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MINIREVIEW

Plasma Tissue Factor in Coronary Artery Disease: Further Step to the Understanding of the Basic Mechanisms of Coronary Artery Thrombosis

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Summary

Tissue factor is a cell surface protein that is expressed constitutively by monocytes, macrophages and fibroblasts, but also by some other cells in response to a variety of stimuli. The main function of the tissue factor is to form a complex with factor VII/VIIa that converts factors IX and X to their active forms. Tissue factor is also involved in the pathophysiology of systemic inflammatory disorders, coagulopathies, atherosclerotic disease, tumor angiogenesis and metastasis. Increased tissue factor expression either locally in the coronary plaques or systematically on circulating blood elements of patients with acute coronary syndromes may be responsible for increased thrombin generation, thus leading to platelet activation and fibrin formation. Tissue factor therefore plays a pivotal role in the initiation of thrombotic complications in patients with coronary artery disease.

Key words

Coronary artery disease • Coronary thrombosis • Tissue factor

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Tissue factor and coagulation

Tissue factor is a cell surface protein that is expressed constitutively by monocytes, macrophages and fibroblasts (Mackman *et al.* 1991, Taubman *et al.* 1993, Kamimura *et al.* 2004, Penn *et al.* 1999). Some authors also describe tissue factor expression by cardiomyocytes (Mumford and McVey 2004). Tissue factor forms a complex with factor VII/VIIa that converts factors IX and X to their active forms. The blood borne tissue factor has been discovered recently. However, TF may also be expressed in some other cell types in response to a variety of stimuli. The initiation phase of thrombus generation begins when the disruption of vessel wall or atherosclerotic plaques exposes tissue factor to circulating factor VII, thus leading to the activation of both the intrinsic and extrinsic blood coagulation cascades (Rosenberg and Aird 1999). The tissue factor also induces cell signaling by stimulating G-protein-coupled protease activated receptors (PARs). Signaling pathways initiated by both TF/VIIa complex protease activation of PARs and phosphorylation of the tissue factor cytoplasmic domain appear to regulate different cellular functions (Rao and Pendurthi 2005). In normal arteries, tissue factor expression is limited to the adventitia except for sporadic expression in the media (Wilcoxon *et al.* 1989). A small quantity of circulating tissue factor is present in both the whole blood and serum of healthy individuals (Giesen and Nemerson 2000). The tissue factor is also involved in the pathophysiology of systemic inflammatory disorders, coagulopathies, atherosclerotic disease, chronic renal failure (Zemanová *et al.* 2003), tumor angiogenesis and metastasis (Price *et al.* 2004). There is a significant positive correlation between tissue factor level and serum creatinine, glycemia, LDL cholesterol, number of cigarettes smoked per day (Sambola *et al.* 2003) and an inverse correlation

between tissue factor level and serum insulin (Zemanová *et al.* 2003).

Tissue factor in coronary artery disease

Inappropriate expression of the tissue factor may result in thrombosis contributing to acute clinical consequences of coronary artery disease. Experimental data show that atheromatous plaques contain a high concentration of tissue factor relative to surrounding tissue (Marmur *et al.* 1996). Abundant tissue factor is also found in atheromatous lesions within foamy macrophages in macrovascular disease in humans such as aortic aneurysms and carotid arteries (Wilcoxon *et al.* 1989). Ruptures of atheromatous plaques (usually multiple) expose tissue factor to circulating factor VII causing the initiation of a clot formation and may consequently lead to an abrupt vessel closure and myocardial infarction. This can be demonstrated by the examination of tissue samples obtained from patients with acute coronary syndromes, where higher levels of tissue factor are present in these lesions. In this way, the tissue factor may play not only a role in the development of acute coronary syndromes but also in the progression of coronary artery disease (Annex *et al.* 1995, Ardissino *et al.* 1997, Mann and Davies 1999, Westmuckett *et al.* 2000) *via* the tissue factor-dependent intramural fibrin deposition after plaque rupture and subsequent progression of the atherosclerotic lesion (Westmuckett *et al.* 2000). Modified ELISA (Westmuckett *et al.* 2000) and a rabbit polyclonal antibody against the solubilized tissue factor activity (Thiruvikraman *et al.* 1996) were used for *in situ* localization of the tissue factor in human atherosclerotic plaques. Quantitation of tissue factor antigen was done to test the hypothesis that thrombin generation takes place directly in the atherosclerotic lesion (Thiruvikraman *et al.* 1996). This findings support the possible role of the tissue factor-dependent coagulation pathway in the intramural fibrin deposition and the progression of the atherosclerotic lesions.

Detached endothelial cells as well as microparticles from endothelial cell monolayers express tissue factor activity and this activity is markedly inhibited by microparticle-associated tissue factor pathway inhibitor (Kushak *et al.* 2005).

