and the ankle brachial index (ABI) of 0.9 or less measured using the established methods.²³ The exclusion criteria were a history of VTE, recent (<6 months) arterial thrombotic event, cancer, liver cirrhosis, renal failure (serum creatinine >177 $\mu\text{mol/l}$), any acute illness, pregnancy, and anticoagulant therapy. None of the patients took clopidogrel at the time of enrollment. Forty age- and sex-matched volunteers from among outpatients and hospital staff with the ABI values above 0.9 served as controls. CAD was defined as a history of MI, coronary revascularization, or coronary stenosis (>50% of the lumen diameter) on coronary angiography. Carotid artery stenosis was diagnosed

if a significant ($\geq 50\%$) occlusion of the common or internal carotid artery was found based on the NASCET protocol (North American Symptomatic Carotid Endarterectomy Trial).²⁴ Multi-site atherosclerosis was diagnosed if PAD was accompanied by CAD and/or carotid artery stenosis. Diabetes and stroke were diagnosed according to the World Health Organization criteria.

The local Chamber of Physicians' Ethical Committee approved the study, and patients provided written informed consent in accordance with the Declaration of Helsinki.

Laboratory investigations Blood was drawn from an antecubital vein with minimal stasis after an overnight fast between 8 to 10 a.m. Glucose, lipid profile, and creatinine were assayed by routine laboratory techniques. Fibrinogen was determined using the Clauss method and high-sensitivity CRP by immunoturbidimetry (Roche, Mannheim, Germany). The commercially available immunoenzymatic assays were used to determine D-dimer, tissue-type plasminogen activator antigen (tPA:Ag) and PAI-1 antigen (PAI-1:Ag) (all, American Diagnostica, Greenwich, Connecticut, United States). All intra-assay and interassay coefficients of variation were below 7%.

Plasma fibrin clot analysis Plasma fibrin clot variables were determined at least in duplicate by technicians blinded to the origin of the samples (intraassay and interassay coefficients of variation, 5% to 8%, for all variables), as described.¹⁹

1 Fibrin clot permeation using a pressure-driven system, with calculation of a permeation coefficient (K_s), which indicates the pore size. Lower K_s values indicate reduced permeability.

2 The lag phase of the turbidity curve, which reflects the time required for initial protofibril formation and maximum absorbance at 405 nm at the plateau phase (ΔAbs), indicating the number of protofibrils per fiber.

3 Fibrinolysis in the presence of recombinant tPA (rtPA, Boehringer Ingelheim, Ingelheim, Germany) added simultaneously with human thrombin was evaluated. Clot lysis time was defined as the time required for a 50% decrease in clot turbidity at 405 nm ($t_{50\%}$), induced by 1 $\mu\text{g/ml}$ rtPA.

4 Fibrin clots formed as described above were perfused with the same buffer containing 0.2 $\mu\text{mol/l}$ rtPA. The lysis rate was determined by measuring the levels of D-dimer, a marker of plasmin-mediated fibrin degradation, every 20 minutes in the effluent. The maximum rates of an increase in D-dimer levels ($D\text{-D}_{\text{rate}}$), and their maximum values ($D\text{-D}_{\text{max}}$) detected at 80 or 100 minutes were analyzed.

Statistical analysis Continuous variables were expressed as mean \pm standard deviation for normally distributed variables and as median and interquartile range for nonnormally distributed variables. Continuous variables were checked

TABLE 1 Characteristics of patients with peripheral artery disease and control subjects

