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ORIGINAL ARTICLE

Selected clotting factors in blood of patients with abdominal aortic aneurysms

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Abstract

Background: Tissue factor (TF), tissue factor pathway inhibitor (TFPI) and vascular endothelial growth factor A (VEGF-A) present in vascular structures take part in blood coagulation and in organ revascularisation. The concentration of thrombin–antithrombin complexes (TAT) in blood of patients with abdominal aortic aneurysms (AAA) reflects thrombin-generation.

Aim: To determine the concentration of TF, TFPI, VEGF-A and TAT complexes in blood of patients with AAA and to consider if these factors after clot formation can play a role in the pathogenesis of abdominal aortic aneurysms.

Methods: Forty eight patients (43 men and 5 women) in the age of 59–80 (mean 72) years with AAA were examined. The blood was drawn in the morning to 3.2% natrium citrate in proportion 9:1. The concentration of TF, TFPI, VEGF-A and TAT complexes were measured in plasma with commercial kits using enzyme immunoassay.

Results: In plasma of patients with AAA the mean concentration of TF was elevated almost twice and TAT complexes were three times higher compared with controls. But the mean levels of TFPI and VEGF-A were similar as in control group.

Conclusions: Increased concentrations of TF and TAT complexes indicate on high thrombin-generation, hypercoagulability and formation in abdominal aortic aneurysm of intraluminal thrombus, which can induce proteolytic processes in aortic wall.

Key words: TF, TFPI, VEGF-A, TAT complexes, abdominal aortic aneurysms

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INTRODUCTION

The number of patients with abdominal aortic aneurysm (AAA) is still increasing in the United States and Western Europe and also in Poland. Aortic wall destructive remodelling leading to loss of its elasticity and strength is believed to play a key role in the pathogenesis of AAA. This results from the decrease in amount of elastin as well as collagen metabolism disturbances characterised by increased generation of collagen precursors. Responsible for the degradation of those proteins are matrix metalloproteinases (MMPs) [1–3]. Wassef et al. [4] and Ailawadi et al. [5] reviewed literature on pathogenesis of AAA from the years 2001–2003. Enzymes that are expressed within both normal and aneurysmal human aortic wall include: fibroblast collagenases — MMP-1 and MMP-13, fibroblast elastases — gelatinase-A (MMP-2) and gelatinase-B (MMP-9), and macrophage elastase (MMP-12). In the pathogenesis of aortic aneurysm, the role of tissue inhibitors of metalloproteinases (TIMPs) that suppress the degradation of elastin and collagen (TIMP-1 and TIMP-2) shouldn't be neglected. Proteolytic enzymes of the aortic wall also include: cysteine proteinases (cathepsins S, K, L and H, and carboxyl proteinase — cathepsin D), serine proteinases (plasmin [PL] and tissue [t-PA] and urokinase plasminogen activators [u-PA]). Increased levels of cytokins IL-1β, IL-6, IL-8, IL-10, TNF-α, and INFγ were found in the aortic wall and in the blood of patients with AAA.

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Some phenotypic traits and genetic predispositions (gene polymorphisms) are related with susceptibility for AAA in humans [4, 5]. Pro-matrix metalloproteinases are activated to metalloproteinases by plasmin which is formed by t-PA and u-P-mediated activation of plasminogen. Reilly (1996) and Gacko (2001) also emphasized the role of fibrinolysis in the pathogenesis of aortic aneurysms [6, 7]. Mural thrombi are always found in the lumen of aortic aneurysms, and the blood tests show hypercoaguable state [7, 9, 10]. Some researchers, for example Gacko et al. (1998), Carrell et al. (2006) and Cnotliwy et al. (2007) pointed out an unquestionable role of mural thrombi in proteolytic degeneration of the aortic wall [7–11].