Apart from the role, which the tissue factor has in the acute coronary syndromes, tissue factor expression has a potential role in vascular remodeling after coronary angioplasty. Cell culture studies have demonstrated that the TF/VIIa complex is critical for smooth muscle cell migration (Siegbahn *et al.* 2000). Furthermore, TF/VIIa-mediated smooth muscle cell migration can be inhibited by the overexpression of the tissue factor pathway inhibitor. The links between lipoproteins and tissue factor expression, as well as tissue factor and vascular remodeling (Singh *et al.* 2001) suggest a potential mechanism for the surprisingly early benefits of high-dose statin therapy in patients with acute coronary syndrome. Alternatively, statin therapy could be beneficial by decreasing CRP levels in these patients, because C-reactive protein has been shown to induce tissue factor expression in monocytes (Cermak *et al.* 1993).

Heparin treatment is associated with a decrease in tissue factor plasma levels and monocyte procoagulant activity (Gori *et al.* 1999) as well as with increased plasma level of the tissue factor pathway inhibitor.

Arnaud *et al.* (2000) investigated whether individual differences in tissue factor gene expression could predispose subjects to thrombosis. They sequenced the 5' domain of the gene and found six different polymorphisms. Genotyping of patients with myocardial infarction in a case-control study involving 2354 subjects showed no association between the polymorphisms and nonfatal coronary thrombosis (Arnaud *et al.* 2000).

Many studies demonstrated elevated tissue factor plasma levels in patients with myocardial infarction (Saigo *et al.* 2001), stable angina (Kim *et al.* 2000), unstable angina and an increased risk for unfavorable outcomes in patients with unstable angina and raised tissue factor levels. Other authors found only a non-significant trend to raised plasma levels of tissue factor in patients with acute myocardial infarction and unstable angina pectoris as compared to patients with stable coronary artery disease and normal subjects (Malý *et al.* 2003). Tissue factor could be useful for the evaluation of the effect of cardiovascular risk intervention, but results in this field are still controversial (Lim *et al.* 2004). Can tissue factor also be used as a prognostic marker in patients with cardiovascular disease? Seljeflot *et al.* (2003) followed patients after myocardial infarction for 4 years. Patients, who had suffered an endpoint event (reinfarction or stroke), had significantly higher tissue factor levels as compared to those who did not. Other authors (Sambola *et al.* 2003, Lim *et al.* 2004) reported higher plasma levels of tissue factor in patients with diabetes than in control subjects. They suggest that higher levels of tissue factor may be the mechanism responsible

for the increased thrombotic complications associated with the presence of other cardiovascular risk factors. No significant differences were found between diabetics and non-diabetics in patients with chronic renal failure (Zemanová *et al.* 2003).

Diagnostic tests for plasma tissue factor

The plasma level of tissue factor may be determined by means of the commercially available Tissue Factor ELISA Kit. The kit employs a murine anti-human tissue factor monoclonal antibody for antigen capture. Plasma samples are incubated in microtest wells precoated with capture antibody. Once captured, the tissue factor is detected using a biotinylated antibody fragment that specifically recognizes the bound tissue factor. The subsequent binding of horseradish peroxidase conjugated streptavidin completes the formation of the antibody-enzyme detection complex. Quantitative data are obtained by measuring the solution absorbance at 450 nm and relating it to the standard curve. The detection limit of this assay is about 10 pg/ml.

Tissue factor may circulate in the blood incorporated in pro-coagulant microparticles shedded as membrane vesicles (Mallat *et al.* 1999). Moreover, a form of human tissue factor generated by alternative splicing has been identified (Bogdanov *et al.* 2003). Alternatively spliced human tissue factor is soluble, circulates in the blood, exhibits pro-coagulant activity when exposed to phospholipids, and is incorporated into thrombi and thus may contribute to thrombus growth (Bogdanov *et al.* 2003). Plasma tissue factor activity not associated with cells or microparticles has also been studied. Tissue factor-dependent generation of factor Xa on cryosections was used to assess the functional activity of the tissue factor (Thiruvikraman *et al.* 1996).

To employ tissue factor as a useful marker of atherothrombosis, a very sensitive diagnostic test for its detection and quantitation is needed. High-affinity antibodies are employed in the improved version of ELISA diagnostic tests for tissue factor plasma level assessment (Chen *et al.* 2005). The new assays provide higher sensitivity with much lower detection limit than currently available diagnostic sets. Data obtained by these assays also show markedly reduced individual variation. Higher sensitivity in tissue factor detection is of critical value especially in the group of patients with normal or slightly elevated tissue factor levels and opens a new field for research. It is also very important to detect accurately also low levels of tissue factor in order to use tissue factor as a potentially useful tool for risk stratification in patients with cardiovascular disease.

However, the question how to interpret an elevated tissue factor levels still remains unanswered. Additional investigations will be necessary for better acceptance of the tissue factor as an independent biomarker for cardiovascular risk that we can incorporate into decision-making algorithms. We should not ignore the limited specificity with elevated tissue factor levels in a variety of different clinical situations. Larger scale clinical studies should confirm the independent significance of the tissue factor.

Conflict of Interest

There is no conflict of interest.