Variables	PAD ≤55 years n = 31	PAD >55 years n = 32	Controls n = 40	<i>P</i> ¹	<i>P</i> ²
age, y	53 (51–55)	62 (59.5–68)	52.5 (49.5–54)	NS	<0.001
male, n (%)	23 (74.2)	25 (78.1)	28 (70)	NS	NS
ABI	0.75 (0.50–0.80)	0.66 (0.56–0.76)	1.00 (0.96–1.06)	<0.001	NS
current smoking, n (%)	15 (48.4)	16 (50)	6 (15)	0.002	NS
pack-years	34.93 ± 14.79	38.12 ± 12.49	NA	NA	NS
hypertension, n (%)	22 (71)	24 (75)	15 (37.5)	0.002	NS
diabetes, n (%)	10 (32.2)	14 (43.7)	4 (10)	0.004	NS
obesity, n (%)	7 (22.6)	14 (43.7)	7 (17.5)	0.04	0.04
BMI, kg/m ²	26.79 ± 4.32	28.70 ± 2.85	26.89 ± 2.85	NS	NS
MI, n (%)	7 (22.6)	12 (37.5)	0 (0)	<0.001	NS
CAD, n (%)	14 (45.2)	21 (65.6)	0 (0)	<0.001	NS
carotid artery stenosis, n (%)	9 (29)	6 (18.8)	0 (0)	0.002	NS
multisite atherosclerosis, n (%)	16 (51.6)	23 (71.9)	0 (0)	<0.001	NS
medications					
aspirin, n (%)	27 (87.1)	23 (71.9)	3 (7.5)	<0.001	NS
statins, n (%)	25 (80.6)	24 (75)	0 (0)	<0.001	NS
β-blockers, n (%)	13 (41.9)	16 (50)	0 (0)	<0.001	NS
ACEIs, n (%)	15 (48.4)	18 (56.2)	0 (0)	<0.001	NS
sartans, n (%)	5 (16.2)	4 (12.5)	0 (0)	0.04	NS
laboratory parameters					
TC, mmol/l	4.88 ± 1.23	5.17 ± 1.16	5.03 ± 1.23	NS	NS
LDL-C, mmol/l	2.90 ± 1.11	3.22 ± 0.91	2.92 ± 0.90	NS	NS
HDL-C, mmol/l	1.46 ± 0.51	1.24 ± 0.31	1.52 ± 0.51	NS	NS
TG, mmol/l	1.26 (0.95–1.74)	1.60 (1.19–1.91)	1.33 (0.69–1.68)	NS	NS
creatinine, μmol/l	79.09 ± 14.48	81.25 ± 13.69	70.62 ± 16.12	NS	NS
glucose, mmol/l	5.50 (5.10–6.30)	6.15 (5.05–7.55)	4.80 (4.45–5.30)	0.008	NS
CRP, mg/l	2.62 (0.89–4.50)	3.39 (2.19–5.40)	1.38 (0.88–1.98)	0.02	NS
PAI-1:Ag, ng/ml	64.0 (50.4–81.4)	76.35 (59.00–85.90)	8.15 (6.90–9.70)	<0.001	NS
tPA:Ag, ng/ml	11.00 (9.80–12.50)	9.20 (8.00–10.65)	5.80 (4.96–6.95)	<0.001	NS
D-dimer, ng/ml	234.03 ± 75.59	265.34 ± 94.61	186.31 ± 80.67	NS	NS
fibrinogen, g/l	3.54 ± 0.65	4.64 ± 1.20	3.45 ± 0.95	NS	<0.001

Values are given as mean ± SD, median (IQR), or number (percentage).

*P*¹ refers to comparisons between premature PAD and controls; *P*² refers to comparisons between premature and older PAD.

Abbreviations: ABI – ankle brachial index, ACEIs – angiotensin-converting enzyme inhibitors, BMI – body mass index; CRP – C-reactive protein, HDL-C – high-density lipoprotein cholesterol, IQR – interquartile range, LDL-C – low-density lipoprotein cholesterol, MI – myocardial infarction, NA – not available, NS – nonsignificant, PAD – peripheral artery disease, PAI-1:Ag – plasminogen activator inhibitor-1 antigen, SD – standard deviation, TC – total cholesterol, TG – triglycerides, tPA:Ag – tissue-type plasminogen activator antigen

for normal distribution by the Shapiro-Wilk statistic and compared by the analysis of variance when normally distributed or by the Kruskal-Wallis test for nonnormally distributed variables. The post-hoc Sheffé test was used to evaluate precise intergroup statistics. Categorical variables were compared by the χ^2 test or Fisher's exact test as appropriate. The Pearson or Spearman rank correlation coefficients were calculated to test the association between 2 variables with a normal or nonnormal distribution, respectively. A covariance analysis adjusted for potential confounders, including cardiovascular risk factors and medications, was used to evaluate intergroup differences in fibrin clot parameters. To identify

independent factors influencing fibrin clot parameters, we used a multiple regression analysis in which a *P* value of 0.05 or less in a simple regression analysis was used as a criterion for entry into the model. A multiple regression analysis was performed with the manual selection of predictors as well as with backward and forward stepwise regression. A *P* value less than 0.05 was considered statistically significant. All statistical analyses were performed with STATISTICA for Windows 10.0.