Aortic wall damage or rupture as well as surgical repair of aortic aneurysm may further increase existing hypercoagulable state by releasing tissue factor (TF) from damaged tissues (endothelial cells, vascular myocytes and macrophages). Being a key activator of blood coagulation process, TF promotes thromboembolic complications and — in case of aortic wall damage — the formation of mural thrombus [12, 13]. We found high concentrations of TF and thrombin–antithrombin (TAT) complexes within atherosclerotic plaques of human carotid arteries [14, 15].

Co-acting with TF, vascular-endothelial growth factor A (VEGF-A) stimulates endothelial cell proliferation, activates physiological and pathological angiogenesis, increases vascular permeability, and takes part in inflammatory processes [16]. Tissue factor pathway inhibitor (TFPI) suppresses TF/VIIa complex and Xa factor and thereby reduces thrombin generation and thrombus formation. Therefore it may also inhibit thrombus formation in the lumen of aortic aneurysms [17, 18]. The intensity of TF-induced thrombin generation is reflected by the concentration of TAT complexes in the blood. The above-mentioned clotting factors are present in vascular structures, which damage may lead to release of those factors into the blood.

The aim of our study was to assess the effect of the blood levels of TF, TFPI, VEGF-A and TAT complexes on mural thrombus formation in patients with unruptured AAA.

METHODS

The study included 48 patients with AAA, 43 men and 5 women, aged 59–80 (mean age 72) years. In all patients, medical interview, physical examination and vascular tests were performed. Aortic aneurysms were diagnosed based on ultrasound examination, computed tomography and arteriography. The size of aneurysms detected in the study participants ranged from 3.5–10.0 cm (mean 5.87 ± 1.39 cm), whereas the size of mural thrombi was 1.2–8.0 cm (mean 3.36 ± 1.64 cm). According to examination results, patients were qualified to elective endovascular procedures or open surgery. Of patients included in the study, 22 (46%) had various forms of ischaemic heart disease, 13 (30%) had hypertension, 6 (12.5%) — chronic respiratory insufficiency, 5 (10.4%) — chronic renal failure, and 3 (6.2%) — diabetes. All patients with AAA were current or ex-smokers. The control group consisted of 50 healthy subjects who were qualified for their first blood donation in the Blood Donation Centre (41 men and 9 women aged 20–56 years). In clinically healthy control subjects, medical history was taken and physical examination as well as laboratory tests including blood type identification, blood count, and viral tests were performed. Subject from control group were younger than patients with AAA, but literature data suggest that age doesn't influence the assessed parameters.

Examinations performed

A 5-mL blood samples for laboratory tests were drawn in the morning, after night fasting, from an antecubital vein, to the test tube with 3.2% natrium citrate in proportion 9:1. Plasma was obtained by centrifugation of blood samples at 2500 g for 15 min. Subsequently, 0.2 mL of the plasma to be tested was pipetted into Eppendorf tubes and stored at –70°C until measurements. Blood sampling was approved by Ethic Committee of Provincial Specialist Hospital in Wroclaw. Plasma concentrations of clotting factors were measured with commercial enzyme immunoassay kits: (1) TF — Imubind TF ELISA Kit (American Diagnostica Inc.). This assay detects TF and TF/VIIa complexes. The manufacturer has not specified the normal range but recommends that each laboratory establish its own reference values. (2) TFPI — Imubind Total TFPI ELISA Kit (American Diagnostica Inc.). This assay detects both full-chain and "truncated" TFPI complexes with either TF or factor VIIa. TFPI suppresses both factor Xa and TF/VIIIa complexes. Manufacturer's normal range: 75–120 ng/mL. (3) VEGF-A — Human VEGF-A Biolisa Med. System Diagnostics GmbH. Due to lack of manufacturer's reference range for citrated plasma, each laboratory should establish its own reference values. (4) Thrombin–antithrombin (TAT) complexes — Enzygnost TAT, Behring. Manufacturer's normal range: 1.0–4.1 μg/L (Me 1.5 μg/L).

