Expert Opinion

The multivalent activity of the tissue factor–thrombin pathway in thrombotic and non-thrombotic disorders as a target for therapeutic intervention

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Importance of the field: Tissue factor (TF) is the key initiator of the coagulation cascade. The exposure of subendothelial TF after vessel injury to blood is a critical step in hemostasis and in the pathogenesis of arterial and venous thrombotic disorders. Moreover, an additional role for TF overexpression and subsequent generation of TF:FVIIa complex, FXa and thrombin have been recently emerged, contributing in non-thrombotic manifestations such as inflammation, cancer growth and fibrosis.
Areas covered in this review: The multivalent role of TF and the above mentioned proteases in disease is reviewed, with focus on their implication in non-thrombotic disorders, as suggested by clinical and experimental data. Moreover, potential therapeutic interventions using anticoagulation agents are discussed.
What the reader will gain: A better understanding of the pathogenic role of the TF–thrombin pathway in the pathogenesis of disease and the effect of anticoagulants in the treatment of such disorders.
Take home message: The TF-thrombin pathway, apart from the initiation of hemostasis and thrombosis, exert intracellular signaling activity through protease-activated receptors, participating in inflammation and tumor biology. Both low-molecular-weight heparins and recently developed anticoagulants rise as candidates for the modification of biological functions associated with disorders like sepsis, ischemia–reperfusion or cancer growth and metastasis.

Keywords: cancer, inflammation, TF, thrombin, thrombosis

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1. Introduction

In 1905 Morawitz stated a clotting theory, proposing a crucial and calcium-dependent role for thrombokinase in the conversion of prothrombin (thrombogen, as he named it) to thrombin. Later, in 1908, the term tissue thromboplastin was used instead of thrombokinase by Nolf, and even later, in 1935, the term tissue factor (TF) was proposed by Howell. Although various theories regarding clotting had been proposed, the clotting theory of Morawitz influenced the research in the field for many decades. In 1977, it was shown that the complex TF–factor VII (FVII) activated both factor X (FX) and IX (FIX), holding a significant role in clot formation. Thus, 90 years later, in 1995, Rapaport and Rao pointed to TF as the main factor able to trigger coagulation *in vivo*. They wrote: "..the binding of factor VII

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Article highlights.

- The increased expression of TF by leukocytes, endothelium or cancer cells initiates the activation of the coagulation cascade that leads to thrombin generation.
- The TF–thrombin pathway is implicated in vascular microangiopathy and in venous and arterial thrombosis.
- The proteases of the extrinsic coagulation system exert signaling through PARs, regulating several inflammatory responses and tumor growth and metastasis.
- Experimental and clinical data provide evidence for the effectiveness of anticoagulation-based strategies in the treatment of inflammatory and malignant disorders.
- Several recently developed agents with favorable safety profile and tolerability constitute promising alternative treatment strategies for the treatment of both thrombotic and non-thrombotic disorders.

This box summarizes key points contained in the article.

to TF and the subsequent reactions so triggered play a 'prima ballerina' role in the initiation of coagulation" [1,2].

During the period 1980 – 2000, the exons/introns organization of the TF gene and the characterization of the structure of TF protein contributed to the resolution of various questions regarding the expression of TF by different cells and tissues, its natural inhibitor, TF pathway inhibitor (TFPI), [2], the function of TF isoforms, the characterization of blood-borne TF and the appropriate or not induction of TF expression under several clinical conditions.

Despite the characterization of TF structure and function, no human disorder that depends on a genetic defect of this factor has been described. However, it is currently established that the activation of TF–FVII complex has, apart from its procoagulant activity, a signaling activity too. Protease-activated receptors (PARs) [3] mediate the regulation of non-thrombotic functions such as inflammation, angiogenesis, tumor growth and fibrosis by the proteases of this pathway, namely TF–FVIIa complex, FXa and thrombin.

Since the TF pathway constitutes a pleiotropic system involved in various disorders, thrombotic or non-thrombotic, it is reasonable for it to be currently considered as a multivalent target for future therapeutic intervention. The field of selective targeting of either TF–VII signaling or TF pathway inducers is rapidly evolving and novel and safe agents are available in experimental trials and clinical use.

2. The physiological role of TF

2.1 TF structure

TF is a 47 kDa transmembrane glycoprotein that demonstrates high homology in secondary and tertiary structure with interferon γ receptors and is a member of the human class II cytokine receptor family [4]. TF is encoded by a 12.4 kb gene on chromosome 1, which is organized into six exons [5]. The main TF mRNA transcript is comprised by 2.3 kb [5], while the existence of larger transcripts [6,7] with no physiological relevance has been reported [8]. Recently, a functional alternative spliced variant of TF (asTF) has been identified by Bogdanov and colleagues [9]; asTF transcript lacks exon 5 which encodes for the transmembrane domain, resulting in a soluble shorter protein of 206 amino acids [9].

2.2 TF expression

The plethora of activating binding sites of TF promoter region indicates multipotency in gene expression, permitting TF expression in a variety of cells under different stimuli and conditions (**Figure 1**). In addition, methylation of the promoter could prevent expression in certain cells such as T and B lymphocytes. More specifically, TF is expressed in cells of the vascular wall, astrocytes, cells lining organs, fibroblasts, smooth muscle cells and adipocytes [10,11]. TF is not expressed in endothelial cells under physiological conditions [12], preventing the unintended and potentially dangerous activation of blood coagulation. However, various subendothelial cells express significant amounts of TF, able to activate the extrinsic coagulation cascade after the disruption of vessel wall integrity. Inflammatory molecules (TNF-α, IL-1β, IL-8, C5a) [13-17] and mitogens (VEGF) [18] are able to induce TF expression in endothelial cells, monocytes and peripheral polymorphonuclear leukocytes (**Figure 1**). The promoter region of TF possesses two binding sites for the transcription factor activator protein-1 (AP-1), one nuclear factor κB site, three early growth response gene-1 (Egr-1) and five specific protein-1 (Sp1) sites [19-21]. The presence of a CpG island on the first part of the TF promoter sequence provides a methylating site [5].