RESULTS Premature peripheral artery disease vs. controls The 2 groups did not differ with regard to demographics (TABLE 1). Hypertension,

TABLE 2 Fibrin clot characteristics

Variables	PAD ≤55 years n = 31	PAD >55 years n = 32	Controls ≤55 years n = 40	<i>P</i>	Adjusted <i>P</i>
lag phase, s	40 (37–42)	40 (38–42)	44 (36–47)	NS	NS
ΔAbs, 405 nm	0.89 ± 0.06	0.85 ± 0.21	0.81 ± 0.09	NS	NS
K_s , 10 ⁻⁹ cm ²	5.60 (5.00–6.30)	6.25 (5.05–7.00)	8.25 (6.80–9.30)	<0.001	<0.001
$t_{50\%}$, min	10.30 (9.5–11.1)	10.30 (9.65–10.85)	9.7 (8.6–10)	0.004	0.007
D-D _{max} , mg/l	4.64 (4.25–4.87)	4.41 (4.13–4.83)	3.54 (3.33–3.71)	<0.001	<0.001
D-D _{rate} , mg/l/min	0.068 ± 0.01	0.067 ± 0.005	0.071 ± 0.01	NS	NS

Values are given as mean ± SD or median (IQR).

P refers to comparisons between premature PAD and controls in the post-hoc Sheffé test; values were adjusted for hypertension, diabetes, smoking, multisite atherosclerosis, and medications, including aspirin, β-blockers, ACEIs, and statins

Abbreviations: ΔAbs (405 nm) – maximum absorbance of a fibrin gel at 405 nm determined by using turbidimetry, D-D_{max} – maximum D-dimer levels measured in the lysis assay, D-D_{rate} – maximum rate of increase in D-dimer levels in the lysis assay, K_s – permeability coefficient, lag phase – time required for initial protofibril formation, $t_{50\%}$ – half-lysis time, others – see **TABLE 1**

diabetes, current smoking, and history of MI as well as carotid or coronary stenosis were more frequent in premature PAD patients. Atherosclerotic plaques in the arteries of the lower limbs were found in the iliac or femoral arteries in 25 premature PAD patients (80.6%) and in the distal segments (popliteal artery and below) in 2 premature PAD patients (6.5%). In 4 patients (12.9%), significant occlusions were found both in the proximal and distal segments of the lower limb arteries.

Glucose and CRP were higher in premature PAD group. Plasma PAI-1 was 8-fold higher ($P < 0.001$) and tPA 90% higher in premature PAD ($P < 0.0001$). Plasma D-dimer was similar in both groups (**TABLE 1**). Compared with controls, premature PAD patients had 32% lower clot permeability ($P < 0.001$), 7% longer clot lysis time ($P = 0.004$), and 31% higher maximum D-dimer levels ($P < 0.001$) (**TABLE 2**). Other fibrin clot variables did not differ between patients and controls. After adjustment for risk factors and medications, the intergroup differences in K_s , $t_{50\%}$, and D-D_{max} remained significant.

Younger vs. older patients with peripheral artery disease The groups did not differ with regard to sex and body weight (**TABLE 1**). All PAD patients had a history of smoking but current smokers represented about 50% of the subjects. Fibrinogen was 31% lower in premature PAD patients ($P < 0.001$). There were no differences between premature and older PAD patients in the prevalence of MI, carotid artery stenosis, or multisite atherosclerosis or in the medications used (**TABLE 1**). Twelve premature PAD patients (39%) underwent revascularization procedures in the lower limb arteries.

As shown in **TABLE 2**, all fibrin clot parameters were similar in premature and older PAD patients despite a large intergroup difference in age ($P < 0.001$).

Associations In premature PAD patients, there was a positive correlation between CRP and ΔAbs ($r = 0.72$, $P < 0.001$), an inverse correlation

between CRP and K_s ($r = -0.43$, $P = 0.02$), and no correlations between CRP and the remaining fibrin variables. No associations were observed between clot variables and the body mass index. Interestingly, there were no correlations between the ABI or pack-years and fibrin clot parameters in premature PAD. We observed no differences between patients with multisite atherosclerosis and the remainder, between diabetic and nondiabetic patients, or current vs. former smokers (data not shown). As expected, plasma fibrinogen correlated with ΔAbs ($r = 0.71$, $P < 0.001$), lag phase ($r = -0.41$, $P = 0.03$), K_s ($r = -0.53$, $P = 0.002$), $t_{50\%}$ ($r = 0.45$, $P = 0.01$), and D-D_{rate} ($r = -0.63$, $P < 0.001$) in premature PAD. Age correlated with $t_{50\%}$ ($r = 0.51$, $P = 0.004$).

