Clinical Significance of Tissue Factor–Exposing Microparticles in Arterial and Venous Thrombosis

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Abstract

Microparticles (MP) are small extracellular vesicles (30–1,000 nm) that are released from activated cells or platelets. Exposure of negatively charged phospholipids and tissue factor (TF) renders MP procoagulant. Normal plasma levels of intravascular TF-exposing MP (TFMP) are low, but their number may rise in pathological conditions, including cancer and infectious disease. Emerging evidence indicates an important role for these circulating TFMP in the pathogenesis of thrombotic complications such as venous thromboembolism and disseminated intravascular coagulation, whereas their contribution to arterial thrombosis is less studied. Despite serious limitations of the currently available assays for measuring TFMP levels or the procoagulant activity associated with TFMP with respect to sensitivity and specificity, the scientific interest in TFMP is rapidly growing because their application as prognostic biomarkers for thrombotic complications is promising. Future advances in detection methods will likely provide more insight into TFMP and eventually improve their clinical utility.

Keywords
- tissue factor
- microparticles
- venous thromboembolism
- arterial thrombosis
- extracellular vesicles

Normal hemostasis is a tightly regulated process that keeps the blood in a fluid state, yet permits the rapid closure of damaged blood vessels. Imbalances in this system may result in thrombosis, a common disease characterized by intravascular blood clotting leading to partial or complete obstruction of an artery or vein. Arterial thrombosis, including myocardial infarction and ischemic stroke, is the leading cause of death worldwide.[1] Its counterpart, venous thromboembolism (VTE), comprising deep vein thrombosis (DVT) of the leg and pulmonary embolism (PE), is also a major contributor to global disease burden.[2]

Although arterial and venous thrombosis both involve the occlusion of blood vessels, their pathophysiology is different. Arterial thrombosis is often the ultimate complication of atherosclerotic lesions that have formed over the course of many years.[3] Upon disruption of an atherosclerotic plaque, tissue factor (TF) exposed by macrophages and subendothelial structures triggers thrombin generation and platelet activation. These activated platelets play a predominant role in this process because of their ability to aggregate under high shear stress conditions. Classical risk factors for atherosclerosis and subsequent arterial thrombosis are dyslipidemia, hypertension, smoking, and diabetes mellitus.

In contrast, VTE is believed to occur as a result of changes in blood flow, hypercoagulability, or activation of the endothelium. This is known as Virchow triad, named after the German pathologist who in the 19th century postulated his theory of causative factors for VTE.[4] Indeed, pathological conditions that are risk factors for the development of VTE either induce hypercoagulability such as cancer and hereditary thrombophilia, or endothelial damage such as surgery or trauma, or venous stasis such as immobility and venous insufficiency.

The most likely sites of venous thrombus initiation are the valve pockets, which are characterized by a turbulent blood

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flow and low oxygen tension.[5] The local flow conditions are assumed to facilitate thrombus formation by concentrating various blood-borne, procoagulant factors, whereas hypoxia leads to activation of the endothelium. The specific triggers that ultimately shift the balance toward undesirable blood clotting under these conditions have not been fully elucidated. However, emerging evidence suggests a key role for microparticles (MP)-exposing TF in the pathogenesis of some forms of VTE. In this review, we will focus on the association between TF-exposing MP (TFMP) and (1) VTE in otherwise healthy patients, (2) VTE in cancer patients, (3) VTE in patients with bacterial infections, and (4) patients with arterial thrombosis.

Extracellular Vesicles

MP are small (30–1,000 nm) extracellular membrane vesicles which are released from cells, such as endothelial cells and leukocytes, or activated platelets.[6] In this review, we will use the term MP as a collective term for all extracellular vesicles present in the blood, which may also include exosomes and other types of vesicles. The capacity of cells and platelets to release MP, that is, vesiculation, was already acknowledged in the 1960s, although MP were initially thought to be inert cellular trash.[7] Advances in the detection and characterization of MP over the past two decades have revealed that vesiculation is an important physiological process that contributes to highly specific intercellular communication. MP contribute to coagulation, inflammation, angiogenesis, and cellular waste management but also play a role in a variety of pathological conditions such as infections, autoimmune diseases, and cancer.[8] Hence, MP have gained scientific as well as clinical interest as potential biomarkers for disease detection or prediction.

