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MINIREVIEW

The Role of Tissue Factor in Thrombosis and Hemostasis

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Summary

The tissue factor (TF) is one of the most important regulators of arterial thrombosis. Because arterial thrombosis is the pathophysiologic background of acute coronary syndrome, the possible impact of blocking the arterial thrombosis on its onset is a challenging problem. The investigations of TF brought a new concept of "cell-based coagulation model" which highlighted the question of blood-borne TF as a source of TF in circulating blood. In this review we summarize essential information on the pathophysiology, molecular structure, expression and distribution of TF and we propose a novel concept of blood-borne TF, suggesting the possibilities of inhibition of the coagulation cascade with newly synthetized drugs.

Key words

Tissue factor • Thrombosis • Hemostasis • Coagulation • Microparticles

Introduction

Tissue factor (TF) is a major regulator of normal hemostasis and thrombosis (Nemerson 1988). TF, which is a membrane-bound molecule, acts as an essential cofactor with activated factor VIIa to form a complex that cleaves factors IX and X and activates the whole coagulation cascade (Banner *et al.* 1996). Recent investigations suggest the existence of a blood-borne pool of TF that may play a critical role in the propagation of thrombosis (Giesen *et al.* 1999). These investigations revealed that TF bound to procoagulant microparticles, which are present in the circulating blood under pathophysiological conditions and might exert a specific thrombogenic potential (Nieuwland *et al.* 1997). Consequently high levels of shed apoptotic microparticles and TF activity were found in extracts from atherosclerotic plaques (Marmur *et al.* 1996, Mallat *et al.* 1999). Increased levels of microparticles with a procoagulant potential were found in the peripheral circulating blood of patients with acute coronary syndrome (ACS) (Mallat *et al.* 2000).

Pathophysiology

TF (also known as coagulation factor III or tissue thromboplastin) is a transmembrane glycoprotein responsible for the onset of blood coagulation. The classical view of blood coagulation as a cascade of activated coagulation factors consists of intrinsic and extrinsic pathways. After vascular injury, TF, like the

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serine protease receptor for the coagulation factor VIIa (FVIIa), binds circulating FVII which then turns by allosteric activation into an activated form FVIIa. Besides this activation, TF enhances proteolytic and amidolytic activity of FVIIa and TF/FVIIa complex, which subsequently turns factor X (FX) into FXa. FXa as a prothrombinase complex (FXa/FVa) cleaves prothrombin into thrombin and thrombin then cleaves fibrinogen into fibrin, completing the extrinsic pathway of blood coagulation. TF/FVIIa complex is also capable of turning zymogens FVII and at slower rate also factor IX (FIX) into their active forms FVIIa and FIXa (Ruf 1998). FIXa together with its cofactor VIIIa (FVIIIa) activate FX into FXa, thus explaining the role of TF in the intrinsic pathway of blood coagulation. Factor V (FV) and factor VIII (FVIII) are transformed into their active forms by thrombin, thus providing a back-activation loop.

At present, blood coagulation is evolving into more complex aspects uniting the whole process and emphasizing the role of cells and activated platelets. A cell-based model involves a series of reactions between the coagulation factors divided into three steps: 1) initiation, 2) amplification and 3) propagation occurring on two principal cell surfaces – the TF-bearing cells and the platelets (Walsh 2004). After the exposure of TF-bearing cells into the bloodstream, TF/FVIIa complex is formed and FX is turned into FXa. However, the process is rapidly stopped by an endogenous inhibitor – tissue factor pathway inhibitor (TFPI) – which forms an inactive quaternary TF/FVIIa/FXa/TFPI complex. In this way, only small amounts of FXa are produced and only traces of thrombin are generated, incapable to cleave enough fibrinogen into fibrin. Nevertheless, the generated thrombin is sufficient to activate FV and FVIII, which then serve as cofactors and strongly enhance the production of FXa by FIXa/FVIIIa complex and subsequently the production of thrombin by FXa/FVa complex. Furthermore, thrombin activates factor XI (FXI) into FXIa, which provides another route of activation of FIX apart from that of TF/FVIIa complex. This is in accordance with findings that people, deficient in contact factors (FXII, prekallikrein, high molecular weight kininogen) which were claimed to be responsible for FXI activation, do not suffer from bleeding, while patients deficient in FXI do, proving that there must be another route of FXI activation. TF seems to serve as a kind of starter to produce minute amounts of thrombin before its pathway is switched off and the process then continues thanks to back-activation loops leading from thrombin to FIX, FVIII and FV (Walsh 2004, Frederick *et al.* 2005).