In older patients with PAD, ABI was strongly positively correlated with K_s ($r = 0.91$, $P < 0.001$) and D-D_{rate} ($r = 0.64$, $P < 0.001$) and negatively with $t_{50\%}$ ($r = -0.66$, $P < 0.001$), ΔAbs ($r = -0.47$, $P = 0.006$), and D-D_{max} ($r = -0.72$, $P < 0.001$). There was a positive correlation between ΔAbs and pack-years in that group ($r = -0.52$, $P = 0.002$). There were no correlations between CRP and fibrin clot parameters in older PAD patients. We observed no differences between patients with multisite atherosclerosis and the remainder, as well as between diabetic and nondiabetic patients in this group (data not shown). In older patients with PAD, current smokers had lower ΔAbs compared with former smokers (0.77 ± 0.27 vs. 0.92 ± 0.06 , $P = 0.045$). In older PAD patients, fibrinogen correlated with ΔAbs ($r = 0.55$, $P < 0.001$), K_s ($r = -0.72$, $P < 0.001$), $t_{50\%}$ ($r = 0.70$, $P < 0.001$), D-D_{max} ($r = 0.78$, $P < 0.001$), and D-D_{rate} ($r = -0.59$, $P < 0.001$).

In all patients with PAD, there were inverse correlations between ABI and $t_{50\%}$ ($r = -0.42$, $P < 0.001$), D-D_{max} ($r = -0.30$, $P = 0.02$), PAI-1 ($r = -0.40$, $P = 0.001$), plasma D-dimer ($r = -0.32$, $P = 0.01$), and fibrinogen ($r = -0.28$; $P = 0.002$), while the ABI was positively correlated with K_s ($r = 0.52$, $P < 0.001$). There was a positive correlation between CRP and ΔAbs ($r = 0.53$, $P < 0.001$) and $t_{50\%}$ ($r = 0.28$, $P = 0.03$) and a negative correlation

TABLE 3 Correlations between fibrin clot parameters and selected variables in 31 patients with premature peripheral artery disease (panel A) and in 32 older patients with peripheral artery disease (panel B)

A

Variables	ABI		Pack-years		CRP	
	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>
$t_{50\%}$, min	−0.22	NS	−0.23	NS	0.30	NS
K_s , 10^{-9} cm ²	0.27	NS	−0.21	NS	−0.43	0.02
lag phase, s	0.08	NS	0.07	NS	−0.30	NS
Δ Abs, 405 nm	−0.08	NS	0.20	NS	0.72	<0.001
D-D _{max} , mg/l	−0.04	NS	−0.18	NS	0.04	NS
D-D _{rate} , mg/l/min	−0.12	NS	−0.08	NS	−0.28	NS

B

Variables	ABI		Pack-years		CRP	
	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>
$t_{50\%}$, min	−0.66	<0.001	−0.30	NS	0.32	NS
K_s , 10^{-9} cm ²	0.91	<0.001	0.15	NS	−0.31	NS
lag phase, s	−0.08	NS	0.02	NS	−0.32	NS
Δ Abs, 405 nm	−0.47	0.006	−0.52	0.002	0.30	NS
D-D _{max} , mg/l	−0.72	<0.001	−0.24	NS	0.21	NS
D-D _{rate} , mg/l/min	0.64	<0.001	0.15	NS	−0.15	NS

Abbreviations: see TABLES 1 and 2

between CRP and the lag phase ($r = -0.26$, $P = 0.04$) and K_s ($r = -0.34$, $P = 0.006$)

The multiple regression model incorporating all 103 patients with the lag phase as dependent variable showed no independent predictors. The multiple regression model with Δ Abs as dependent variable showed premature PAD and plasma fibrinogen as independent predictors (TABLE 4). The multiple regression model with K_s as dependent variable revealed that this fibrin variable was predicted by premature PAD and ABI (TABLE 5). Multisite atherosclerosis was identified as the only independent predictor of $t_{50\%}$ (coefficient beta = -0.65 , $P = 0.03$).