2.3 Blood-borne TF

Until recently, unstimulated peripheral blood cells were considered unable to express TF, supporting the theory of the haemostatic envelope of blood vessels. However, emerging evidence indicates the presence of circulating TF in blood (also called blood-borne TF). Blood-borne TF originates from three possible sources: peripheral blood cells [10,13,14,16,17], circulating microparticles (MP) containing TF [22] and the soluble alternative spliced variant of TF [8]. Peripheral blood monocytes and neutrophils can express TF when stimulated by different factors such as LPS, TNF-α, and C5a [13,14,16,17]. Furthermore, small lipoprotein structures formed by vesiculation of cell membrane of endothelial and peripheral blood cells (microparticles (MP)) were found to contain TF [22]. Additionally, asTF constitutes a significant part of blood-borne TF [9]. Despite the fact that its procoagulant activity is not clearly defined [9,23], as TF seems to be a functional variant that can activate FVII for further signalling while its contribution in angiogenesis has been recently described [24].

2.4 Haemostatic activity of TF

TF is released in blood from tissues in the event of vessel wall injury. Furthermore, activated endothelial cells and peripheral blood cells express TF after stimulation through several

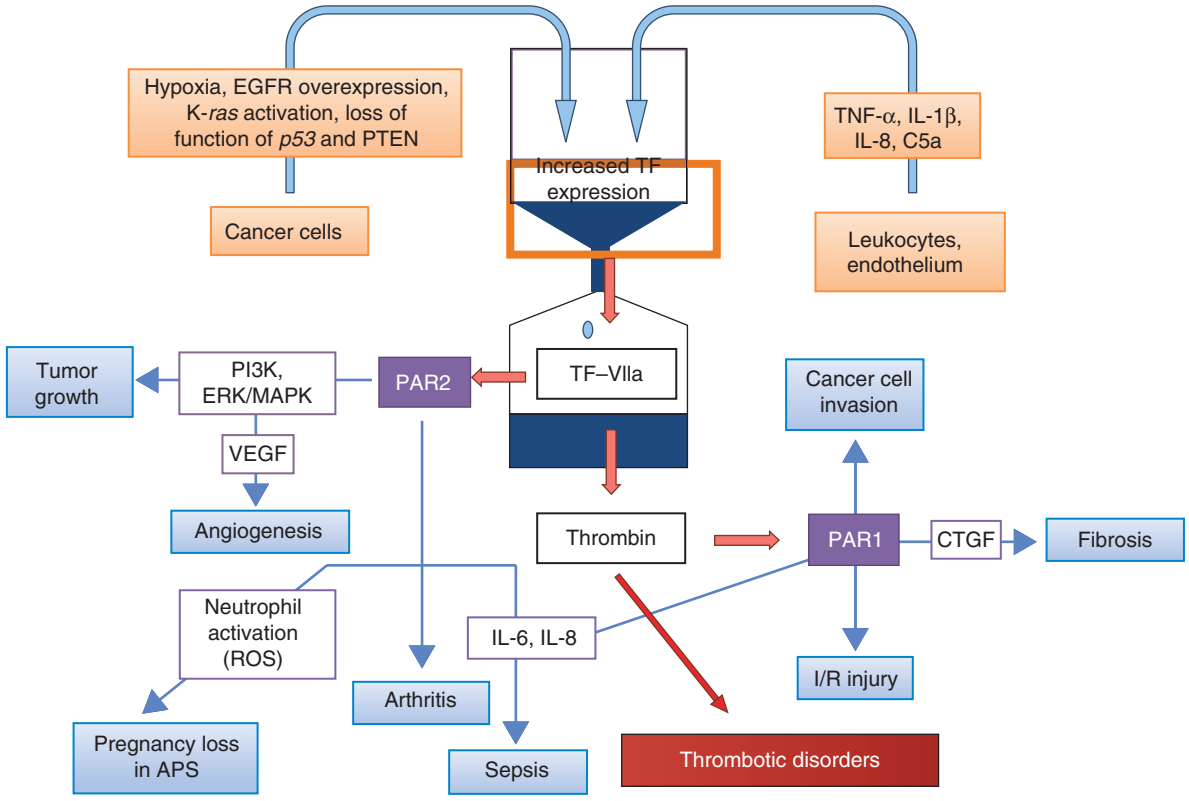

Figure 1. TF in the bottleneck of disease pathogenesis. Several stimuli induce the expression of TF in endothelial cells and/or blood cells (leukocytes, platelets). Moreover, cancer cells express high levels of TF, in response to hypoxia or due dysregulation of oncogenes and/or tumor suppressor genes. The increased levels of TF result in the activation of the extrinsic coagulation cascade, leading to venous thrombosis. In addition, the proteases of this cascade activate PARs, participating in the pathogenesis of inflammation, tumor growth, angiogenesis and fibrosis.

APS: Antiphospholipid syndrome; CTGF: Connective tissue growth factor; ERK: Extracellular-signal-regulated kinase; I/R: Ischemia–reperfusion; PAR: Protease activated receptor; PTEN: Phosphatase and tensin homologue; ROS: Reactive oxygen species.

mediators. TF acts as the main *in vivo* initiator of coagulation, when it comes into contact with blood [25]. The activation of the extrinsic coagulation system in a sequential cascade of events plays a critical role in hemostasis. The extrinsic coagulation cascade is initiated when TF, localized on cell surface, binds and activates FVII. The TF–FVIIa complex further activates both vitamin-K-dependent zymogens, FIX and FX. FXa in turn, and in conjunction with FVa, converts prothrombin to thrombin [26]. The generated thrombin, even though not sufficient for the formation of fibrin, amplifies the initial thrombin signal. Thrombin binds to platelets that adhere at the site of tissue injury and expose on their membranes phosphatidylserine, an anionic phospholipid involved in the further activation of the coagulation cascade. The recruitment of platelets enables the assembly of the intrinsic tenase (FIXa/FVIIIa) and prothrombinase (FXa/FVa) complexes, further activating FX and resulting in the conversion of prothrombin into thrombin, respectively [27]. FVII, FX and thrombin belong to the family of serine proteases and signal through proteolytic cleavage/activation of other proteins/receptors, participating in several functions other than thrombosis (**Figure 2**) [28]. The coagulation cascade is regulated by natural anticoagulants such as protein C, protein S and TFPI.