Molecular structure of TF

TF is encoded by a 12.4 kb gene organized into six exons separated by five introns located on chromosome 1, at locus 1p22-23. The promoter region exhibits two binding sites for the transcription factor activator protein-1, one κB-binding site, three Egr-a binding sites and five Ap1 sites (Mackman *et al.* 1989). A binding site for nuclear factor of activated T cells was identified as well, overlapping the κB site (Armesilla *et al.* 1999).

TF is constitutively expressed in several cell types such as adventitial fibroblasts, smooth muscle cells and epithelial cells but extensive cell culture studies showed that TF promoter is capable of inducing TF expression after stimulation (by LPS, IL-1β, TNF-α) on other cell types as well (e.g. monocytes) (Eilertsen and Bjarne 2004).

TF is composed of an extracellular domain consisting of two fibronectin type III repeats (N-terminal domain TF1 and C-terminal domain TF2), a transmembrane domain and a short cytoplasmic tail. The crystal structure of soluble tissue factor (sTF) showed the interdomain angle to be about 120° (Harlos *et al.* 1994, Muller *et al.* 1994) and a structural relationship between TF and the cytokine receptors (Bazan 1990). A close structural similarity of TF with cytokine receptors explains a receptor function of TF with intracellular signaling capacity. Several studies addressing this aspect of TF function prove the view that TF plays a role in biological actions unrelated to coagulation, such as tumor metastasis and angiogenesis (Rickles *et al.* 2003, Versteeg *et al.* 2003, Bromberg *et al.* 1995, Zhang *et al.* 1994).

Expression of TF

TF is expressed on cells in the vessel wall (Wilcox *et al.* 1989) and is normally not found on cells that are in direct contact with the bloodstream (Kirchhofer and Banner 1997). Various endogenous (e.g. TNF-α) and exogenous agonists (e.g. lipopolysacharides) can induce TF expression on endothelial cells and monocytes (Camerer *et al.* 1996). This TF upregulation may play a role in thrombotic disorders. Furthermore, induction of TF expression on smooth muscle cells was observed in experimental restenosis, implicating tissue factor activity in thrombotic complications after angioplasty (Marmur *et al.* 1993, Speidel *et al.* 1995), and also in neointima proliferation (Jang *et al.* 1995). TF expression in atherosclerotic plaques was first described by Wilcox *et al.* (1989). Subsequent studies identified extensive TF expression in human atherectomy

specimens (Annex *et al.* 1995, Marmur *et al.* 1996), suggesting a function of the TF pathway in the development of coronary artery diseases.

Blood-borne TF

Several studies show that an additional source of TF, known as blood-borne TF or plasma TF, may also contribute to thrombosis. According to them, TF antigen is present in plasma of healthy subjects at mean levels ranging from 149 to 172 pg/ml (Koyama *et al.* 1994, Albrecht *et al.* 1996). Some studies have shown that levels of blood-borne TF are increased in various disease states, such as atherosclerosis, sepsis, diabetes, and sickle cell disease (Semenza *et al.* 1990, Misumi *et al.* 1998, Soejima *et al.* 1999, Mallat *et al.* 1999, Nieuwland *et al.* 2000, Diamant *et al.* 2002). It was also shown that there is a correlation between levels of blood-borne TF and acute myocardial infarction (Nieuwland *et al.* 1997, Misumi *et al.* 1998, Seljeflot *et al.* 2003). Furthermore, inhibition of TF in a rabbit model of venous thrombosis reduced thrombus propagation (Himber *et al.* 1997).

The role of blood-borne TF was strongly supported by a study measuring the rate at which FX in a well-mixed clot supernatant permeates the clot and is converted to FXa on various *in vitro* prepared platelet-fibrin clots on TF/VIIa-coated surfaces. Paradoxically, the growing thrombus acted as a barrier, restricting the convective and diffusive exchange of substrates and coagulation products between the blood and reactive vessel wall, thus limiting the role of TF from the vessel wall played in thrombus growth. The apparent diffusion coefficients of FX(a) in fibrin and platelet-fibrin clots at 37 °C was 2.3×10^{-7} and 5.3×10^{-10} cm^2/s, respectively, indicating that the mean time required for FX(a), and likely FIX(a), to diffuse 1 mm in a fibrin clot is 4 hours, and 3.6 months in the presence of platelets. As complete human thrombotic occlusion has been observed within 10 min, an alternative source of procoagulant activity that can be localized to the outer surface of growing thrombi (such as platelet factor XI or blood-borne TF) appears to be essential for rapid thrombus growth (Hathcock and Nemerson 2004).