DISCUSSION Our study demonstrates that plasma fibrin clot characteristics are unfavorably altered in premature PAD patients compared with control subjects, and that there are no differences between premature and older PAD patients in plasma fibrin clot parameters, although we had expected a more prothrombotic phenotype in PAD occurring at a relatively young age. However, the lack of differences in plasma clot permeability and lysis associated with the age of PAD patients clearly indicates that the early occurrence of large atherosclerotic burden in the lower extremities is characterized by particularly abnormal clot characteristics unseen at that age, similar to those observed in MI. It is known that fibrin clot parameters tend to aggravate with age in healthy individuals.²⁵ The effect of age on clot phenotype is likely abolished in premature PAD patients by high prevalence of cardiovascular risk factors that have already been proved to negatively affect fibrin clot characteristics, i.e., smoking, diabetes, and arterial hypertension.²

Altered fibrin clot structure was demonstrated in patients with advanced CAD and survivors of MI.¹⁴ A subset of patients with PAD and CAD displayed lower clot permeability, longer clot lysis, and faster polymerization compared with individuals without CAD symptoms.²⁰ We failed to observe such differences likely due to different patient characteristics and a small size of our subsets. One might suspect that PAD is potent enough to abolish additional prothrombotic alterations in fibrin clot phenotype induced by CAD. Likewise, in PAD patients, the coexistence of diabetes did not affect plasma fibrin clot properties although diabetes itself substantially worsens clot phenotype.^{2,14,15}

Similarly, abnormal fibrin clot characteristics were observed in patients with premature CAD.^{17,20} The characteristics of patients with premature CAD were similar to the present premature PAD group with high prevalence of former or current cigarette smokers.¹⁷

Our study confirms the role of systemic inflammation in PAD through negative changes in fibrin clot parameters. We demonstrated that in younger PAD patients, CRP correlated with clot permeability and maximum absorbance of a fibrin gel while ABI did not influence any fibrin clot parameters. In older PAD patients, ABI correlated with most fibrin clot parameters while CRP did not. Those differences are an unexpected and novel observation. It might be speculated that low clot permeability and susceptibility to lysis, mostly driven by elevated PAI-1, enhance the progression of PAD, leading to potent associations of ABI with plasma fibrin properties. At a younger age, systemic inflammation, reflected by CRP, accounts for unfavorable alterations in plasma fibrin clot

TABLE 4 Multiple regression model in 103 patients with Δ Abs as dependent variable

	Coefficient beta	Standard error beta	−95% CI	+ 95% CI	P
premature PAD	0.09	0.04	0.01	0.17	0.03
fibrinogen	0.05	0.01	0.02	0.08	0.002
CRP	0.002	0.002	−0.002	0.005	NS
tPA:Ag	−0.004	0.008	−0.02	0.01	NS
PAI-1:Ag	0.0009	0.0007	−0.0005	0.002	NS
ABI	0.13	0.12	−0.09	0.34	NS

Abbreviations: CI – confidence interval, others – see TABLES 1 and 2.

TABLE 5 Multiple regression model in 103 patients with K_s as dependent variable

	Coefficient beta	Standard error beta	−95% CI	+ 95% CI	P
ABI	2.24	0.96	0.32	4.12	0.02
PAI-1:Ag	−0.01	0.007	−0.02	0.003	NS
CRP	−0.02	0.02	−0.05	0.01	NS
smoking	0.35	0.25	−0.14	0.85	NS
premature PAD	−0.85	0.40	−1.63	−0.06	0.03
fibrinogen	−0.18	0.13	−0.44	0.08	NS
age	−0.03	0.02	−0.08	0.02	NS

Abbreviations: see TABLES 1 and 4

characteristics and this effect weakens with time. It should be noted that the analysis of the whole PAD group showed the effect of both ABI and CRP on clot permeability and lysability, the 2 major variables describing clot phenotype.

Our study provides additional evidence that elevated CRP is linked to atherosclerosis in all arteries, especially in middle-aged individuals, in part through unfavorable fibrin clot phenotype.^{14,16,21} Probably, smoking elevates plasma CRP concentrations, which is associated with pathologic clot properties, promoting thrombotic events.²⁶

Of note, the present study provided evidence that patients with premature PAD share common unfavorable clot features with those with premature CAD.^{17,20} This indicates that abnormal fibrin clot phenotype may characterize all patients with premature atherosclerotic vascular disease, highlighting a major, though as yet poorly described, role of abnormal fibrin formation and degradation in this clinical setting.

There were marked intergroup differences in plasma PAI-1 and tPA levels with mean PAI-1 levels 8-fold higher in premature PAD patients compared with controls. High plasma PAI-1 levels were reported in middle-aged and elderly patients with PAD^{9,21} and in those with premature CAD.¹⁷ Increased PAI-1 contributes to hypofibrinolysis that might result in impaired removal of thrombi from the vessels and stimulate growth of atherosclerotic plaques. Our findings, particularly high PAI-1, characterize subjects with PAD diagnosed below 55 years of age. This finding merits further investigation.