Procoagulant Microparticles

The role of MP in venous and arterial thrombosis is predominantly determined by the nature of their surface rather than their contents. Since most MP have a diameter of less than 100 nm, circulating MP provide a surprisingly large surface area, whereas their concurrent volume is small.[9] The membrane of circulating MP is thought to have procoagulant properties due to exposure of negatively charged phosphatidylserine (PS), an essential cofactor for the formation of various complexes of clotting factors. Because the exposure of PS is easily affected by blood collection and handling conditions, and increases during imposed conditions such as centrifugation and storage, to which extent circulating MP expose PS in vivo is still debated.[6] The procoagulant potential of MP increases dramatically when they expose the transmembrane protein TF, which may be inherited from various parent cells such as leukocytes, endothelial cells, and tumor cells (►**Fig. 1**), or perhaps from fusion between MP via specific receptor-ligand interactions.[10] The TF exposure provides the MP with the capacity to actually perform the initial step of the coagulation activation. Thus, TFMP both initiate and support the coagulation activation process, whereas MP that do not expose TF constitutively (such as erythrocyte-derived MP) may only support the coagulation process via PS exposure.

Under normal conditions, the blood levels of circulating TFMP are low, and TF is almost exclusively present in subendothelial tissue. Upon vascular injury, however, subendothelial TF becomes exposed to the blood stream and rapidly forms a complex with circulating coagulation factor (F) VII or its activated form, FVIIa, thus initiating coagulation.[11] Because coagulant and noncoagulant forms of TF are known, circulating TFMP can also be present in a dormant, noncoagulant form.[10]

Under pathological conditions, the release of coagulant TFMP can increase in a response to a stimulus, such as bacterial lipopolysaccharide (LPS) in the case of leukocyte-derived TFMP, or spontaneously, for example, tumor-derived TFMP. The presence of intravascular coagulant TFMP is unlikely to cause instantaneous blood clotting because such MP probably will have to accumulate locally in sufficient quantities for the coagulation process to be initiated. One has to bear in mind, however, that various physiological mechanisms will try to inhibit intravascular coagulation, thereby reducing the risk of thrombus formation. These mechanisms include the presence of plasma coagulation inhibitors, such as TF pathway inhibitor (TFPI), and efficient MP clearance mechanisms. It has been postulated that TFMP cause blood clotting by local deposition of TFMP to the surface of activated platelets at sites of endothelial damage (►**Fig. 2**).[12,13] Alternatively, TFMP accumulation at low-flow sites, such as the pockets of venous valves, may be sufficient to overcome the natural thresholds of the anticoagulant systems (►**Fig. 2**). In summary, high levels of circulating MP-exposing coagulant TF may predispose patients to thrombotic complications, such as disseminated intravascular coagulation (DIC) or cancer-associated VTE.

Measuring TF-Exposing MP

Measurement of intravascular TFMP is challenging because of the low concentration, even under pathological conditions. Furthermore, TF can be present but in a dormant, noncoagulant form, whereas the ability of coagulant TF to initiate clot formation is so strong that only minute amounts of TF are sufficient. Methods to quantify TFMP are either antigen- or activity-based.

To measure the quantity of exposed (full length) TF antigen associated with MP, TF-specific antibodies have to be used in, for example, enzyme-linked immunosorbent assay (ELISA) or flow cytometry. Apart from detection limitations, which we will briefly discuss, the specificity of the antibodies is of utmost important. However, antibodies against (human) TF are often poorly characterized, hampering interpretation of such assays.

ELISA will provide information about the total concentration of TF that is associated with MP, but the detection limit of currently used ELISAs is higher than the concentration of TF that is required to initiate coagulation. In addition, ELISAs are not able to distinguish dormant TF from coagulant TF and hence will not provide insight into the concentration of *coagulant* TF that is associated with MP.