One controversial issue is the form of blood-borne TF. Many studies have shown that TF circulates in the blood in the form of cell-derived microparticles (Nieuwland *et al.* 1997, Combes *et al.* 1999, Berckmans *et al.* 2001, Sturk-Maquelin *et al.* 2003). Other groups suggest that TF is present in platelets (Siddiqui *et al.* 2002, Camera *et al.* 2003, Muller *et al.* 2003). Finally, it was recently proposed that an alternatively spliced form of soluble TF is the major form of blood-borne TF (Bogdanov *et al.* 2003). Monocytes were shown to be capable of shedding microparticles exposing TF and phosphatidylserine together with adhesion molecules CD14, CD11a and CD18 after LPS stimulation (Eilertsen and Osterud 2005). It was found that this TF on microparticles became incorporated into spontaneous human thrombi, suggesting that TF may be transferred to platelets by interaction of CD15 with P-selectin exposed on activated platelets (Giesen *et al.* 1999). TF itself plays a role of adhesion molecule necessary for this transfer. These findings were supported by the fact that anti-CD15 antibodies abolish 80 % of the LPS-induced TF activity in monocytes of cell suspensions recombined with platelet-rich plasma (Halvorsen *et al.* 1993). Furthermore, mice lacking P-selectin or PSGL-1 developed platelet-rich thrombi with minimal TF and fibrin (Falati *et al.* 2003). Leukocyte-platelet interactions associated with the release of TF and subsequently thrombin are suspected to play an important role in various hyperthrombotic states in patients with high LDL cholesterol concentrations, cigarette smoking and diabetes mellitus, thus emphasizing the importance of not only the vulnerable or high-risk atherothrombotic lesions but also the relevance of hyperreactive or vulnerable blood. This explains a possible mechanism of thrombus formation in those cases of acute coronary syndromes that are based only on a superficial erosion of the fibrotic plaque and not on a disruption of a lipid-rich plaque exposing the lipid core with high levels of TF.

Small amounts of microparticles with TF probably originate from apoptotic cells. The role of apoptosis in atherothrombosis is important both at the site of the plaque and in the circulation (Viles-Gonzalez *et al.* 2005). Apoptosis within atheromas involves all cell types and it has been demonstrated that it coexists with high levels of TF within the plaque. TF activity is dependent on the presence of phosphatidylserine, exposure of which is associated with apoptosis. The prothrombotic potential of apoptotic cells was also shown to be present in the circulation because one group found high levels of endothelial membrane microparticles in the circulating blood of patients with acute coronary syndrome (ACS), inferring that they might participate in the generation and perpetuation of intracoronary thrombi (Mallat *et al.* 2000).

Another significant issue with blood-borne TF is how to measure its functional levels. It is clear that the levels of blood-borne TF are very low compared with TF levels in the vessel wall, particularly in healthy subjects (Mackman 2004). In addition, microparticles in the blood have TF-dependent and TF-independent procoagulant activity (Berckmans *et al.* 2001, Aras *et al.* 2004). A

recent study described a novel assay that overcomes this problem by measuring TF activity of selectively captured, TF-positive microparticles. This study also showed that elevation of procoagulant microparticles, a subset of which contained TF, restored hemostasis in hemophilic mice (Hrachovinová *et al.* 2003). Moreover, FVIIa has been proven to be a very effective hemostatic agent in hemophilic patients and in trauma patients (Hedner 2000). The mechanism of action of FVIIa has been proposed to be independent of TF (Hoffman *et al.* 1994). However, the presence of blood-borne TF suggests that this conclusion should be re-evaluated. It seems to be possible that the hemostatic protection provided by FVIIa may be, in part, caused by its binding to blood-borne TF (Mackman 2004).