The cutoff point for premature PAD in the current study was set at 55 years of age or younger, which requires some comments. De Bruijne¹³ defined age for premature PAD at 45 years or

younger in men and 55 years and younger in women. Collet et al.¹⁷ recruited patients aged less than 45 years while evaluating premature coronary atherothrombosis. Bartholomew et al.³ stated that premature PAD occurs in individuals aged 50 years or younger. Age less than 55 years at first cardiovascular event in men has been used as the cutoff point for positive family history in the European Society of Cardiology guidelines.²⁷ Given the low incidence of PAD below 45 years, the present approach appears pragmatic.

The current study has several limitations. First, the size of the study population is limited, which may have introduced type II errors, especially in calculations of correlation coefficients. Secondly, our analysis was based on a determination of each variable at a single time point and changes in clot variables with time cannot be excluded. Third, the scanning electron microscopy of fibrin clots has not been performed. However, functional plasma-based assays without clot dehydration may provide more relevant information on fibrin clot alterations. Our experimental approach did not allow to analyze the effect of blood cells and platelets on fibrin clot structure and function. Additional fibrin clot modifiers that have not been investigated in the current study include oxidative stress and proteins released from stimulated platelets.²⁸ Moreover, a potential impact of statins and antihypertensive agents on fibrin clot properties^{2,14,15} has not been determined, especially that therapeutic effects of those interventions in clinical practice could be suboptimal.²⁹ Finally, significant associations do not necessarily mean the cause-effect relationship.

In conclusion, our findings demonstrate that plasma fibrin clots in premature PAD patients are less permeable and are lysed at a reduced rate

compared with those made from plasma of control subjects. Of note, there are no significant differences between premature and older PAD patients in plasma fibrin clot parameters indicating potent effects of large atherosclerotic burden on fibrin clot phenotype regardless of age. This study adds new information on the links between atherosclerosis, inflammation, and coagulation, suggesting a major role of fibrin formation and degradation characteristics in the progression and vascular complications of vascular atherosclerotic disease involving the lower limbs in younger and older patients.

Acknowledgment This work was supported by a grant from Jagiellonian University School of Medicine (K/ZDS/002 936, granted to A.U.).