Statistical analysis

Data are presented as means and standard deviations (SD). When SD was higher than 50% of the mean, median (Me) and lower (Q1) and upper (Q3) quartiles were calculated. Statistical significance of between-group differences was assessed using Student's t-test. Differences were considered statistically significant at $p < 0.05$. Correlations of mural thrombus size with aneurysms diameter and concentrations of TAT complexes and TF were determined by calculating correlation r coefficients and their p-values. Due to non-normal distribution of variables, Spearman's correlation coefficient was used.

Table 1. Blood coagulation parameters in patients with abdominal aortic aneurysm (AAA)

Assessed parameters	AAA (n = 48)	Control group (n = 50)	P
TF [pg/mL]	Me: 211.0; Q1: 149.8; Q3: 261.5	Me: 125; Q1: 78.5; Q3: 186.5	0.003
TFPI [ng/mL]	90 ± 23 (51–174)	84 ± 19 (52–124)	NS
VEGF [pg/mL]	Me: 27.3; Q1: 19.0; Q3: 40.9	Me: 22.0; Q1: 15.5; Q3: 30.6	NS
TAT complexes [ng/mL]	Me: 5.24; Q1: 2.70; Q3: 8.77	Me: 1.39; Q1: 0.69; Q3: 2.21	0.0003

TF — tissue factor; TFPI — tissue factor pathway inhibitor; VEGF-A — vascular endothelial growth factor A; TAT — thrombin–antithrombin complexes; ( ) — dispersion of measured values; Me — median; Q1, Q3 — quartile; n — number of patients; p — statistical significance of between-group comparisons; $p < 0.05$

Table 2. Number of abdominal aortic aneurysm (AAA) patients with normal, decreased or increased levels of coagulation parameters

Studied/analysed parameters (normal range)	Patients with AAA (n = 48)					
	Normal		Decreased		Increased	
TF [pg/mL]; (18–290)*	40	83%	–	–	8	17%
TFPI [ng/mL]; (75–125)**	30	62.5%	13	27.5%	5	10.5%
VEGF-A [pg/mL]; (8.3–44.4)*	39	81.25%	–	–	9	18.75%
TAT [ng/mL]; (1.0–4.1)**	18	37.5%	4	8.3%	26	54.2%

*Our own normal range, **manufacturer's normal range

RESULTS

Table 1 presents measured concentrations of TF, TFPI, VEGF-A and TAT complexes in the blood of patients with AAA compared to those of healthy control subjects. As demonstrated by our study, TF concentration in the blood of patients with AAA was significantly (almost two-fold) higher than in the control group. Conversely, mean blood level of TFPI in AAA patients, that was 90 ± 23 ng/mL, was similar to that in control group and within the manufacturer's normal range. Although VEGF-A concentration was slightly higher in AAA patients than in control subjects, the difference wasn't statistically significant. On the other hand, the concentration of TAT complexes were significantly different between groups ($p < 0.0003$). Although in AAA patients mean values of TF and TAT complexes' concentrations were significantly higher than and TFPI and VEGF-A concentrations were similar to those in the control group, the results were not consistent across all study participants. Table 2 presents the number of patients with normal, decreased or increased levels of assessed parameters.

Our analysis showed strong significant correlation between the size of aortic aneurysms and mural thrombi ($r = 0.706$, $p < 0.0001$). Conversely, there was no statistical correlation between mural thrombi and the concentration of TAT ($r = –0.253$, $p = 0.09$). Any correlations were found neither between aortic aneurysm size and TF concentration ($r = 0.063$, $p = 0.706$) nor between the concentrations of TF and TAT complexes ($r = 0.0589$, $p = 0.7007$).