3. The extrinsic coagulation cascade in thrombosis

Inappropriate TF expression is implicated in the pathogenesis of arterial and venous thrombotic disorders. It is suggested that the exposure of subendothelial TF to blood and/or the induction of TF expression in endothelial and blood cells is the critical step for the pathologic activation of coagulation that characterizes arterial and venous thrombosis, respectively (**Figures 1** and **2**) [26].

3.1 Atherothrombosis

Cardiovascular disorders constitute the leading cause of mortality in the developed world, having a major effect on public health. The rupture of an atherosclerotic plaque and the

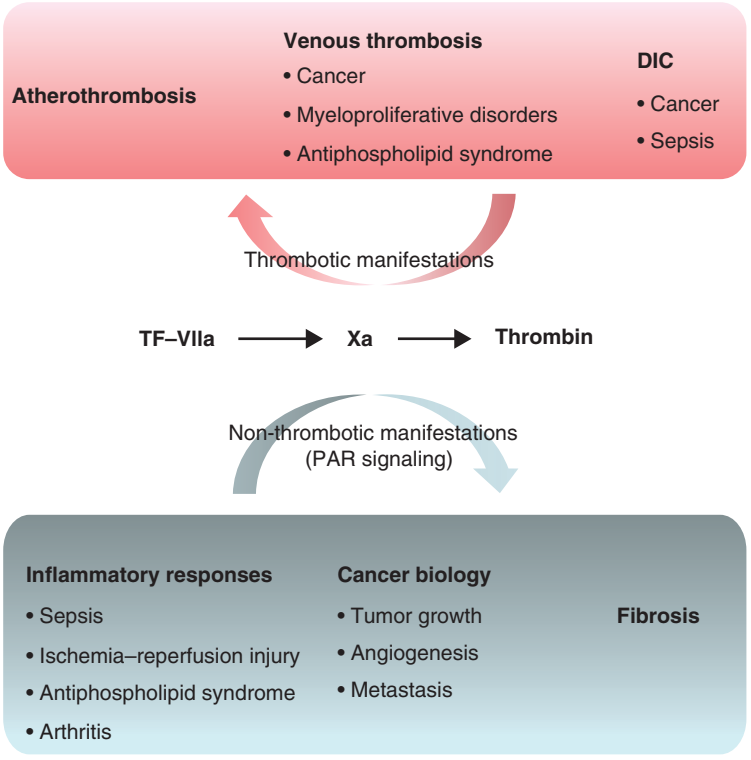

Figure 2. The TF-thrombin pathway is implicated in thrombotic and non-thrombotic disorders. The activation of the extrinsic coagulation cascade and the generation of thrombin is the key event in the pathogenesis of venous thrombosis and disseminated intravascular coagulation (DIC). This cascade is further implicated in arterial thrombosis by stabilizing the initially formed platelet-rich thrombi. Moreover, TF–FVIIa complex, FXa and thrombin signaling through protease activated receptors (PARs) is involved in the pathogenesis of inflammatory disorders, including sepsis, ischemia–reperfusion injury, antiphospholipid syndrome and arthritis, cancer (participating in metastasis, tumor growth and angiogenesis), and probably in fibrotic disorders.

formation of platelet rich thrombi represent the initial event in atherothrombosis. The subsequent release of TF, which is highly expressed in the subendothelial tissue [12,29] activates blood coagulation and results in fibrin deposition on the platelet surface and stabilization of thrombus [30]. Whether blood-born TF, localized on blood cells or MP, contributes in this process is still a matter of debate [20]. Even though the levels of circulating TF have been associated with acute myocardial infarction [31], data extracted from *ex vivo* studies or *in vivo* animal models of arterial thrombosis remain controversial. TF derived from the vessel wall and not from blood cells was involved in thrombosis in healthy mice after carotid artery injury [32], while a laser-induced injury model of mouse cremaster arterioles implicated circulating TF in thrombosis [33].

3.2 Venous thrombosis

Available evidence points towards aberrant TF expression among the main pathogenic mechanisms for the majority of deep vein thrombosis (DVT) and pulmonary embolism (PE) events, while the inherited disorders of coagulation and fibrinolytic systems are currently considered as predisposing factors. Circulating TF, expressed on MP derived from activated or apoptotic cells or blood cells, is thought to play the key role in venous thrombosis, since vessel-wall damage is not observed in thrombotic events in this compartment of systemic circulation [26]. Concerning procoagulant MP, the identification of increased levels of TF-positive MP in patients with cancer [34-37] or myeloproliferative disorders (MPD) [38] suggests their implication in thrombosis.

3.2.1 *Cancer and thrombosis*

Thromboembolic disease is a common complication in patients with cancer. It constitutes a leading cause of death in patients with adenocarcinoma, while about 20% of the total burden of venous thromboembolism is associated with malignancies [39]. Several experimental studies tried to elucidate the pathogenic mechanism of thrombosis in cancer since the original description of the association between cancer and coagulation by Armand Trousseau.

Increased TF expression in both stromal and neoplastic cells [40] has been demonstrated in many malignancies, including colorectal [41], pancreatic [42], prostate [37] and breast cancer [43], and is involved in the prothrombotic

state of patients with malignancies [39]. Enhanced TF expression by cancer cells depends on hypoxia or genetic transformation, including overexpression of the oncogenic EGFR, activation of K-ras oncogene or loss of function of the tumor suppressors p53 and phosphatase and tensin homologue (PTEN) [40].