Tissue-specific TF distribution

TF exhibits a non-uniform tissue distribution with high levels in the brain, lung, and placenta, intermediate levels in the heart, kidney, intestine, uterus and testes, and low levels in the spleen, thymus, skeletal muscle and liver (Bach 1988, Drake *et al.* 1989, Hartzell *et al.* 1989, Faulk *et al.* 1990, Mackman *et al.* 1993). Immunohistochemical and *in situ* hybridization studies demonstrated that high levels of TF were expressed by astrocytes in the brain, alveolar cells in the lung, trophoblasts in the placenta, epithelial cells surrounding organs and at body surfaces, adventitial fibroblasts surrounding blood vessels, and cardiac myocytes in the heart (Drake *et al.* 1989, Fleck *et al.* 1990, Eddleston *et al.* 1993, Mackman *et al.* 1993, Flossel *et al.* 1994, Luther *et al.* 1996). This cell type-specific distribution suggested that TF provides a "hemostatic envelope" to limit bleeding after vessel injury (Drake *et al.* 1989). The higher levels of TF in the brain, lung, placenta, heart, and uterus would provide additional hemostatic protection to these vital organs. In contrast, tissues that express low levels of TF, such as skeletal muscles and joints, rely on the FVIIIa/FIXa complex of the intrinsic pathway to prevent bleeding. Indeed, this model explains why hemophilia patients deficient in either FVIII or FIX frequently bleed into joints and soft tissues (Bolton-Maggs and Pasi 2003).

TF is constitutively expressed in cardiac myocytes but not in skeletal myocytes (Drake *et al.* 1989). The likely function of TF in the heart is to provide additional hemostatic protection. Low-TF mice had very low levels of TF in their hearts compared with the level sof TF in the hearts of wild-type mice, suggesting reduced TF expression in cardiac myocytes. Importantly, low-TF mice and low-FVII mice exhibited hemosiderin deposition and fibrosis in their hearts. It is believed that the hemosiderin is derived from erythrocytes hemorrhaging into the myocardium and phagocyte digestion of the hematin. Indeed, occasional hemorrhages in the hearts of low-TF mice were observed (Pawlinski *et al.* 2002). Taken together, these results suggest that low-TF and low-FVII mice have impaired heart hemostasis. It has been found that overexpression of murine TF in the cardiac myocytes abolishes fibrosis in the hearts of low-TF mice. These results indicate that TF expression in cardiac myocytes plays a key role in the heart, most likely by providing additional hemostatic protection to this vital organ that may be prone to mechanical injury of the vessels. In contrast, FIX-deficient mice have normal hearts (Mumford and McVey 2004). These results suggest that the TF/FVIIa complex, but not the FVIIIa/FIXa complex, plays a critical role in heart hemostasis.

FIX and other factors of the amplification stage of blood coagulation normally provide an important back-activation loop, thus enhancing thrombin generation. However, some computer-generated models showed that high concentrations of TF (for example in the conditions of sepsis or endotoxemia) provide enough FXa and enough thrombin to generate a fibrin clot without this back-activation loop (Pawlinski and Mackman 2004).

Role of TF in thrombosis

Aberrant TF expression triggers intravascular thrombosis associated with various diseases, such as atherosclerosis, cancer and sepsis (Rao 1992, Creasey *et al.* 1993, Tremoli *et al.* 1999, Rickles *et al.* 2003). Importantly, inhibition of TF/FVIIa complex activity reduced coagulation and decreased mortality in animal models of sepsis (Taylor *et al.* 1991, 1998). In atherosclerosis, TF is expressed (apart from other cells) by macrophage-derived foam cells within atherosclerotic plaques (Wilcox *et al.* 1989). These results strongly suggest that high levels of TF exposed upon plaque rupture trigger thrombosis and myocardial infarction. Thus, the classical view of TF is that it is expressed locally within an atherosclerotic lesion. Inhibition of TF would be expected to reduce thrombosis associated with a variety of diseases.

The role of TF and FVII in coronary no-reflow was also studied. The data obtained suggest that active TF is released from dissected coronary atherosclerotic plaque and is one of the factors causing the no-reflow phenomenon (Bonderman *et al.* 2002).

Functional inhibition of TF with an anti-rabbit TF monoclonal antibody in a rabbit coronary ligation

model has been shown to improve coronary blood flow after myocardial ischemia/reperfusion (I/R) injury. At-risk areas of myocardium showed increased TF expression in the sarcolemma of cardiomyocytes, which was associated with a low level of extravascular fibrin deposition. Infarct size was reduced by 61 % and 44 %, respectively (Erlich *et al.* 2000).