DISCUSSION

Although parietal thrombi in the lumen of aneurysms and hypercoagulable state are always present in patients with AAA, no research on coagulation system and its potential role in the pathogenesis of aortic aneurysms were conducted for a long time. According to Ross's theory, primary event in the development of atherosclerotic plaque of aortic wall is damage of endothelial and smooth muscle cells by various pathogenetic factors, including high blood pressure, oxygenated LDL, homocysteine, viruses, bacteria, immune or mechanical factors and others [19]. Similar mechanism may be supposed in the development of aortic aneurysms. Aortic wall damage exposes TF, a receptor for plasma factor VII. Subsequently, TF/VIIIa complexes activate coagulation process, which results in intense thrombin generation and increase in TAT complexes concentration. Higher levels of TF and TAT complexes are suggestive of hypercoagulable state and parietal thrombus formation in the lumen of aortic aneurysm. Only a certain proportion of patients had increased values of assessed parameters.

Given the normal range for TF 18–290 pg/mL, only in 8 of 48 AAA patients TF concentrations were higher than normal. In the rest of AAA patients, TF levels, although within the normal range, were markedly higher than in the control group. Thus, our study demonstrated the presence of TF expressed as a concentration in the blood both of AAA patients and healthy controls. Our findings are consistent with the study performed by Hobbs et al. [20], who estimated TF

activity in the blood of patients with ruptured and unruptured AAA.

The presence of TF in blood plasma indicated the extrinsic pathway of blood coagulation, which is initiated by TF. Furthermore, we showed strong correlation between the diameter of aortic aneurysm and the thickness of mural thrombus. Conversely, we haven't found significant correlation between thrombus thickness and the levels of TAT complexes or TF. The majority of TF molecules that are released from arterial wall can be found not only in the plasma but also in the intraluminal thrombi. High activity of TF within mural thrombi located in the lumen of aortic aneurysms was described by Gacko [7] and Cnotliwy et al. [11]. The levels of TAT complexes higher than upper limit of normal (that is 4.1 ng/mL), were observed in 26 patients with markedly increased rate of thrombin generation. In 2004, Jelenska et al. [21] noticed that phenomenon while evaluating increased levels of PF_{1+2}. We found increased levels of TF, TFPI, VEGF and TAT complexes not only in the blood of AAA patients, but also in atherosclerotic plaques of carotid arteries [14, 15]. Steffel et al. [12] demonstrated that age, sex and risk factors for atherosclerosis like hypertension and hyperlipidaemia don't influence TF levels in plasma of atherosclerotic patients. On the other hand, TFPI is the principle inhibitor of early phase of coagulation that suppresses TF/VIIa complex and Xa factor. Furthermore, it modulates endothelial cell proliferation and is considered by many authors as a marker of endothelial dysfunction. Some publications indicate that by inhibiting thrombin generation and thrombus formation, TFPI also prevents postoperative restenosis [17]. Our previous study revealed markedly increased levels of TFPI in the blood of patients with atherosclerotic lesions within carotid arteries and lower extremities, which is consistent with the findings of Radziwon et al. [18]. Conversely, in atherosclerotic plaques of carotid arteries only trace concentration of TFPI (5.0 ± 3.5 ng/mL) was detected [15].

Unfortunately, scant literature data are available on TF and TFPI levels in the blood of patients with AAA. The authors of those papers examined mainly TF and TFPI activity [20, 22], and they evaluated thrombin generation by measuring the levels of prothrombin fragments 1 +2 (PF_{1+2}) [21, 22]. Some papers compared the levels of clotting and fibrinolytic factors in patients with ruptured and unruptured aneurysms, before and after open surgical or endovascular reparative procedures [22, 23]. Though VEGF-A is involved in angiogenesis, we haven't found any publications reporting measurements of its blood levels in AAA patients. It seems that the first research on coagulation and fibrinolytic parameters, particularly TF and TFPI, in patients with AAA was that performed by Hobbs et al. [20] in 2007. In that study, TF, TFPI, t-PA and TAFI blood activities were analysed in 27 patients with AAA during elective (17) and emergency (10) open surgery repair procedures. In patients with ruptured aneurysms, significantly higher levels of assessed parameters were found compared with those with unruptured aneurysms [20]. Like in our study, no between-group differences in TFPI levels were found [20]. In 2002, Adam et al. [22] observed increased fibrinolytic activity in patients with AAA, which resulted from reduced inhibition of fibrinolysis. The authors concluded that measurements of prothrombin fragments 1+2 (PF_{1+2}) concentration and PAI-1 activity in the blood of patients may enable differentiation between ruptured and unruptured aneurysms [22]. In 2008 Skagius et al. [23] also observed higher blood concentrations of t-PA ag and D-dimmers in AAA patients with ruptured *vs*. unruptured aneurysms.