In contrast to ELISA, flow cytometry is capable of detecting *single* MP-exposing TF. Again, apart from the problem

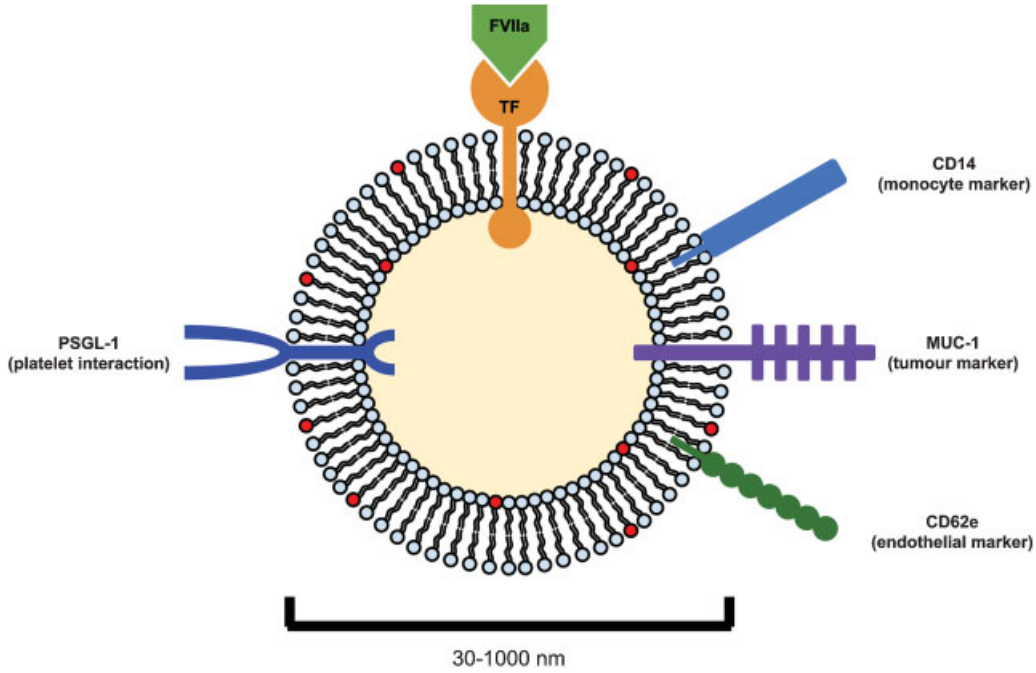

Fig. 1 Coagulant properties ofMP. MP are submicron extracellular membrane vesicles with can have procoagulant properties due to (1) exposure of negatively charged phosphatidylserine in the outer membrane (red dots) and (2) the presence of TF, which initiates coagulation upon binding to its natural ligand FVIIa. MP may interact with platelets via binding of PSGL-1 to P-selectin exposed on activated platelets. MP that expose TF (TFMP) are typically derived from monocytes, tumor cells, or endothelial cells. The cellular origin of TFMP can determine by flow cytometry using antibodies for specific markers (CD14 for monocytes, MUC-1 for tumor cells, and CD62e for endothelial cells). In this figure, all markers are shown on a single MP, but some MP will expose TF, whereas many others will not, and the markers CD14, MUC-1, and CD62e will only be exposed on the MP from parent cells carrying those molecules. FVIIa, factor VII(a); MP, microparticles; PSGL, P-selectin glycoprotein; TF, tissue factor; TFMP, TF-exposing MP.

whether the detected TF has any coagulant activity, the sensitivity of flow cytometers is hampered by the small size of most MP (< 100 nm), which are below the detection range. For conventional flow cytometry, the minimum detectable MP size is estimated at 270 to 600 nm.[14] Consequently, most currently available flow cytometers will detect only approximately 1% of the total MP population.[15] More recent techniques, including dedicated flow cytometry, can detect MP as small as 150 nm, but even then the smallest TFMP remain undetected.[14] Finally, it is likely that the number of TF molecules exposed on small TFMP (< 100 nm) is too low to generate sufficient fluorescence signal, even when all TF molecules would bind a specific fluorescent-probe labeled TF antibody. One should bear these limitations in mind when interpreting the currently available literature.

Functional assays that measure the procoagulant activity (PCA) associated with TFMP are preferred above antigen-