Local inhibition of TF by both recombinant tissue factor pathway inhibitor and a polyclonal antibody against human TF reduces the thrombogenicity of disrupted human atherosclerotic plaques in an *in vitro* model of human atherosclerotic and normal arterial segments exposed to heparinized blood at flow conditions modeling medium-grade coronary stenosis (Badimon *et al.* 1999). The antithrombotic effects of the specific inhibition of plaque TF was assessed by reduction in the deposition of radiolabeled platelets and fibrinogen and immunohistochemical analysis of perfused arteries.

TF role in embryonic development

As there are no known TF-deficient humans, some groups studied the effect of knocked-out murine TF gene and they all reported high rate of lethality of embryos (approximately 90 %) (Bugge *et al.* 1996, Carmeliet *et al.* 1996). Defect of hemostasis was considered to be the cause of death, but flawed development of the yolk sac vasculature was also noted (Toomey *et al.* 1996). Controversial were findings that FVII deficient embryos survive the embryonic development (Rosen *et al.* 1997). Using a transgenic approach the role of several coagulation factors and PARs (protease-activated receptors) in the embryonic development was studied with the conclusion that the high rate of lethality of TF-deficient embryos may be caused by a loss of both hemostatic and non-hemostatic pathways and an absence of maternal rescue of this transmembrane receptor (Carmeliet *et al.* 1996*)*.

Inhibition of early steps of the coagulation cascade

The study of TF role in blood coagulation leads to new ideas in anticoagulation therapy. New drugs are expected to be found, with less side effects and stronger anticoagulation potential when acting at the beginning of the coagulation cascade.

The possible influence of TF transcription was reported by a group demonstrating that activation of liver X receptors (LXRs) LXRα and LXRβ suppresses TF expression. Treatment of mouse peritoneal macrophages with synthetic LXR agonist T0901317 or GW3965 reduced TF expression induced by pro-inflammatory stimuli. LXR agonists also suppressed TF expression and its activity in human monocytes. Human and mouse TF promoters contain binding sites for the transcription factors AP-1, NFκB, Egr-1 and Sp1, but no LXR-binding sites could be found. Cotransfection assays with LXR and TF promoter constructs revealed that LXR agonists suppressed LPS-induced TF promoter activity. Analysis of TF promoter also showed that inhibition of TF promoter activity by LXR was at least in part mediated by inhibition of the NFκB signaling pathway. In addition, LXR agonists reduced *in vivo* TF expression within aortic lesions in an atherosclerosis mouse model as well as in the kidney and lung of mice stimulated with LPS. These findings indicate that activation of LXR results in the reduction of TF expression, which may influence atherothrombosis in patients with vascular disease (Terasaka *et al.* 2005).

A lot of effort has been made to inhibit the TF gene expression at the translational level using various approaches involving ribozymes, antisense technologies and RNA interference (Zhang *et al.* 1996, Armesilla *et al.* 1999, Cavusoglu *et al.* 2002). However these techniques seem to have limited efficiency and still need to develop an effective and safe delivery mechanism (Armesilla *et al.* 1999).

Direct blocking of the TF, FVIIa or TF/FVIIa complex activity can be achieved by various inhibitors. One way of blocking the TF-mediated start of coagulation is using a specific antibody. In a recently published study Morrow *et al.* (2005) used Sunol-cH36, a chimeric monoclonal antibody to TF, which blocks binding of factor X to the TF/VIIa complex, for a trial in humans. The safety and pharmacokinetics of Sunol-cH36 were assessed in an open-label, dose-escalating trial among subjects with stable coronary artery disease. No major bleeding (≥ 2 g/dl hemoglobin decline) was reported. Spontaneous minor bleeding was observed with a dose-related pattern. The majority of spontaneous bleeding episodes were clinically consistent with platelet-mediated bleeding (e.g. gum, tongue) without thrombocytopenia. Sunol-cH36 exhibited dose-dependent anticoagulant effects and the mucosal bleeding observed with this potent inhibitor of thrombin generation may reflect antiplatelet effects resulting from networking between the coagulation cascade and platelet pathways that could prove clinically relevant with this novel class of anticoagulants (Morrow *et al.* 2005).