Based on results of our own research and literature data, it seems that aortic wall damage leading to the exposure of TF, which in turn activates coagulation process and aortic mural thrombus formation, may play a role in the activation of fibrinolysis by t-PA and u-PA within the thrombus and the aortic wall, accordingly. Plasmin generated as a result of those processes may then activate pro-matrix metalloproteinases to matrix metalloproteinases which are responsible for the proteolytic degradation of the aortic wall.

CONCLUSIONS

In patients with AAA, markedly elevated levels of TF and TAT are suggestive of hypercoagulable state.

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References

1. White JW, Haas K, Phillips S, Comeruta AJ. Adventitionalelastolysis is a primary event in aneurysm formation. J VascSurg, 1993; 17: 371–380.
2. Dobrin PB, Markvicka R. Failure of elastin or collagen as possible critical connective tissue alterations underlying aneurysmal dilatation. Cardiovasc Surg, 1994; 2: 484–488.
3. Gacko M. Metaloproteinazy macierzy międzykomórkowej. Post Hig Med. Dośw, 1997; 51: 577–589.
4. Wassef M, Baxter BT, Chisholm RL et al. Pathogenesis of abdominal aortic aneurysms: a multidisciplinary research program supported by the National Heart, Lung and Blood Institute. J Vasc Surg, 2001; 34: 730–738.
5. Ailawadi G, Eliason JL, Upchurch GR, Mich AH. Current concepts in the pathogenesis of abdominal aortic aneurysm. J Vasc Surg, 2003; 38: 584–588.
6. Reilly JM. Plasminogen activators in abdominal aortic aneurysm disease. Ann NY Acad Sci, 1996; 800: 151–156.
7. Gacko M. Tkankowy i osoczowy układ hemostatyczny w tętniaku aorty. Postępy Nauk Med, 2001; 2: 30–38.
8. Carmeliet P, Moons L, Lijnen R. Urokinase generated plasmin activates matrix metalloproteinasesduring aneurysm formation. Nat Genet, 1997; 17: 439–444.