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Time-Course of Tissue Factor Plasma Level in Patients with Acute Coronary Syndrome

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Summary

Enhanced expression of tissue factor (TF) may result in thrombosis contributing to acute clinical consequences of coronary artery disease. Several studies demonstrated elevated plasma levels of TF in patients with acute coronary syndrome (ACS). The aim of our study was to compare the concentrations of TF in coronary sinus (CS), proximal part of the left coronary artery (LCA) and peripheral vein (PV) of patients with ACS and stable coronary artery disease (SCAD). Time course of the TF plasma levels in PV was followed on day 1 and day 7 after index event of ACS presentation and was compared to day 0 values. No heparin was given prior to the blood sampling. Twenty-nine patients in the ACS group (age 63.6±10.8 years, 20 males, 9 females) and 24 patients with SCAD (age 62.3±8.1 years, 21 males, 3 females) were examined. TF plasma level was significantly higher in patients with ACS than in those with SCAD (239.0±99.3 ng/ml vs. 164.3±114.2 ng/ml; p=0.016). There was no difference in TF plasma levels in PV, CS and LCA (239.0± 99.3 ng/ml vs. 253.7±131.5 ng/ml vs. 250.6±116.4 ng/ml, respectively). TF plasma levels tended to decrease only non-significantly on the day 7 (224.4±109.8 ng/ml). Significant linear correlation between TF and high sensitivity CRP (hs-CRP) levels on day 0 was found. In conclusion, TF plasma levels are elevated in patients with ACS not only locally in CS but also in systematic circulation. Our data support the relationship between TF production and proinflammatory mediators.

Key words

Coronary artery disease • Coronary thrombosis • Tissue factor

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Introduction

Excessive expression of tissue factor (TF) may result in thrombosis contributing to acute clinical consequences of coronary artery disease. Several studies demonstrated elevated plasma levels of TF in patients with acute coronary syndrome (ACS) (Saigo *et al.* 2001, Kim *et al.* 2000, Seljeflot *et al.* 2003). The aim of our study was to compare the concentrations of TF in coronary sinus (CS), proximal part of the left coronary artery (LCA) and peripheral vein (PV) of patients with ACS and stable coronary artery disease (SCAD) and to assess its relations to the traditional biomarker of inflammation – hs-CRP levels (Nomoto *et al.* 2004).

Patients and Methods

Patients referred for coronary angiography were selected for the study and they were included into one of the two study groups. The first group comprised patients with STE and non-STE ACS. Patients with stable coronary artery disease (SCAD), mainly stable angina pectoris class II - III CCS were included into the second group. Subjects with cardiogenic shock, malignant arrhythmias and inflammatory diseases were excluded from the study.

Twenty-nine patients in the ACS group (age 63.6±10.8 years, 20 males, 9 females) and 24 patients in

the SCAD group (age 62.3±8.1 years, 21 males, 3 females) were examined. Characteristics of both groups are given in Table 1. Informed consent was signed by all patients before the study. The study was approved by the local hospital Ethical Committee.

Table 1. Demographic data for the studied cohorts of patients.

	ACS		SCAD	
	n	***%***	**n**	***%***
N	29		24	
- males	20	69	21	88
- females	9	31	3	12
Age (years, mean ± SD)	63.6±10.8		62.3±8.1	
Risk factors				
- positive family history	4	14	6	25
- BMI > 25	22	76	22	92
- arterial hypertension	18	62	16	67
- hyperlipoproteinemia	19	66	23	96
- diabetes mellitus	10	34	10	42
Insulin dependent	2	7	2	8
- smoking	2	7	6	25
Previous history of CAD	10	34	13	54
Acute coronary syndrome				
- STEMI	13	45		
- NSTEMI/UAP	16	55		
LV EF (% ± SD)	50.9±10.0		51.8±14.5	
Medical treatment:				
- ASA	26	90	24	100
- ASA>7D	9	31	24	100
- thienopyridines	8	28	7	29
- β-blockers	21	72	24	100
- ACEI/ATII.bl.	11	38	20	83
- Ca-channel blockers	6	21	5	21
- diuretics	8	28	16	67
- statins	10	34	22	92
- statins>7D	11	38	21	88

ACS – patients with acute coronary syndrome. SCAD – patients with stable coronary artery disease. BMI=body mass index. CAD – coronary artery disease. STEMI – acute myocardial infarction with ST segment elevations. NSTEMI – acute myocardial infarction without ST segment elevations. UAP – unstable angina pectoris. LV EF – left ventricular ejection fraction. ACEI – angiotensin converting enzyme inhibitors. ATII.bl – angiotensin II receptor blockers. ASA – acetylsalicylic acid. ASA>7D – chronic treatment of acetylsalicylic acid. Statins>7D – chronic statin treatment.