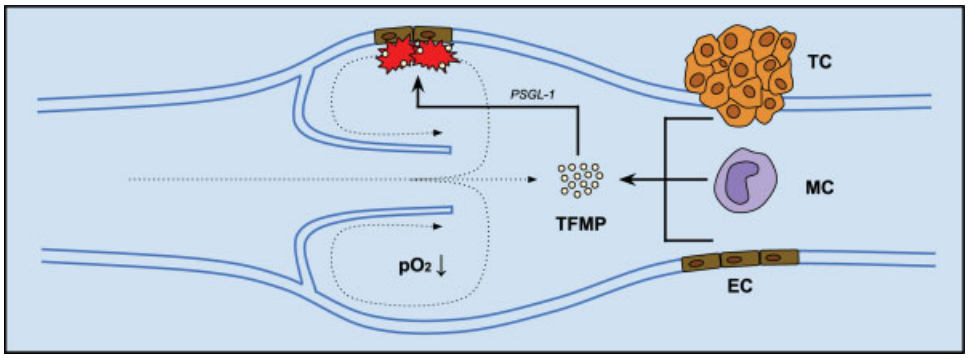

Fig. 2 TFMP in the pathogenesis of deep venous thrombosis. TFMP are primarily released by TC, MC, or EC upon various stimuli. These TFMP may accumulate at sites of initial vascular injury by binding to activated platelets, thereby promoting coagulation locally. Sites particularly prone for development of thrombosis are the pockets of venous valves. Relative stasis due to turbulent flow and hypoxia-induced endothelium activation facilitate the formation of a venous thrombus. EC, endothelial cells; MC, monocytes; PSGL, P-selectin glycoprotein TC, tumor cells; TFMP, tissue factor-exposing microparticles.

based assays, because the functional assays are, in general, more sensitive, not affected by dormant TF, and measure the overall TF coagulant activity, even when part of such TF activity would be provided by the small (< 100 nm) MP. Because coagulation can also be initiated by contact activation, the contribution of coagulant TF can be demonstrated only by performing coagulation tests in the absence and presence of antibodies that inhibit either TF or FVIIa. Clearly, also here the specificity of applied antibodies will affect the outcome.

The most widely used functional assay is a two-stage clotting assay that measures total TF-dependent FXa generation.[16] In short, MP are isolated by centrifugation and then incubated with FVIIa, FX, and either an anti-TF antibody or control antibody. After recalcification, total generated FXa is measured by adding a chromogenic substrate for FXa. The TF-dependent FXa generation is then determined by subtracting the amount of FXa generated in the presence of the anti-TF antibody from the amount of FXa generated in the presence of the control antibody. Other functional assays determine either TF-dependent FXa generation or TF-dependent fibrin generation, for example, the "fibrin generation test," in the presence and absence of an anti-FVIIa antibody.[17,18] In a head-to-head comparison applied to frozen/thawed plasma samples from cancer patients and controls, the TF-dependent FXa generation test and the fibrin generation test showed no differences with regard to sensitivity and specificity for the occurrence of VTE.[19] Recently, a commercial ELISA-based TFMP activity assay became available, but this assay had lower sensitivity and specificity compared with the TF-dependent FXa generation test.[20]

In this relatively young research field, the differences across the currently available quantitative and functional assays are not to be ignored, and there is no consensus regarding a gold standard. Preanalytic variables, including blood handling, centrifugation, and MP preparation, may all have a large impact on TFMP level and activity measurements, although attempts for standardization have been made.[21] A major factor may be analyses of fresh versus stored MP, with the latter involving freezing and thawing of the MP samples. In addition, most tests use essentially different methods to measure TFMP, all with their own strengths and limitations, hampering valid comparisons between the assays at present. In the near future, comparative studies will be essential to standardize measurements between laboratories, which in turn are a prerequisite for acceptance of MP as biomarkers.

TF-Exposing MP in Healthy and Diseased Individuals

Despite the technological advances in TFMP detection methods, it is still under debate whether TFMP circulate under normal conditions. TFMP are detected in blood of healthy individuals using conventional flow cytometry,[17,19,22,23] but it is not clear whether this actually reflects a pool of coagulant TFMP or merely is the result of a background signal generated by the TF antibody. With respect to functional TFMP assays, TFMP PCA in healthy subjects is usually below the detection limits of current methods, hampering accurate measurements.[24] In the absence of solid evidence for the constitutive presence of blood-borne TFMP, one could only speculate on their biological significance. If present, they may exhibit a coagulant effect only when they adhere to activated platelets hence providing an extra source of TF when the initial clot has already covered the exposed subendothelial TF. Conversely, low levels of TFMP could also protect healthy subjects from thrombosis by promoting low-grade thrombin generation and subsequently the activation of the natural anticoagulant protein C by thrombin. In view of these hypotheses, thrombosis caused by TFMP under pathological conditions could be regarded as derailment of an otherwise physiological process.