9. Gacko M, Głowiński S. Activities of proteases in parietal thrombus of aortic aneurysm. Clin Chim Acta, 1998; 271: 171–177.
10. Carrell TWG, Burnand KG, Booth NA. Intraluminal thrombus enhances proteolysis in abdominal aortic aneurysms. Vascular, 2006; 14: 1–8.
11. Cnotliwy M, Jastrzębska M, Wienicki I, Chełstowski K. Coagulation and fibrinolysis in the abluminal layer of the thrombus within abdominal aortic aneurysm. Acta Angiol, 2007; 13: 114–121.
12. Steffel J, Thomas F, Lüscher MD, Felix C, Tanner MD. Tissue factor in cardiovascular diseases. Circulation, 2006; 113: 73–75.
13. Kotschy M, Kotschy D, Witkiewicz W. Rola czynnika tkankowego w procesie krzepnięcia krwi oraz w powikłaniach zakrzepowych. Kardiol Pol, 2010; 68: 1158–1162.
14. Migdalski A, Jawień A, Kotschy M, Knapik-Bieniek A. Selected haemostatic factors in carotid bifurcation plaque of patients undergoing carotid endarterectomy. Eur J Vasc Endovasc Surg, 2004; 27: 172–177.
15. Migdalski M, Kotschy M, Jawień A. Tissue factor, tissue factor pathway inhibitor and vascular endothelial growth factor-A in carotid atherosclerotic plaques. Eur J Vasc Endovasc Surg, 2005; 30: 41–47.
16. Ferrara N. Vascular endothelial growth factor. basic science and clinical progress. Endocrine Rev, 2004; 25: 581–611.
17. Bajaj MS, Birktoft JJ, Steer SA, Bajaj SP. Structure and biology of tissue factor pathway inhibitor. Thromb Haemost, 2002; 86: 959–972.
18. Radziwon P, Bielawiec M, Kłoczko J et al. Tissue factor pathway inhibitor (TFPI) in patients with occlusive arterial diseases in consideration with risk factors and conservative treatment of the diseases. Angiology, 2001; 7: 43–54.
19. Ross R. The pathogenesis of atherosclerosis, a perspective for the 1990s. Nature, 2003; 362: 801–809.
20. Hobbs SD, Haggart P, Fegan C, Bradbury AW, Adam DJ. The role of tissue factor undergoing open repair of ruptured and nonruptured abdominal aortic aneurysms. J Vasc Surg, 2007; 46: 682–686.
21. Jeleńska MM, Szmidt J, Bojakowski K, Grzela T, Palester-Chlebowczyk M. Compensated activation of coagulation in patients with abdominal aortic aneurysm: effects of heparin treatment to elective surgery. Thromb Haemost, 2004; 92: 997–1002.
22. Adam DJ, Haggart PC, Ludlam CA, Bradbury AW. Haemostatic markers before operation in patients with acutely symptomatic nonruptured and ruptured infrarenal abdominal aortic aneurysms. J Vasc Surg, 2002; 35: 661–665.
23. Skagius E, Siegbahn A, Berggvist D, Henricsson AE. Fibrinolysis in patients with an abdominal aortic aneurysm with special emphasis on rupture and shock. J Thromb Haemost, 2008; 1: 147–150.

Wybrane czynniki krzepnięcia we krwi chorych z tętniakami aorty brzusznej

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Streszczenie

Wstęp: Czynnik tkankowy (TF), jego inhibitor (TFPI) i naczyniowo-śródbłonkowy czynnik wzrostu A (VEGF-A) są obecne w strukturach naczyń krwionośnych i biorą udział w procesie krzepnięcia krwi oraz unaczynieniu narządów. Metodą ilustrującą trombinogenezę jest oznaczanie stężenia kompleksów trombina–antytrombina (TAT).

Cel: Celem pracy była ocena stężenia TF, TFPI, VEGF-A i kompleksów TAT we krwi chorych z tętniakami aorty brzusznej i próba odpowiedzi na pytanie, czy czynniki te, odpowiedzialne za wytworzenie przyściennego zakrzepu, mogą uczestniczyć w patogenezie tętniaków aorty.

Metody: Badaniem objęto 48 pacjentów z tętniakami aorty brzusznej, w tym 43 mężczyzn i 5 kobiet w wieku 59–80 (śr. 72) lat. Krew do badań pobierano z żyły łokciowej do 3,2-procentowego roztworu cytrynianu sodu w proporcji 9:1. W osoczu krwi oznaczano stężenia TF, TFPI, VEGF-A i kompleksów TAT metodami immunoenzymatycznymi przy użyciu komercyjnych zestawów.

Wyniki: W osoczu chorych z tętniakami aorty brzusznej stwierdzono prawie 2-krotnie wyższe średnie stężenia TF i ok. 3-krotnie większe stężenia kompleksów TAT w porównaniu z grupą kontrolną. Natomiast średnie wartości TFPI i VEGF-A były podobne jak w grupie kontrolnej.

Wnioski: Podwyższone stężenia TF i kompleksów TAT świadczą o zwiększonej generacji trombiny, nadkrzepliwości krwi i wytworzeniu w obrębie tętniaków aorty brzusznej przyściennych zakrzepów, prawdopodobnie wywołujących procesy proteolityczne w ścianie aorty.

Słowa kluczowe: TF, TFPI, VEGF-A, kompleksy TAT, tętniaki aorty brzusznej

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