Increased levels of TF-bearing MP have been detected in patients suffering from malignancy, especially pancreatic and breast cancer [34-37]. Interestingly, circulating TF has been proposed as a biomarker for the identification of patients at increased risk for thromboembolism [44]. Moreover, a correlation between TF-bearing MP levels and overt disseminated intravascular coagulation (DIC) associated with malignancy has been recently suggested [35]. A recent study in an animal model further demonstrated that TF-bearing MP derived from human cancer cells induced the activation of the coagulation cascade [34]. Moreover, MP derived from human pancreatic and lung cancer cells promoted thrombus formation after injection in an *in vivo* murine model, while endogenous cancer-derived MP contributed to the thrombogenic state in mice that developed ectopic tumors [45].

In summary, it is currently established that increased TF expression from cancer cells and the release of TF-bearing MP are implicated in the development of thromboembolic complications in patients with cancer.

3.2.2 *Myeloproliferative disorders (MPD)*

MPDs are a group of clonal hematopoietic disorders, characterized by increased production of leukocytes, erythrocytes and/or platelets. Essential thrombocythemia (ET) and polycythemia vera (PV) are included in this group of syndromes. These disorders are frequently associated with arterial and venous thrombotic complications. The JAK2 (V617F) mutation, which is detectable in 90 – 95% of cases of PV and in only 50 – 60% of ET cases, has been correlated with an increased risk for thrombotic events in patients with PV and ET [46,47]. Moreover, increased TF expression in monocytes [48], platelets [49] and polymorphonuclear cells [50] from patients with MPD was associated with thrombotic complications. A correlation between TF expression and JAK2 (V617F) mutation was further suggested [48,49]. It is of interest that treatment with hydroxyurea reduced the levels of TF in neutrophils from patients with ET and PV, providing a further linkage between circulating TF expression and disease pathogenesis [50]. A recent study also demonstrated increased TF-bearing MP derived from platelets in patients with ET [38], even though a direct correlation between thrombotic events and levels of MP was not indicated.

3.2.3 *Antiphospholipid syndrome (APS)*

Arterial and venous thrombotic events and recurrent miscarriages are common disease manifestations of APS. Even though the pathogenesis of this syndrome is not fully elucidated, accumulating evidence suggests a critical role for increased TF expression by blood cells in the prothrombotic tendency of APS [16,51]. It has been proposed that complement activation by antiphospholipid antibodies results in the generation of the anaphylatoxin C5a, which in turn induces TF production [16,51]. The enhanced circulating TF expression activates the coagulation cascade, resulting in thrombosis. On the other hand, TF expression in neutrophils has been associated with the obstetric manifestations of APS [52], as discussed in the following section.

3.3 Sepsis

DIC is a detrimental complication of sepsis and is characterized by the formation of thrombi in the microvasculature, resulting in multi-organ failure. Induction of circulating TF expression, due to the proinflammatory microenvironment that characterizes sepsis, has been associated with DIC [53]. Several experimental models of endotoxemia provide evidence for this linkage. Increased levels of TF-bearing MP have been observed in a human experimental model of endotoxemia [54]. Similar results were extracted by an analogous murine model, suggesting the use of TF-bearing MP as a biomarker for the evaluation of the risk for DIC [55]. Recently, a study in a non-human primate model of *Escherichia. coli*-driven sepsis provided evidence for the implication of complement activation in this process. Complement inhibition reduced the activation of the coagulation cascade and the progression to DIC by decreasing the expression of TF [56].

4. Non-thrombotic effect of TF-thrombin pathway

Several lines of evidence suggest a role for the TF–thrombin pathway in a growing spectrum of biological processes. It has been suggested that TF overexpression and the subsequent activation of serine proteases contribute to inflammation, tumor biology or fibrosis, implicating the coagulation cascade in the pathogenesis of various disorders. Signaling through PARs has been proposed as the mediating mechanism for the above mentioned effects. Four members (PAR1 – PAR4) constitute this family of G-protein-coupled receptors and are expressed in numerous cell types. The proteases of the extrinsic coagulation cascade can activate all four PARs. Thrombin is considered as a major activator of PAR1, PAR3 and PAR4, while TF–FVIIa targets PAR2 and FXa activates PAR1 and PAR2 [3]. Several intracellular pathways are activated downstream of PAR1 and PAR2 activation, including PI3K, Src family tyrosine kinases and the extracellular signal-regulated kinase (ERK)/MAPK pathway for PAR1 and PI3K, ERK/MAPK and the G-proteins Rac and Rho-A for PAR2 [57]. PAR activation results in the expression and release of several cytokines and chemokines and is implicated in a variety of biological functions [3].

In the following section clinical observations and experimental data supporting the non-thrombotic functions of the TF–thrombin pathway are presented (**Figures 1** and **2**).

4.1 Inflammatory responses

The TF–thrombin pathway has emerged as a significant player in inflammatory responses. The activation of this cascade seems to enhance inflammation in disease models including sepsis, ischemia–reperfusion (I/R) injury, arthritis and antiphospholipid syndrome (APS).

4.1.1 *Sepsis*

The crosstalk between the coagulation cascade and inflammation in sepsis is bidirectional. The inflammatory milieu in sepsis is able to induce TF expression, resulting in the activation of coagulation cascade [53,54,56]. The subsequent fibrin deposition plays a key role in DIC, as described in the previous section [55,56]. On the other hand, the activation of TF-thrombin pathway further induces inflammation, creating a vicious cycle.