In contrast, body fluids exposed to the "milieu extérieur" such as seminal fluid,[25] synovial fluid,[26] urine,[18] and saliva[18] of *healthy* human subjects all contain high levels of coagulant TFMP. A likely reason for the presence of TFMP in these fluids is that they provide a permanent, additional source of extravascular TF to facilitate hemostasis upon injury, thereby minimizing blood loss and reducing the risk of infection. In contrast, under normal, physiological conditions, no TFMP coagulant activity is detectable in plasma from healthy humans.[27]

Notwithstanding, the biological significance of TFMP under normal conditions in body fluids other than blood, the occurrence of TFMP so far has been studied mostly in blood under *pathological* conditions, including cancer,[28] bacterial infections,[29] human immunodeficiency virus infection,[30] trauma,[31] diabetes,[10] obesity,[23] acute respiratory distress syndrome,[32] and recurrent pregnancy loss.[33] The association of circulating TFMP with these diseases suggests that their presence is not without risk, and they have been linked to hypercoagulability and thrombosis. Indeed, infusion of human TFMP in rats showed massive thrombus formation in the vena cava inferior, which could be blocked by an antibody against human TF, directly illustrating the risk of such circulating TFMP within the blood.[34]

Role of TF-Exposing MP in the Pathogenesis of Unprovoked Venous Thromboembolism

A substantial proportion of patients experiencing VTE have no apparent risk factors at the time of diagnosis, that is, unprovoked VTE. Thaler et al recently investigated whether circulating TFMP contribute to the pathogenesis of VTE in a prospective cohort study of 30 patients presenting with an acute, unprovoked DVT. At baseline, no differences were observed in TF antigen levels and TFMP PCA between subjects and healthy controls. Moreover, TFMP PCA in subjects remained unchanged during 1 year of follow-up.[35] Similarly, TFMP PCA was not associated with confirmed PE in a case–control study of 159 patients with clinically suspected PE.[36] Campello et al observed higher TFMP levels in 30 patients with unprovoked VTE compared with controls using conventional flow cytometry but did not perform a functional assay to confirm these findings.[37] Taken together, the contribution of circulating TFMP to the development of VTE in otherwise healthy patients seems limited, although the shortcomings of the various TFMP

assays and the case–control design of the studies preclude any definite conclusions.

TF-Exposing MP and Cancer-Associated VTE

The association between TFMP and VTE is by far best studied in patients with an active malignancy.[28] Cancer patients have a four- to sevenfold increased risk of VTE, partly as a result of hypercoagulability induced by circulating tumor-cell–derived TFMP.[38] TF is aberrantly expressed in a variety of cancers and is thought to drive angiogenesis and tumor growth through its PCA but also its capacity to induce intracellular signaling.[39] Consequently, plasma of cancer patients contains increased levels of TFMP and has higher TFMP PCA compared with healthy controls, as has consistently been shown (►**Table 1**).[19,22,40,41] Moreover, the cancer patients with the highest TFMP PCA are those with the poorest survival, probably reflecting the stimulating effects of TF on cancer progression.[42–44]

In the first report linking TFMP directly to cancer-associated VTE, a significantly higher TFMP PCA was observed in 7 cancer patients presenting with VTE compared with 43 cancer patients without VTE.[17] These results were later confirmed in various other case–control studies, all showing either increased TFMP counts or TFMP PCA in patients with cancer-associated VTE (►**Table 1**). It is still to be determined whether TFMP measurements could also be used as a predictive tool for cancer-associated VTE. In the largest study to date, Thaler et al did not find an association between high TFMP PCA at baseline and future symptomatic VTE in 348 cancer patients during 2 years of follow-up.[42] The results of this study conflict with those of others demonstrating increased TFMP PCA[19,43] or increased TFMP counts[45] in cancer patients developing symptomatic VTE compared with those not developing VTE (►**Table 1**). Following the results of the latter study, the group of Zwicker et al conducted a small trial evaluating the (a)symptomatic VTE incidence in 66 advanced cancer patients with either low or high TFMP levels as measured by impedance-based flow cytometry.[46] Indeed, the VTE incidence at 2 months in the group with high TFMP levels was 27.3% compared with 7.2% in patients with low TFMP levels, but the low number of events in the observation arms that were primarily asymptomatic ($N = 5$) preclude any firm conclusions. Again, one has to bear in mind that differences in the experimental approach to detect TFMP may be a major cause for discrepant findings between research groups.