REFERENCES

- Brevetti G, Giugliano G, Brevetti L, Hiatt WR. Inflammation in peripheral artery disease. *Circulation*. 2010; 122: 1862-1875.
- Undas A. Acquired dysfibrinogenemia in atherosclerotic vascular disease. *Pol Arch Med Wewn*. 2011; 121: 310-319.
- Batholomew JR, Olin JW. Pathophysiology of peripheral arterial disease and risk factors for its development. *Cleve Clin J Med*. 2006; 73: S8-S14.
- Balogopal P, de Ferranti SD, Cook S, et al.; American Heart Association Committee on Atherosclerosis Hypertension and Obesity in Youth of the Council on Cardiovascular Disease in the Young; Council on Nutrition, Physical Activity and Metabolism; Council on Epidemiology and Prevention. Nontraditional risk factors and biomarkers for cardiovascular disease: mechanistic, research, and clinical consideration for youth: a scientific statement from the American Heart Association. *Circulation*. 2011; 123: 2749-2769.
- Selvin E, Erlinger TP. Prevalence of and risk factors for peripheral arterial disease in the United States: results from the national Health and Nutrition Examination Survey, 1999–2000. *Circulation*. 2004; 110: 738-743.
- Wildman RP, Muntner P, Chen J, et al. Relation of inflammation to peripheral arterial disease in the national health and nutrition examination survey, 1999–2002. *Am J Cardiol*. 2005; 96: 1579-1583.
- Lane JS, Vittinghoff E, Lane KT, et al. Risk factors for premature peripheral vascular disease: results for the National Health and Nutritional Survey, 1999–2002. *J Vasc Surg*. 2006; 44: 319-324.
- Unlü Y, Karapolat S, Karaca Y, Kiziltunç A. Comparison of levels of inflammatory markers and hemostatic factors in the patients with and without peripheral arterial disease. *Thromb Res*. 2006; 117: 357-364.
- Mota AP, de Castro Santos ME, Lima e Silva FC, et al. Hypercoagulability markers in patients with peripheral arterial disease: association to ankle-brachial index. *Angiology*. 2009; 60: 529-535.
- Tzoulaki I, Murray GD, Price JF, et al. Hemostatic factors, inflammatory markers, and progressive atherosclerosis: the Edinburgh Artery Study. *Am J Epidemiol*. 2006; 63: 334-341.
- Vig S, Chitolie A, Sleight S, et al. Prevalence and risk of thrombophilia defects in vascular patients. *Eur J Vasc Surg*. 2004; 28: 124-131.
- Cieśla-Dul M, Gawęda B, Wypasek E, et al. Residual iliofemoral thrombosis in a 31-year-old woman on oral contraceptives possessing heterozygous factor V Leiden, factor XIII Val34Leu allele and alpha-fibrinogen Thr312Ala allele: case report. *Pol Arch Med Wewn*. 2009; 119: 822-825.
- de Bruijne EL, Gils A, Rijken DC, et al. High thrombin activatable fibrinolysis inhibitor levels are associated with an increased risk of premature peripheral arterial disease. *Thromb Res*. 2011; 127: 254-258.
- Undas A, Ariëns RA. Fibrin clot structure and function: a role in the pathophysiology of arterial and venous thromboembolic diseases. *Arterioscler Thromb Vasc Biol*. 2011; 31: e88-e99.
- Scott EM, Ariëns RA, Grant PJ. Genetic and environmental determinants of fibrin structure and function. Relevance to clinical disease. *Arterioscler Thromb Vasc Biol*. 2004; 24: 1558-1566.
- Undas A, Szuldrzyński K, Stępień E, et al. Reduced clot permeability and susceptibility to lysis in patients with acute coronary syndrome: effects of inflammation and oxidative stress. *Atherosclerosis*. 2008; 196: 551-558.
- Collet JP, Allali Y, Lesty C, et al. Altered fibrin architecture is associated with hypofibrinolysis and premature coronary atherothrombosis. *Arterioscler Thromb Vasc Biol*. 2006; 26: 2567-2573.
- Undas A, Podolec P, Zawilska K, et al. Altered fibrin clot structure/function as a novel risk factor for cryptogenic ischemic stroke. *Stroke*. 2009; 40: 1499-1501.
- Undas A, Zawilska K, Cieśla-Dul M, et al. Altered fibrin clot structure/function in patients with idiopathic venous thromboembolism and in their relatives. *Blood*. 2009; 114: 4272-4278.
- Guimarães AH, de Bruijne EL, Lisman T, et al. Hypofibrinolysis is a risk factor for arterial thrombosis at young age. *Br J Haematol*. 2009; 145: 115-120.
- Undas A, Nowakowski T, Cieśla-Dul M, al Sadowski J. Abnormal plasma fibrin clot characteristics are associated with worse clinical outcome in patients with peripheral arterial disease and thromboangitis obliterans. *Atherosclerosis*. 2011; 215: 481-486.
- Bhasin N, Ariens RA, West RM, et al. Altered fibrin clot structure and function in the healthy first-degree relatives of subjects with intermittent claudication. *J Vasc Surg*. 2008; 48: 1497-1503.
- Criqui MH, McClelland RL, McDermott MM, et al. The ankle-brachial index and incident cardiovascular events in the MESA (Multi-Ethnic Study of Atherosclerosis). *J Am Coll Cardiol*. 2010; 56: 1506-1512.
- Carpenter JP, Lexa FJ, Davis JT. Determination of duplex Doppler ultrasound criteria appropriate to the North American Symptomatic Carotid Endarterectomy Trial. *Stroke*. 1996; 27: 695-699.
- Mills JD, Ariëns RA, Mansfield MW, Grant PJ. Altered fibrin clot structure in the healthy relatives of patients with premature coronary artery disease. *Circulation*. 2002; 106: 1938-1942.
- Undas A, Topór-Madry R, Tracz W, Pasowicz M. Effect of cigarette smoking on plasma fibrin clot permeability and susceptibility to lysis. *Thromb Haemost*. 2009; 102: 1289-1291.
- Perk J, De Backer G, Gohlke H, et al. European Guidelines on cardiovascular disease prevention in clinical practice (version 2012). The Fifth Joint Task Force of the European Society of Cardiology and Other Societies on Cardiovascular Disease Prevention in Clinical Practice (constituted by representatives of nine societies and by invited experts). Developed with the special contribution of the European Association for Cardiovascular Prevention & Rehabilitation (EACPR). *Eur Heart J*. 2012; 33: 1635-1701.
- Szuldrzyński K, Zalewski J, Machnik A, Zmudka K. Elevated levels of 8-iso-prostaglandin F2alpha in acute coronary syndromes are associated with systemic and local platelet activation. *Pol Arch Med Wewn*. 2010; 120: 19-24.
- Sliz D, Mamcarz A, Filipiak KJ, et al. 3ST-POL trial: standards of statin use in Poland in the context of the European Society of Cardiology guidelines. *Pol Arch Med Wewn*. 2010; 120: 328-333.