Early studies in animal models demonstrated that inhibition of the TF–FVIIa complex by either active site-inhibited FVIIa (FVIIai), or tissue factor pathway inhibitor-1 (TFPI-1) resulted in decreased expression of the proinflammatory cytokines IL-6 and IL-8 and reduced mortality [57,58]. Activation of PARs was further implicated in the crosstalk between coagulation cascade and inflammatory responses. In an animal model for endotoxemia, both TF deficiency and combined inhibition of thrombin and deficiency in PAR2 reduced inflammation [59].

The beneficial effect of the TF–thrombin pathway inhibition in abolishing inflammation and preventing DIC in animal septic models led to the investigation of the effect of pharmaceutical intervention with the natural anticoagulants, TFPI-1, antithrombin-III (ATIII), and activated protein C (APC), in animal models of sepsis [57,58,60-62]. The promising results obtained from these studies prompted the initiation of clinical trials for the assessment of the effectiveness of these natural anticoagulants in sepsis, as discussed in a following section.

4.1.2 *Ischemia–reperfusion (I/R) injury*

In addition to the key role of the coagulation cascade in atherothrombotic disease, the TF-thrombin pathway is involved in the inflammatory process associated with I/R, contributing in both morbidity and mortally of acute myocardial infarction and stroke. A study in an animal model revealed that the inhibition of TF and thrombin reduced the size of myocardial infarction in a manner independent of fibrin deposition. This study proposed that TF and thrombin mediate their effect through enhanced inflammation [63]. Moreover, fibrin-derived products have been implicated in I/R injury pathogenesis in a rat model of myocardial infarction [64]. Studies in murine models of myocardial I/R and cerebral ischemia further supported this suggestion, demonstrating the implication of PAR1 in myocardial remodeling and left ventricular hypertrophy [65] and in infract size, respectively [66]. Considering the effect of TF–FVIIa complex and PAR2 in this process, there are controversial reports. It has been recently shown that the inhibition of TF–FVIIa complex with FVIIai significantly reduced I/R injury in a murine model of myocardial I/R injury [67]. However, PAR2 activation has been shown to provide beneficial results in a rat model of myocardial I/R injury [68]. Despite the above experimental data, there no reported clinical data assessing the role of anticoagulation with the natural anticoagulants in I/R injury.

4.1.3 *Antiphospholipid syndrome (APS)*

Recurrent miscarriages frequently complicate the disease course of APS. Pregnancy morbidity was originally thought to depend on thrombosis of the placenta. However, recent experimental data suggest a role for TF–FVIIa-complex-dependent induction of inflammation in this process.

Experimental evidence indicates that complement activation due to antiphospholipid antibodies results in the overexpression of TF in the surface of neutrophils [16,52]. TF–FVIIa signaling through PAR2 in neutrophils was demonstrated to induce oxidative stress. The resultant production of reactive oxygen species after PAR2 activation was further suggested to lead to trophoblast oxidative injury and pregnancy loss [69]. An interesting observation is that statins reduced both TF and PAR2 expression and prevented pregnancy loss [69]. However, it has to be demonstrated whether the use of statins is beneficial in clinical practice.

4.1.4 *Arthritis*

Clinical and experimental evidence also implicates the TF–thrombin pathway in the pathogenesis of arthritis. Several studies demonstrate the activation of coagulation and fibrinolysis in synovial fluid from patients with rheumatoid arthritis [70]. Furthermore, increased functional TF activity was observed in synovial membranes from patients with rheumatoid arthritis compared with those from patients with osteoarthritis. This study further associated inhibition of TF signaling by FVIIai with decreased synovial inflammation as detected by the measurement of synovial thickness and articular cartilage damage in murine antigen-induced arthritis [71]. Signaling through PAR2 may be implicated in this process due to the observed inhibition of joint inflammation in PAR2-deficient mice in an adjuvant monoarthritis model of chronic inflammation [72].

4.2 Tumor biology

Accumulating evidence correlates TF expression by malignant cells with many aspects of the pathogenesis of cancer. Increased TF expression has been associated with aggressive and metastatic disease in breast [44], colorectal [42] and pancreatic cancer [43,73]. Apart from the implication of TF in venous thrombosis in cancer patients, a key role for TF–FVIIa and thrombin in metastasis, angiogenesis and tumor growth has been suggested. Enhanced TF expression [41-44,73] and ectopic synthesis of FVII [74] by cancer cells has been reported and implicated in tumor progression.

The vast majority of experimental data in various models of malignancy indicates that TF–FVIIa–PAR2 signaling contributes in tumor neoangiogenesis mainly due to the induction of VEGF. TF expression was correlated with VEGF expression and microvessel density in resected pancreatic cancer [43], while silencing of TF gene retarded (although not abrogated) tumor growth *in vivo* due to inhibition of angiogenesis in colorectal cancer [75]. Moreover, TF inhibition and PAR2 deficiency reduced proliferation and migration of malignant glioma cells *in vitro* [76], while TF–FVIIa inhibition impaired hepatic metastasis in colorectal cancer models [77]. Finally, it has been demonstrated that the administration of an inhibitory antibody of direct TF–FVIIa signaling attenuated tumor growth in a murine model, while PAR2 deficiency resulted in similar inhibition of the TF–FVIIa complex [78]. asTF has also been reported to induce angiogenesis independent of PAR2 activation but dependent on integrin function [24].

While the TF–FVIIa complex contributes primarily to tumor growth and angiogenesis, thrombin generation seems to be implicated in the progression to metastatic disease. Thrombin generation results in the formation of fibrin and platelet-rich envelopes that protect metastatic cancer cells from clearance by natural killer cells [79]. Experimental data demonstrated that endogenous thrombin generation enhances the metastatic capacity of cancer cells through PAR1 signaling [80] or cathepsin D upregulation [81]. PAR1 silencing has been further reported to attenuate metastasis in malignant melanoma [82]. The implication of PAR1 in the metastatic process is also suggested by the increased expression of PAR1 in cancer cells [83] and in neoplastic cells from patients with metastatic melanoma cells compared with dysplastic nevi and primary melanoma [84].