Role of TF-Exposing MP in Coagulopathies Associated with Infectious Disease

DIC is a common manifestation in bacterial sepsis. This syndrome is characterized by systemic activation of the coagulation system and is associated with an unfavorable prognosis.[51] Already more than three decades ago, it was demonstrated that endotoxin-stimulated monocytes induce DIC in a TF-dependent manner.[52] Later studies showed that in fact high levels of coagulant monocyte-derived TFMP, rather than monocytes, are the main cause of the hemostatic abnormalities observed in patients with meningococcal sepsis.[29,53] Indeed, a single infusion of LPS was associated with a transient rise in TFMP PCA in healthy volunteers.[54] Accordingly, high levels of bacterial LPS in septic patients correlated with high TFMP PCA and this was associated with a worse outcome.[55] Also in patients with *Escherichia coli* urinary tract infection, higher TFMP PCA was observed in patients with confirmed bacteremia compared with patients with a localized infection, linking a prothrombotic state to clinical disease severity.[56] To our knowledge, at present, no prospective studies have been performed trying to identify patients with infectious disease at high risk of developing thrombotic complications.

TF-Exposing MP and Arterial Thrombosis

In contrast to the emerging role for TFMP in development of VTE, evidence for a contribution of TFMP to the pathogenesis of arterial thrombosis is still scarce and more controversial. Higher TFMP levels have been demonstrated in patients with acute stroke, stable angina, and acute myocardial infarction (AMI) as compared with healthy subjects.[57–59] Conversely, two other studies found that AMI patients had in fact lower TFMP levels than controls.[60,61] These authors hypothesized that adherence of TFMP to the coronary thrombus might lead to a decreased number of circulating TFMP, which is in line with earlier findings of increased levels of TFMP within occluded coronary arteries compared with peripheral blood.[62] Others have demonstrated that it is in fact the atherosclerotic plaque itself that contains significantly elevated levels of, mainly leukocyte-derived, TFMP compared with plasma.[63] Moreover, these TFMP were highly thrombogenic compared with plasma-derived TFMP, suggesting that they may contribute to rapid thrombus formation upon plaque rupture.

Based on the findings mentioned earlier, both circulating and atherosclerotic lesion-associated TFMP could be a therapeutic target to prevent arterial thrombotic events. Candidate inhibitors of local or systemic TFMP release in patients with cardiovascular disease are statins, a group of widely prescribed lipid lowering drugs. Emerging evidence indicates that statins also exhibit antithrombotic properties.[64,65] Patients experiencing arterial thrombotic events often have dyslipidemia including increased levels of circulating oxidized low-density lipoprotein (oxLDL).[66] Because oxLDL—but not LDL itself—induces TF expression in monocytes and increases TFMP release, monocytes may be a main source of TFMP in plasma or atherosclerotic plaques of patients with cardiovascular disease.[67] Statins may decrease the prothrombotic tendency in hyperlipidemia patients by inhibiting monocytes TF expression and subsequent TFMP release, as was shown in animal models.[68] These results are promising, but confirmation in human studies is needed.

Knowledge Gaps and Future Applications of TF-Exposing MP

The evidence for TFMP as a causal factor in thrombotic complications is growing. Future improvements in detection techniques will undoubtedly improve our understanding of

Table 1 Observational studies evaluating tissue factor-exposing microparticles in cancer patients and its association with venous thromboembolism