A recombinant version of the truncated, extracellular form of TF (hTFAA) has also been reported to function as an antagonist of membrane-bound TF (Kelley *et al.* 1997). Promising investigation was presented of a selective small-molecule TF inhibitor

PHA-927F, which is capable efficiently inhibit or prevent acute thrombosis without any increase in the risk of bleeding thanks to its great selectivity against TF when compared to other coagulation factors as a result of extensive crystal structure studies of similar molecules (Suleymanov *et al.* 2003, Frederick *et al.* 2005).

Stone *et al.* (2005) showed the effect of active site-inhibited FVIIa (FVIIai) binding to TF. FVIIai blocks binding of the corresponding enzyme (FVIIa) or zymogen (FVII) forms of FVII and inhibits coagulation. Although several studies have suggested that FVIIai may have superior anticoagulation effects *in vivo,* a challenge for use of FVIIai is the cost of its production. The study reported the properties of dimeric forms of FVIIai that are cross-linked through their active sites. Dimeric wild-type FVIIai was at least 75-fold more effective than monomeric FVIIai in blocking FVIIa association with TF. The dimer of a mutant FVIIai with higher membrane affinity was 1600-fold more effective. Anticoagulation by any form of FVIIai differed substantially from agents such as heparin and showed a delayed mode of action. Coagulation proceeded normally for the first minutes, and the inhibition increased when equilibrium binding was established. It is suggested that association of FVIIa(i) with TF in a collision-dependent reaction gives equal access of inhibitor and enzyme to TF. Assembly was not influenced by the higher affinity and slower dissociation of the dimer. As a result, anticoagulation was delayed until the reaction reached equilibrium. Different dissociation experiments suggested that dissociation of FVIIai from TF occurred by a two-step mechanism. The first step was a separation of TF/FVIIa(i) while both proteins remained bound to the membrane. The second step was a dissociation of the FVIIa(i) from the membrane. These results suggest novel actions of FVIIa(i) that distinguish it from most of the anticoagulants that block later steps of the coagulation cascade (Stone *et al.* 2005).

Some studies of a novel class of peptide exosite inhibitors of FVIIa (like E-76, A-183 or extended A-183X) have been performed. Among these, A-183X is capable of an almost complete *in vitro* inhibition of TF/FVIIa activity. The NAPc2 (Nematod Anticoagulant Protein c2) isolated from the canine hookworm *Ancylostoma caninum*, binds to FXa and then to TF/FVIIa complex and forms an inactive quaternary complex (Walsh 2004, Frederick *et al.* 2005).

Roque *et al.* (2000) investigated the effect of TF inhibition by recombinant tissue factor pathway inhibitor (rTFPI) on thrombus formation and intimal hyperplasia in a porcine model of coronary balloon angioplasty. Tissue factor pathway inhibitor (TFPI) is the principal physiological inhibitor of the TF-factor VII/VIIa complex and is found mainly in endothelial cells. TFPI therapy has been proven as beneficial in deep vein thrombosis (Holst *et al.* 1994), in preventing arterial reocclusion after fibrinolysis (Abendschein *et al.* 1995) and in reducing intimal hyperplasia in experimental models of arterial injury (Jang *et al.* 1995, Oltrona *et al.* 1997). Using an *ex vivo* perfusion system it has previously been shown that specific inhibition of TF with TFPI reduces thrombogenicity of disrupted human aortic atherosclerotic plaques (Badimon *et al.* 1999).

The potential use of TFPI after angioplasty was also studied in a rabbit model. When the TFPI gene in a liposome or in an adenoviral vector was locally administered after iliac and carotid artery angioplasty, restenosis was markedly reduced (Zoldhelyi *et al.* 2001, Yin *et al.* 2002).

Conclusions

In this review we have summarized essentials of the physiology and the pathophysiology of TF in terms available literary sources. There are still many questions to be answered: the source and the level of TF in healthy subjects, the method how to measure the “thrombogenic” potential of TF because its role in many biological processes, “normal” levels and the possible impact of risk factors and possibility of risk stratification of “healthy” subjects according to the levels of TF as markers of the blood with a procoagulant potential. Answering these questions and the impact of TF blocking as a therapeutical approach in acute coronary syndromes is a challenging field of investigation in blood coagulation.

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