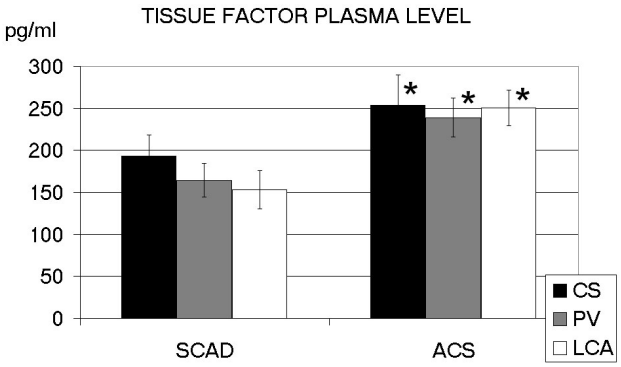

Fig 1. TF plasma levels in patients with ACS and SCAD in coronary sinus (CS), ostium of the left coronary artery (LCA) and peripheral vein (PV). * p<0.05

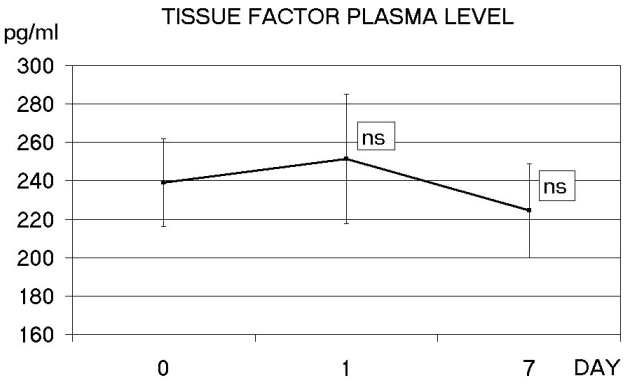

Fig 2. Time course of TF plasma levels in peripheral vein in patients in with ACS.

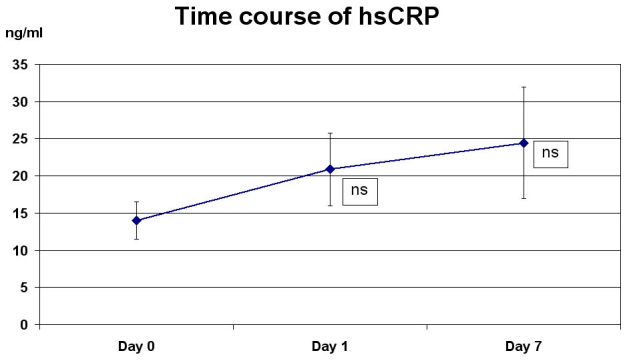

Fig 3. Individual values of TF plasma levels in patients with ACS on day 0, 1 and 7.

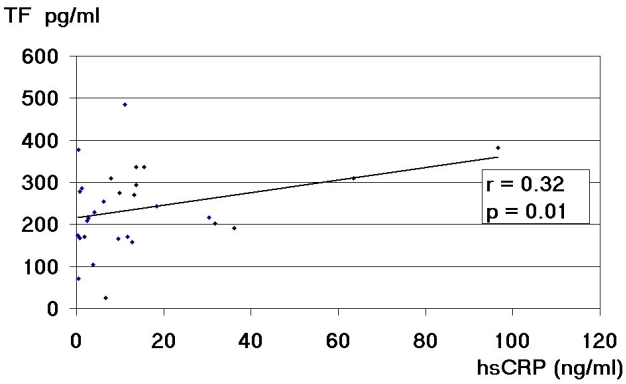

Fig 4. Correlation between hs-CRP and TF plasma levels.

Tissue factor, tissue factor pathway inhibitor (TFPI) and high sensitivity C-reactive protein (hs-CRP) plasma levels were measured in the blood drawn from CS, LCA and PV of patients with ACS and SCAD. Time course of the TF, TFPI and hs-CRP peripheral blood plasma levels was followed on day 1 and day 7 after the first measurement and was compared to the day 0 values. Basic clinical data, resting ECG and biochemistry specimens were obtained in all patients.

Blood samples for TF, TFPI and hs-CRP measurements were drawn in fasting state (apart from several patients with an acute myocardial infarction) at the beginning of the heart catheterization. No heparin was given at least 24 h prior to the blood sampling. Mild sedation was introduced using 10 mg of diazepam and 4 mg of bisulepin. Amplatz right coronary catheter 5F or 6F (Cordis®) was inserted under fluoroscopic control from right femoral vein into the coronary sinus and blood samples were withdrawn. Venous blood sampling from femoral vein was then carried out. Finally, left diagnostic 6F Judkins coronary catheter was positioned into the ostium of the left coronary artery and blood samples were obtained. Blood samples were placed into the ethylenediamine tetra-acetic acid (EDTA) tubes.

The plasma levels of TF, TFPI and hs-CRP were measured using commercially available kits.

Tissue factor and tissue factor pathway inhibitor

For TF and TFPI plasma level measurement, blood sample has been collected into 3.8 % trisodium citrate solution in the proportion of 9 volumes of blood to 1 volume of anticoagulant solution. The sample has been centrifuged at 3.000 rpm for 10 min and stored frozen until the measurement. The plasma has been thawed at 37 ^{o}C for 15 min before the final measurement.

Quantitation of TF was performed by ELISA, employing a murine anti-human TF monoclonal antibody for antigen capture (IMUBIND® Tissue Factor ELISA Kit, American Diagnostica, Inc.). TF levels were determined by measuring solution absorbances at 450 nm and comparing the values with those of standard curve.