Nieprawidłowe właściwości skrzepu fibrynowego u pacjentów z przedwczesną miażdżycą tętnic kończyn dolnych

Agnieszka Okraska-Bylica¹, Tadeusz Wilkosz¹, Lidia Słowik³,
Marta Bazanek¹, Małgorzata Konieczńska¹, Anetta Undas²

¹ Ośrodek Diagnostyki, Krakowski Szpital Specjalistyczny im. Jana Pawła II, Kraków

² Instytut Kardiologii, Uniwersytet Jagielloński, Collegium Medicum, Kraków

³ Poradnia Chorób Naczyń, Uniwersytet Jagielloński, Collegium Medicum, Kraków

SŁOWA KLUCZOWE

D-dimer, miażdżycza
tętnic kończyn, skrzep
fibrynowy, wskaźnik
kostkowo-ramienny

STRESZCZENIE

WPROWADZENIE Wykazano, że u pacjentów w podeszłym wieku z miażdżycą tętnic kończyn dolnych (*peripheral artery disease* – PAD) obserwuje się powstawanie bardziej zbitych i trudniej rozpuszczalnych skrzepów fibrynowych.

CELE Celem pracy było sprawdzenie hipotezy, że u pacjentów z przedwczesną PAD występuje bardziej prozakrzepowy fenotyp skrzepów fibrynowych.

PACJENCI I METODY U 31 pacjentów z PAD (wskaźnik kostkowo-ramienny [*ankle brachial index* – ABI] mediana 0,75; przedział międzykwartylowy 0,50–0,80) w wieku 55 lat lub mniej oraz u 32 pacjentów z PAD (ABI 0,66; 0,56–0,76) w wieku powyżej 55 lat oceniono *ex vivo* przepuszczalność, zmętnienie i podatność na rozpuszczanie osoczowych skrzepów fibrynowych. Dobrani pod względem płci i wieku ochotnicy bez PAD ($n = 40$) stanowili grupę kontrolną.

WYNIKI W porównaniu do grupy kontrolnej u pacjentów z przedwczesną PAD wykazano o 32% mniejszą przepuszczalność skrzepu (K_s) ($p < 0,001$), dłuższy o 7% czas lizy skrzepu ($t_{50\%}$) ($p = 0,004$) oraz większe o 31% stężenie D-dimeru uwalnianego ze skrzepu fibrynowego ($D-D_{max}$) ($p < 0,001$). Powyższe różnice pozostały znamienne statystycznie po uwzględnieniu czynników ryzyka miażdżycy i leków. Żaden z analizowanych parametrów skrzepu fibrynowego nie różnił się między młodszymi i starszymi pacjentami z PAD. Stwierdzono korelacje pomiędzy parametrami skrzepu fibrynowego a CRP u pacjentów z przedwczesną PAD oraz z ABI u starszych pacjentów z PAD. W wieloczynnikowym modelu regresji przedwczesna PAD i ABI niezależnie przewidywały K_s , a przedwczesna PAD i fibrynogen w osoczu maksymalną absorbancję żelu fibrynowego.

WNIOSKI Skrzepy fibrynowe u pacjentów z przedwczesną PAD wykazują podobnie nieprawidłowy, prozakrzepowy fenotyp jak u starszych chorych. Jednak różne czynniki wpływają na parametry skrzepu fibrynowego w tych grupach pacjentów. Przedwczesna PAD była niezależnym predyktorem przepuszczalności skrzepu fibrynowego i maksymalnej absorbancji żelu fibrynowego.

Adres do korespondencji:
dr med. Agnieszka Okraska-Bylica,
Krakowski Szpital Specjalistyczny
im. Jana Pawła II, ul. Piłsudskiego 80,
31-202 Kraków,
tel./fax: 12-614-25-56, e-mail:
aokraska@szpitalip2.krakow.pl
Praca wpłynęła: 13.11.2012.
Przyjęta do druku: 11.12.2012.
Publikacja online: 11.12.2012.
Nie zgłoszono sprzeczności
interesów.
Pol Arch Med Wewn. 2012;
122 (12): 608-615
Copyright by Medycyna Praktyczna,
Kraków 2012