4.3 Fibrosis

The activation of the extrinsic coagulation cascade has been implicated in fibrosis as well. PAR-1 signaling has been shown to induce connective tissue growth factor (CTGF) expression. In accordance with this, the *in vitro* activation of lung fibroblasts from patients with systemic sclerosis for CTGF production and collagen deposition in a thrombin-dependent manner has been recently demonstrated. This effect was attenuated in fibroblasts treated with the thrombin inhibitor dabigatran [85].

Enhanced TF pathway activity has been reported in patients suffering from pulmonary fibrotic disorders [13,86]. The involvement of the coagulation pathway in pulmonary fibrosis is further indicated by the beneficial effect of anticoagulant therapy in patients with idiopathic pulmonary fibrosis [87].

Moreover, TF–FVIIa complex participates in wound healing, as reported by a recent study in a murine *in vivo* model [88]. In addition, impaired wound healing has been shown in a murine model of hemophilia B, which was partially restored after administration of FIX or FVIIa [89].

5. Anticoagulation therapies

In this section, moving on from the well established antithrombotic effect of anticoagulation regimens, we will discuss the clinical benefit of such treatment in non-thrombotic manifestations of the previously mentioned disorders (**Figure 3**) (**Table 1**). On the other hand, treating physicians need to take into account the risk of bleeding with anticoagulation therapy, and administer anticoagulant agents when the benefits outweigh the risks.

5.1 Natural anticoagulants

Beneficial effects have been documented for the natural anticoagulants TFPI, ATIII, and recombinant human activated protein C (APC) in animal models of sepsis [56,58,61] and in Phase II clinical trials [90-92]. However, these effects were confirmed only for human recombinant APC in a Phase III clinical trial [93]. This regimen is currently approved for the treatment of adult patients with severe sepsis with multiple organ failure in the USA and Europe. However, several concerns have been raised regarding the approval and the post-marketing surveillance of APC in the management of severe sepsis [94]. It thus remains to be further evaluated for which patient and at which specific time-point during the septic process APC is beneficial for the management of septic patients.

5.2 TF–FVIIa inhibitors

Nematode anticoagulant protein c2 (NAPc2) is a potent anticoagulant that targets the TF–FVIIa complex, abrogating thrombin generation. It was originally isolated from a haematophagous hookworm, *Ancylostoma caninum* [95]. The safety and the effectiveness of the recombinant form of NAPc2, rNAPc2, in the treatment in patients undergoing elective coronary angioplasty and for the prevention of venous thromboembolism after total knee arthroplasty has been evaluated in Phase I clinical trials [96,97]. Moreover, rNAPc2 provided beneficial results in the treatment of patients with non–ST-segment elevation acute coronary syndrome, while it was well tolerated [98].

Studies in experimental models evaluated the effect of the inhibition of TF–FVIIa complex signaling by rNAPc2 in cancer biology and inflammation. rNAPc2 has shown effectiveness in the inhibition of tumor growth and metastasis in an animal experimental model of colorectal cancer by inhibiting TF [99]. It is of interest that the administration of this agent in a primate model of Ebola hemorrhagic fever prolonged the survival of infected animals by attenuating the coagulation and proinflammatory response [100]. However, rNAPc2 failed to ameliorate the host defense in a murine model of pneumococcal pneumonia and a similar model of abdominal sepsis, despite the proper inhibition of TF–FVIIa activity [101,102].

TF–FVIIa complex is also inhibited by Ixolaris, a tick salivary protein with a function similar to TFPI. Experimental

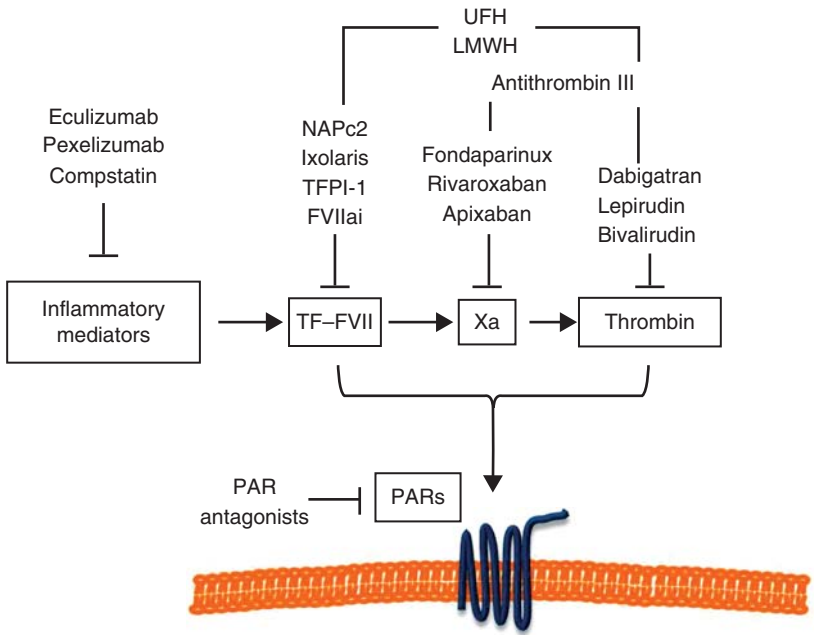

Figure 3. Targeting the TF–thrombin axis in non-thrombotic disorders. Several agents that interfere with the components of TF-thrombin axis have been developed. Clinical evidence suggests an antimetastatic effect of unfractionated heparin (UFH) and low-molecular-weight heparins (LMWH), while the data for their anti-inflammatory effect are inconclusive. Antithrombin III, TFPI and FVIIa inhibitors (FVIIai) have shown an anti-inflammatory potential in experimental models, which was not confirmed by clinical studies for the treatment of sepsis. Recombinant Nematode anticoagulant protein c2 (rNAPc2) and ixolaris are inhibitors of the TF–FVIIa complex, which have shown effectiveness as antimetastatic agents in experimental models. The anti-inflammatory and anti-metastatic effects of the recently developed factor Xa and thrombin inhibitors have not been extensively evaluated yet. The administration of agents that prevent the upregulation of TF expression by blocking complement activation or inhibit protease activated receptor (PAR) signaling may emerge as future strategies that target the TF-thrombin axis.