Quantitation of TFPI was performed by „sandwich" ELISA employing a rabbit anti-human tissue factor pathway inhibitor polyclonal antibody (IMUBIND® Total TFPI Elisa Kit, American Diagnostica, Inc.). TFPI was detected using a monoclonal

antibody specific for Kunitz domain 1 of TFPI. TFPI levels were determined by measuring sample solution absorbance at 450 nm and comparing them against those of standard curve.

High-sensitivity CRP

High-sensitivity CRP was measured by chemiluminescent immunometric assay (DPC, USA). Samples were stored frozen at –40 ^{o}C. Repeated thawing and freezing of samples was avoided.

Statistical analysis

Statistical evaluation included the analysis of variance and Student's t test for the assessment of differences of continuous variables. Correlations were analyzed using linear regression. $P<0.05$ value was considered significant.

Results

TF plasma level was significantly higher in patients with ACS than in those with SCAD (239.0±99.3 ng/ml vs. 164.3±114.2 ng/ml; p=0.016). There was no difference in TF plasma levels in PV, CS and LCA (239.0±99.3 ng/ml vs. 253.7±131.5 ng/ml vs 250.6±116.4 ng/ml, respectively) (Fig. 1). TF plasma levels tended to decrease on the day 7 (224.4±109.8 ng/ml) (Figs 2 and 3). There were no differences between plasma TF levels neither in acute myocardial infarction nor in NAP. hs-CRP levels were also significantly higher in ACS group than in SCAD (14.7±20.9 ng/ml vs. 3.99±7.05 ng/ml; p=0.013) and tended to increase on day 1 (20.9±26.6 ng/ml) and day 7 (24.4±42.7 ng/ml). Significant linear correlation between TF and hs-CRP levels on day 0 was found (Fig. 4). There were no differences in TFPI plasma levels between patients with ACS and SCAD.

Discussion

Recent years have brought a gradual change in the understanding of the origin of acute coronary syndrome. The traditional interpretation of unstable forms of coronary disease as a cascade: unstable coronary plaque – unstable coronary artery – unstable patient, was proposed originally by Ambrose *et al.* (1986) and later generally accepted, resulted in an intensive search for a definite "culprit" lesion. It has been shown, however, that the stenosis responsible for an acute thrombotic occlusion of the artery need not always be the critical one, and that its histological characteristics are specific. Sophisticated methods have been developed with time to identify the plaques likely to cause an acute occlusion of coronary arteries. Yet, preventive sealing of such lesion by coronary angioplasty does not necessarily lead to the stabilization of the patient. Moreover, it has been documented that unstable coronary plaques are mostly multifocal (Nissen *et al.* 2001, Asakura *et al.* 2001, Goldstein *et al.* 2000, Rioufol *et al.* 2002) and that the inflammatory process in coronary circulation is widespread (Spagnoli *et al.* 2002, Buffon *et al.* 2002). The question therefore arises, whether the cellular and enzymatic interactions in the circulating blood – rather than local plaque rupture – are responsible for triggering the thrombotic process in the coronary circulation. TF is one of the key players in the initiation of thrombus formation in patients with acute coronary syndromes.

TF is a transmembrane lipoprotein responsible for the initiation of the coagulation cascade and it serves also as a signaling receptor. TF has a short cytoplasmatic and transmembrane domains and long extracellular domain. The latter forms with factor VII/VIIa a complex that directly converts factor X and also factor IX to their active forms. TF is expressed constitutively by monocytes, macrophages and fibroblasts. TF, however, may be expressed also in some other cell types in response to variety of stimuli. Thus smooth muscle cells have been shown to produce TF-positive microparticles after growth factor stimulation (Schecter *et al.* 1997). A binding of CD40 ligand also induced TF expression by smooth muscle cells *in vitro* (Schönbeck *et al.* 2000).

In normal arteries, TF expression is limited to the adventitia except for sporadic expression in the media (Wilcox *et al.* 1989). A small quantity of circulating TF is present in both whole blood and serum of healthy individuals (Giesen *et al.* 2000).

Apoptotic cells and microparticles in the atherosclerotic plaque have high procoagulant activity, which corresponds to the TF activity (Mallat *et al.* 1999). Moreover, activated cells shed fragments of their membrane into the extracellular space and these microparticles posses cytoplasmatic and cell membrane components with procoagulant activity due to the TF presence. Microparticle-bound TF originates from monocytes, polymorphonuclear cells, lymphocytes, platelets and endothelial and smooth muscle cells (Doshi *et al*. 2002).

Recently, the importance of blood-borne TF has been shown. TF antigen and activity was found in the whole blood and plasma samples in different subsets of patients (Doshi *et al*. 2002). Neutrophils and monocytes were identified by immunostaining as the main source of circulating TF. Recent data also indicate that circulating TF may be more important for initiation and propagation of the thrombus formation than TF originating from the vessel wall. Platelet-derived microvesicles are the prevalent location of the plasma TF. This was confirmed by the presence of full-length TF in the microvesicles acutely shedded from activated platelets. Blood TF activity is thus dependent on the microvesicle and activated platelet TF and enables the entire coagulation system to proceed on a restricted cell surface (Müller *et al.* 2003). These platelet-derived microvesicles possibly transfer platelet-released TF to monocytes (Scholz *et al.* 2002). Chou *et al.* (2004) and Niemetz (2005) showed that blood-borne TF associated with hematopoietic cell-derived microparticles contributes considerably to the thrombus formation.

Excessive expression of TF may initiate the thrombotic process contributing to acute clinical consequences of coronary artery disease. Experimental data show that atheromatous plaques contain a high concentration of TF relative to surrounding tissue (Marmur *et al.* 1996). Abundant TF was also found in atheromatous lesions within foamy macrophages in macrovascular disease of the aorta and carotid arteries (Wilcox *et al.* 1989). Modified ELISA (Westmuckett *et al.* 2000) and a rabbit polyclonal antibody against the solubilized TF antigen (Thiruvikraman *et al.* 1996) were used for *in situ* localization of TF in human atherosclerotic plaques. Quantitation of TF antigen was done to test the hypothesis that thrombin generation takes place directly in the atherosclerotic lesion (Thiruvikraman *et al.* 1996).

Several studies demonstrated elevated TF plasma levels in patients with myocardial infarction (Saigo *et al.* 2001, Seljeflot *et al.* 2003), stable angina (Kim *et al.* 2000), unstable angina as well as an increased risk for unfavorable outcomes in patients with unstable angina and raised TF levels (Soejima *et al.* 1999). Other authors found only an insignificant trend to raised plasma levels of TF in patients with acute myocardial infarction and unstable angina pectoris as compared to patients with stable coronary artery disease and normal subjects (Malý *et al.* 2003). TF could be useful for the evaluation of the effect of cardiovascular risk intervention, but results in this field are still controversial (Lim *et al.* 2004). Some authors (Lim *et al.* 2004, Sambola *et al.* 2003) reported higher plasma levels of TF in patients with diabetes than in control subjects. They suggest that higher levels of TF may be the mechanism responsible for the increased thrombotic complications associated with the presence of other cardiovascular risk factors. No significant differences were found between diabetics and non-diabetics in patients with chronic renal failure (Zemanová *et al.* 2003). High levels of serum TF were not independently associated with an increased risk of future coronary artery disease in healthy individuals (Keller *et al.* 2006).

In our study, TF plasma level was significantly elevated in patients with ACS and this elevation persisted for at least 7 days after acute clinical presentation. Moreover, we did not find any differences between the local TF antigen plasma levels in the coronary circulation and in the peripheral vein. These findings would support the notion that blood-born TF may be clinically important for the development of ACS in the predisposed patients. Our data also corroborated the relationship between TF production and proinflammatory mediators.

The plasma level of TF may be determined by means of several commercially available ELISA kits, one-stage clotting or chromogenic assays or quantitative immunostaining technique (In-Cell Western). In our series the IMUBIND® Tissue Factor ELISA Kit (American Diagnostica, Inc.) has been used. The kit employs a murine anti-human TF monoclonal antibody for antigen capture. However, this technique also measures degraded TF with epitopes recognized by antibodies but biologically inactive. Alternatively, plasma TF activity as TF-dependent generation of factor Xa can be used to assess the functional activity of TF (Westmuckett *et al.* 2000, Thiruvikraman *et al.* 1996).

The limitation of our study as well as most previously reported clinical series is that TF antigen was measured, and this may not precisely reflect the true biological activity of tissue factor. Increased plasma TF activity and antigen level may be regarded as a marker of early stage of TF-induced coagulation and its measurement may provide some information on potential trigger of the clotting in different clinical settings. Routine assessment of TF in patients with coronary artery disease is, however, limited by the cost, low specificity and lack of long-term clinical data. The decision-making in patients with acute coronary syndrome in the present time is still based on the

traditional biomarkers (e.g. markers of myocardial necrosis – troponins, markers of inflammation – hs-CRP). Nevertheless, TF activity assessment may be helpful in the future for possible clinical application of the inhibition of the earliest stages of the coagulation cascade – the TF/FVIIa pathway (Eilertsen *et al.* 2004).

Conflict of Interest

There is no conflict of interest.

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Increased platelet residual activity in patients treated with acetosalicylic acid is associated with increased tissue factor and decreased tissue factor pathway inhibitor plasma levels

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There are several reasons for an inadequate suppression of platelet aggregation ranging from a patient's non-compliance, inadequate dosage of the drug, poor absorption from gastrointestinal tract, and interaction with other drugs to genetic polymorphisms. An important role may also play the interaction with other factors including tissue factor (TF) [1] and tissue factor pathway inhibitor (TFPI).

The aim of our study was to assess the possible association between the adequacy of platelet inhibition by acetosalicylic acid (ASA) and TF and TFPI plasma levels. The relationship between TF and TFPI plasma level and the platelet aggregability was analyzed prospectively in the consecutive cohort of patients with coronary artery disease (both chronic and acute forms) treated by ASA who were hospitalized at the Cardiology Department of the University Hospital.