TFPI: TF pathway inhibitor.

data demonstrated a potent antithrombotic activity for ixolaris [103]. Moreover, a study in an experimental glioblastoma model provided evidence for the effectiveness of this agent in the inhibition of angiogenesis and tumor growth [104].

5.3 Heparins

Heparins can be divided according to their structure and pharmacologic profiles into different groups: unfractionated heparin (UFH), low-molecular-weight heparins (LMWH) and the synthetic pentasaccharides fondaparinux and idraparinux [105,106].

Although UFH is highly efficacious in the treatment of venous thromboembolism (VTE), its clinical use has been restricted due to the intravenous route of administration, the need for continuous laboratory monitoring and the relatively common occurrence of heparin-induced thrombocytopenia (HIT) [105].

LMWH have replaced UFH in the treatment and prevention of venous and arterial thromboembolism. LMWH show similar efficacy to UFH, while they are administered subcutaneously in weight-adjusted fixed dosages with no need for laboratory monitoring, rendering feasible the administration in the outpatient setting. However, the risk for HIT is still present with LMWH, even though in a significantly lower percentage of patients compared with UFH [105].

Fondaparinux and idraparinux are synthetic pentasaccharides that promote the inactivation of FXa by antithrombin. Fondaparinux is approved for the prevention and treatment of DVT and PE following orthopedic and abdominal surgery and for the treatment of acute coronary syndromes. Major bleeding events occurred infrequently, in a rate similar to that observed in patients under treatment with LMWH. Idraparinux has a very long half-time and it is administered subcutaneously once-weekly. Clinical trials have evaluated the efficacy of idraparinux in the treatment and secondary prophylaxis of DVT and PE and the long-term prevention of stroke in patients with atrial fibrillation [106].

Apart from their antithrombotic activity, an anti-inflammatory role has been suggested for heparins. Experimental data demonstrated a beneficial effect of heparins in animal models of chronic inflammation, like colitis [107] or arthritis [108]. On the other hand, there are contradictory clinical data for the efficacy of UFH and LMWH in inflammatory disorders [109,110]. The anti-inflammatory effect

Table 1. Potential non anti-thrombotic effects of anti-coagulants.

	Targeted factors	Anti-metastatic effect	Anti-inflammatory effect
Heparins			
UFH	IIa, Xa, IXa, XIa, XIIa	Experimental and clinical evidence [113-115]	Experimental and clinical evidence [108]
LMWH	IIa, Xa	Experimental and clinical evidence [112-115]	Experimental evidence, contradictory clinical data [107,109-111]
Fondaparinux	Xa	No	No
Vitamin K inhibitors	II, VII, IX, X	No	No
New oral anticoagulants			
Dabigatran	IIa	Not assessed	Not assessed (experimental evidence for anti-fibrotic action) [85]
Rivaroxaban	Xa	Not assessed	Not assessed
Apixaban	Xa	Not assessed	Not assessed
TF–FVIIa inhibitors			
NAPc2	TF–FVIIa	Experimental data [99]	Contradictory experimental data [101-102]
Ixolaris	TF–FVIIa	Experimental data [104]	No

LMWH: Low-molecular weight heparins; NAPc2: Nematode anticoagulant protein c2; UFH: Unfractionated heparin.

of heparins is independent from their anti-coagulant activity and has been attributed to their ability to interact with selectins and to inhibit complement activation [111].

The interaction between the polysaccharide chain of heparins and selectins is suggested to mediate the anti-metastatic effect of heparins [112]. Treatment with LMWH has been shown to improve the survival in cancer patients [113]. Apart from the reduction of thrombosis-dependent morbidity and mortality, an independent-to-anticoagulation effect was suggested by the prolonged survival of patients with non-metastatic cancer under treatment with LMWH compared with those receiving vitamin K antagonists. This effect was not observed in patients with metastatic cancer, suggesting an anti-metastatic effect for LMWH [113]. These clinical observations were confirmed by experimental studies in animal models [114,115]. The specificity of the interaction between heparins and selectins in their anti-metastatic potential is further indicated by the inability of fondaparinux, which lacks a polysaccharide chain, to attenuate metastasis in an experimental model [112].

5.4 Vitamin K antagonists (VKA)

Warfarin and other VKA are effective in the primary and secondary prevention and treatment of VTE. However, the necessity for frequent laboratory monitoring, the drug interactions and the severe bleeding adverse events constitute limitations to their clinical use [116]. Moreover, an anti-metastatic effect was not demonstrated for VKA, as previously mentioned [113]. This observation, along with an increase in bleeding events in cancer patients receiving VKA and antineoplastic agents, render LMWH as a preferable treatment option in patients with malignancy [113].

It is of interest that a study in patients with idiopathic pulmonary fibrosis demonstrated a beneficial effect for warfarin and LMWH in the progression of the disease [87]. The latter has to be further evaluated.

5.5 New oral anticoagulants

The limitations in the clinical use of heparins and VKA prompted the development of new oral anticoagulants: dabigatran that specifically inhibits thrombin, and rivaroxaban and apixaban that inhibit FXa [106].

Phase III clinical trials have documented the efficacy of dabigatran in the prevention of VTE after orthopedic surgery [117] and in long-term treatment and secondary prevention of VTE [118]. Furthermore, in a Phase III clinical trial in patients with atrial fibrillation, dabigatran was associated with rates of stroke and systemic embolism that were similar to those associated with warfarin but lower rates of major hemorrhage at a dose of 110 mg, while at a dose of 150 mg was associated with lower rates of stroke and systemic embolism but similar rates of major hemorrhage as compared with warfarin [119]. Even though experimental data suggest a significant beneficial effect of thrombin inhibition in critical functions like inflammation and tumor metastasis, this effect has to be evaluated in clinical studies.