All patients were on regular ASA therapy (100–200 mg/day) for at least 5 days. Compliance to ASA intake was confirmed in all subjects. Altogether 64 patients were included, all Caucasian, 12 females, 52 males, age 43–81 (mean 62.52 ± 8.48) years.

Whole blood was obtained from peripheral vein of each patient under standard conditions. Platelet rich plasma (PRP) and platelet poor plasma (PPP) were prepared by centrifugation for 10 min. The

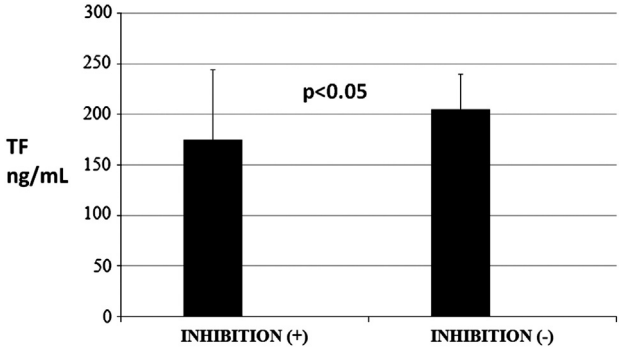

Fig 1. TF plasma levels in patients with an adequate platelet inhibition (INHIBITION +) and in those with high RPR (INHIBITION −). TF=tissue factor plasma level.

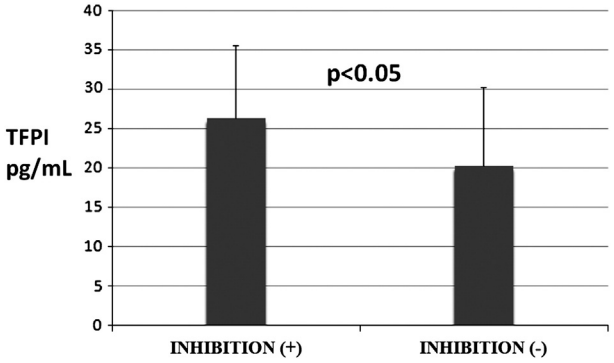

Fig 2. TFPI plasma levels in patients with an adequate platelet inhibition (INHIBITION +) and in those with high RPR (INHIBITION −). TFPI=tissue factor pathway inhibitor plasma level.

determination of aggregation response was performed on the optic aggregometer APACT. The platelet aggregation response was induced by cationic propyl gallate (CPG) (CPG concentration 30 μmol/L). Aggregation curve was constructed and the slope of platelet aggregation onset was assessed in PRP at 37 °C with PPP serving as the reference. Entire patient cohort was subdivided into those with sufficient platelet inhibition (INHIBITION +) and those with inadequate platelet inhibition (INHIBITION −) for cut-off value of 50%/min and 65%/min.

TF and TFPI plasma levels were measured in the blood drawn from the peripheral vein in fasting state. No heparin was given at least 24 h prior to the blood sampling. Quantitation of TF was performed by ELISA, employing a murine anti-human TF monoclonal antibody for antigen capture (IMUBIND® Tissue Factor ELISA Kit, American Diagnostica, Inc.). TF levels were determined by measuring solution absorbances at 450 nm and comparing the values with those of standard curve. Quantitation of TFPI was performed by "sandwich" ELISA employing a rabbit anti-human tissue factor pathway inhibitor polyclonal antibody (IMUBIND® Total TFPI Elisa Kit, American Diagnostica, Inc.). TFPI was detected using a monoclonal antibody specific for Kunitz domain 1 of TFPI. TFPI levels were determined by measuring the sample solution absorbance at 450 nm and comparing them against those of the standard curve.

Plasma level of TF was higher in patients with inadequate platelet inhibitory response to ASA (INHIBITION −) as compared to those with adequate response (INHIBITION +) (204.7 ± 35.2 ng/mL vs 174.9 ± 69.2 ng/mL, respectively, $p<0.05$) (Fig. 1). On the contrary, the plasma level of TFPI was significantly higher in patients with adequate platelet inhibitory response to ASA (INHIBITION +) as compared to those without adequate response (INHIBITION −) (26.3 ± 9.2 pg/mL vs 20.2 ± 10.0 pg/mL, respectively, $p<0.05$) (Fig. 2). The proportion of patients with high RPR was increased in those with plasma levels of TF > 190 ng/mL (14.3% vs 4.3%) ($p<0.05$) and TFPI < 20 pg/mL (17.4% vs 3.6%) ($p<0.001$).

Degree of platelet response to ASA therapy can be characterized as a normal, bell-shaped (Gaussian) distribution and dichotomic labeling as "responder" or "non-responder" is arbitrary and based upon different cut-off points of different platelet function tests. Laboratory documentation of platelet aggregation beyond arbitrary cut-off point, which remains despite ASA administration is termed "high residual platelet reactivity" (RPR) and is different from true documented "aspirin resistance".