Rivaroxaban and apixaban are direct inhibitors of FXa given orally. These agents were superior to LMWH or warfarin in the prevention of VTE after orthopedic surgery [120,121]. Several data also demonstrate their effectiveness in the treatment of VTE [122,123]. Clinical trials are currently evaluating the effectiveness of these agents in the prevention of cardioembolic stroke in patients with atrial fibrillation [124].

5.6 Hirudin analogues

Lepirudin and bivalirudin are included in this group of pharmacological agents. Both agents act as direct thrombin inhibitors.

Lepirudin is a recombinant analogue of the leech protein hirudin. Unlike heparins, it binds directly and irreversibly to thrombin, resulting in its neutralization. Lepirudin is used as an antithrombotic agent in patients with HIT [125].

Bivalirudin is a synthetic peptide which specifically binds and inhibits both circulating and clot-bound thrombin [126]. Clinical studies have demonstrated its effectiveness in the treatment of patients with acute coronary events undergoing percutaneous coronary intervention [127,128]. Moreover, the efficacy of bivalirudin was evaluated in patients with HIT during cardiopulmonary bypass [129].

6. Conclusion

Several experimental and clinical lines of evidence currently suggest a critical role for the TF–VIIa complex in human biology. The description of TF–VIIa complex, FXa and thrombin signaling pathways implicates the coagulation cascade, apart from the well-established role in thrombosis, in the pathogenesis of inflammatory disorders and malignancy. This implication is the springboard for the investigation of the clinical effect of anticoagulation therapies in the treatment of the above mentioned disorders. Moreover, new anti-thrombotic regimens are nowadays available, which offer the opportunity for therapeutic intervention in both thrombotic and non-thrombotic coagulation-cascade-dependent disorders. However, the risk of major bleeding events in patients under anticoagulation treatment is considerable and raises concerns for their use in the treatment for non thrombotic disorders. The development of agents with favorable safety profile and tolerability could enable the broader use of anticoagulants in the treatment of both thrombotic and non thrombotic disorders.

7. Expert opinion

A new era for anti-coagulation therapeutic strategies emerges in view of the novel agents with improved pharmacokinetics and safety profiles. The efficacy of the recently developed regimens in the prevention and treatment of thromboembolism has been evaluated, providing alternative solutions. However, extensive basic and clinical research is needed to evaluate whether FXa and thrombin inhibitors exert possible anti-inflammatory and/or anti-neoplastic effects as presumed from the currently available data that suggest a beneficial effect of thrombin and/or PAR inhibition or deficiency in experimental models of inflammation and cancer. Moreover, the development and application of PAR inhibitors in clinical practice may be an alternative prospective step in the treatment of diseases and syndromes like sepsis, I/R injury or cancer, which constitute a major challenge for the present therapeutic strategies.

Another option for intervening in the activity of the TF–thrombin pathway is the downregulation of the increased TF expression that is responsible for the thrombotic and non-thrombotic manifestations observed in several disorders. The inhibition of the inflammatory mediators implicated in the induction of TF expression is a potential target for therapeutic intervention. In this direction, the inhibition of complement activation by the administration of the recently developed monoclonal antibodies targeting C5, pexelizumab and eculizumab, could be beneficial for a group of disorders whose pathogenesis depends on the cross-talk between C5a and TF. Patients under extra-corporal circulation, like patients undergoing hemodialysis or coronary artery bypass surgery, or patients with APS and sepsis frequently suffer from thrombotic manifestations associated, at least in part, with the increased TF expression driven by the generation of C5a. Recently, a study in a non-human primate model demonstrated that the inhibition of complement activation using compstatin prevented sepsis-induced coagulopathy by downregulating TF and attenuated multi-organ failure [56]. Moreover, a recent study reported that complement activation, induced by the biomaterials of hemodialysis, results in the overexpression of circulating TF, suggesting an implication in the pro-thrombotic state of end-stage renal disease patients. *Ex vivo* and *in vitro* studies suggested that treatment with compstatin precluded TF overexpression [17]. Complement inhibition with compstatin also prevented the induction of TF expression in leukocytes incubated with sera from patients with APS [16]. Considering APS, an animal model suggested a beneficial effect of treatment with statins in the obstetric manifestations of the syndrome via TF downregulation [52], suggesting an additional treatment strategy targeting TF. Moreover, the inhibition of C5a by pexelizumab ameliorated the prognosis in patients undergoing coronary artery bypass surgery, which could be attributed, at least in part, to the induction of TF expression [130]. In addition, the administration of eculizumab in patients with paroxysmal nocturnal hemoglobinuria had a beneficial effect on the thrombotic manifestations of the disease [131]. The imperative need for targeted therapeutic agents for the treatment of disorders with large effects on public health motivates the experimental efforts towards novel directions.

Another significant area of research is the identification of possible additional indications for anti-coagulation agents. Existing experimental and clinical data suggest a role for LMWH in the progression of cancer by attenuating metastasis. The elucidation of the anti-neoplastic mechanism of action of LMWH would break new ground in the investigation of the pathogenesis of metastasis and in the treatment of cancer. It would be of great interest to determine whether the administration of LMWH as an additional to chemotherapy strategy would ameliorate the disease course in all or a subgroup of patients with specific cell type or grade of malignancy, independently or not from the prevention of thrombotic events. The recently introduced anti-coagulation agents could contribute to the expansion of the therapeutic options in cancer patients, if proven effective, especially due to their improved safety profile.

In conclusion, further research is needed for the development of anti-coagulation agents with better safety profile and pleiotropic actions, which would reinforce our arsenal for the treatment of disorders with increasing morbidity and mortality.

Declaration of interest

The authors state no conflict of interest and have received no payment in preparation of this manuscript.

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