The role of inflammation in RPR was studied with inconsistent results. Platelets itself and some of their activators and products may be involved in inflammatory processes [2]. Thus Toll-like receptors 2, which are present at sites of inflammation and interact with immune cells, are expressed on the platelet surface. Their stimulation causes platelet activation and granular protein release [3].

It has been speculated about a possible association between elevated inflammatory markers and high RPR in patients treated by antiplatelet drugs [4]. Poston et al. reported possible relationship between "resistance" to ASA and early saphenous venous grafts failure in patients after off-pump coronary artery surgery. Improved saphenous vein grafts patency after aprotinin administration in these patients was associated with reduced postoperative "resistance" to ASA, decreased platelet response to thrombin and reduced TF activity within saphenous venous grafts segments [5].

Inflammatory mediators may activate coagulation by inducing TF expression—the expression of TF being the part of the inflammatory response signaling process [6]. The prothrombotic effect of inflammation can be thus explained by the increase of TF, with subsequent increase in platelet reactivity [1,6,7]. TF-VIIa complex probably doesn't contribute to platelet activation directly, but rather acts via coagulation factors, such as thrombin. On the other hand, however, recently it has been shown that human platelets synthesize and express functional TF and TF was found on the membrane of resting platelets, in the matrix of α-granules, and in the open canalicular system [8,9]. Translocation and activation of existing TF protein from intracellular compartments to the platelet surface and uptake of TF from other sources like monocytes and possibly polymorphonuclear leukocytes via microparticles have been proposed [8–10]. Moreover, Schwertz et al. reported that quiescent human platelets express TF pre-mRNA in response to activation [11].

The generation of thrombin is physiologically down-regulated by several mechanisms, including the TFPI system. Decreased levels of plasma TFPI have been associated with an increased risk of venous thrombosis. We have previously reported on elevated plasma levels of TFPI in patients with acute coronary syndrome in contrast to the patients with stable coronary artery disease and normal subjects [12–14]. The plasma levels of TFPI in patients with acute coronary syndrome were significantly increased in both the coronary sinus and systemic blood [12].

Although the clinical relevance of circulating TF and TFPI is still a matter of debate [15], the results of our clinical study support the pathophysiological relevance of circulating TF and TFPI in coronary atherothrombosis, particularly with regards to the relationship to the high RPR in patients treated by ASA.

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Brain natriuretic peptide and tumour markers in the diagnosis of non-malignant pericardial effusion

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CA-125
BNP

Pericardial effusion (PE) is a potentially life-threatening condition that occurs in a wide variety of diseases [1–5]. Any infection, neoplasm, autoimmune or inflammatory process that can cause pericarditis can cause PE. However, idiopathic PE is the most common diagnosis [1–5].

Several markers may be used for the differential diagnosis of PE [6–9]. Tumour markers were also measured in suspected malignant disease [9,10] and B-type natriuretic peptide (BNP) in those with heart failure [11]. The aim of this study was to evaluate the levels of BNP and tumour markers in patients with non-malignant PE and to evaluate their relations to the amount of the fluid.

A total of 163 patients with PE were included in the study between January 2003 and November 2007. Etiological evaluation included complete blood count, measurement of troponin I, erythrocyte sedimentation rate, evaluation for viral etiology, thyroid stimulating hormone, rheumatological markers and computerized tomography of thorax in all patients. Diagnostic and therapeutic pericardiocentesis was performed in 44 patients. Pericardial fluid samples were sent for biochemical, microbiological and cytological analyses. The patients with constrictive pericarditis, heart failure and malignancy were excluded from the study.

Forty-five healthy people were selected as the control group for the comparison of the marker levels.

All the patients were informed about the protocol of the study and a written informed consent was obtained from all subjects. The study was approved by the Local Ethical Committee.

Echocardiographic examinations were performed on admission before medical and/or interventional management. They were performed with a standard protocol and a standard device. An anterior–posterior echo-free space at end-diastole under 10 mm is considered as small, 10–20 mm as moderate and over 20 mm is considered as large PE.

Mitral flow velocities were obtained as previously described [12]. The ratio of early-to-late peak velocities (E/A) was calculated and diastolic filling were defined as: normal (EDT 160–240 ms, IVRT 70–90 ms, E/A 1–2, PVs/PVd $\geq$1), abnormal (EDT >240 ms, IVRT >90 ms, E/A <1, PVs/PVd $\geq$1), pseudonormal pattern (EDT 160–200 ms, IVRT <90 ms, E/A 1–1.5, PVs/PVd <1), and restrictive pattern (EDT <160 ms, IVRT <70 ms, E/A >1.5, PVs/PVd <1) [13].

BNP was measured with immunoassay method. CEA, AFP, CA 15-3 and CA 19-9 were measured with electrochemiluminescence immunoassay on Roche Modular E170. CA-125 was measured with quantitative immunoassay technology.

Comparisons of tumour markers and BNP between the study groups were performed by analysis of variance (ANOVA). Correlation between quantitative variables was assessed by Spearman's correlation coefficient. Change in BNP and tumour markers after the follow-

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