	Study details	Cancer patients vs. healthy controls (assay)	VTE vs. non-VTE cancer patients (assay)	VTE vs. no VTE during follow-up in cancer patients (assay)
Hron et al (2007)[22]	Design: case–control Patients: 20 with colorectal cancer	Higher TFMP counts (FC)	–	–
Haubold et al (2009)[47]	Design: case–control Patients: 68 with prostate cancer	Higher TFMP PCA (KC10 coagulation instrument)	–	–
Thaler et al (2014)[41]	Design: case–control Patients: 27 with pancreatic cancer	Higher TFMP PCA (TF-dependent FXa generation)	–	–
Tesselaar et al (2007)[17]	Design: case–control Patients: 23 with pancreatic cancer and 27 with breast cancer VTE: $N = 7$	Higher TFMP PCA (FVII-dependent FXa generation)	Higher TFMP PCA (FVII-dependent FXa generation)	–
Campello et al (2011)[37]	Design: case–control Patients: 60 with various cancers VTE: $N = 30$	Higher TFMP levels	Higher TFMP levels (FC)	–
Tesselaar et al (2009)[44]	Design: case-control Patients: 91 with various cancers VTE: $N = 51$	–	Higher TFMP PCA (FVII-dependent FXa generation)	–
Manly et al (2010)[48]	Design: case–control Patients: 66 with various cancers VTE: $N = 13$	–	Higher TFMP PCA (TF-dependent FXa generation)	–
Khorana et al (2008)[16]	Design: prospective cohort Patients: 11 with pancreatic cancer VTE: $N = 2$ (7 mo follow-up)	Higher TFMP PCA (TF-dependent FXa generation)	–	Higher TF antigen levels (ELISA) Higher TFMP PCA (TF-dependent FXa generation)
van Doormaal et al (2012)[19]	Design: case–control and prospective cohort Patients: 43 with various cancers VTE: $N = 5/43$ (6 mo follow-up)	Higher TFMP levels (FC)	–	No difference in TFMP counts (FC) Higher TFMP PCA (FVII-dependent FXa generation and FGT)
Sartori et al (2013)[49]	Design: prospective cohort Patients: 61 with glioblastoma VTE: $N = 11/61$ (7 mo follow-up)	Higher TFMP levels (FC)	–	Higher levels of tumor-derived TFMP but not other TFMP (FC)
Auwerda et al (2011)[40]	Design: prospective cohort Patients: 122 with multiple myeloma VTE: $N = 15/122$ (follow-up not specified)	Higher TFMP PCA (FVII-dependent FXa generation)	–	No difference in TFMP PCA (FVII-dependent FXa generation)

(*Continued*)

Table 1 (*Continued*)

	Study details	Cancer patients vs. healthy controls (assay)	VTE vs. non-VTE cancer patients (assay)	VTE vs. no VTE during follow-up in cancer patients (assay)
Hernández et al (2013)[50]	Design: prospective cohort Patients: 252 with various cancers VTE: $N = 34/252$ (22 mo follow-up)	No difference in TFMP PCA (Actichrome TF activity; American Diagnostica, Stamford, CT)	–	No difference in TFMP PCA (Actichrome TF activity)
Bharthuar et al (2013)[43]	Design: prospective cohort Patients: 117 with pancreaticobiliairy cancer VTE: $N = 36/117$ (6 mo follow-up)	–	–	Higher TFMP PCA (TF-dependent FXa generation)
Thaler et al (2012)[42]	Design: prospective cohort Patients: 60 with pancreatic cancer, 43 with stomach cancer, 126 with colorectal cancer, and 119 with brain cancer VTE: $N = 49/348$ (2 y follow-up)	–	–	No difference in TFMP PCA (TF-dependent FXa generation)
Zwicker et al (2009)[45]	Design: case–control and prospective cohort Patients: 90 with various cancers VTE: $N = 30$ VTE: $N = 4/60$ (1 y follow-up)	Higher TFMP levels (impedance FC)	Higher TFMP levels (impedance FC)	Higher TFMP levels

Abbreviations: ELISA, enzyme-linked immunosorbent assay; FC, flow cytometry; FVII, factor VII; FXa, factor Xa; PCA, procoagulant activity; TFMP, tissue factor-exposing microparticles; VTE, venous thromboembolism.

TFMP. However, there are still many knowledge gaps that yet have to be filled. For instance, not all TFMP expose coagulant TF. Various groups have found that higher TFMP levels do not necessarily translate into higher TFMP PCA.[17,19,47] Possible explanations include (1) the presence of encrypted, nonfunctional TF on MP rather than coagulant TF, (2) TF inhibition by its natural anticoagulant protein TFPI, (3) the need for concentration of TF in membrane lipid rafts before it may exhibit coagulant activity, and (4) dependence of TF activity on the concurrent exposure of PS surrounding the TF molecule in the membrane to become active. Furthermore, as mentioned, currently used quantification techniques such as flow cytometry detect only the tip of the (TF)MP iceberg. The most sensitive, dedicated flow cytometers to date are still not able to detect and characterize the MP sized less than 150 nm, while these MP are thought to form the largest bulk of circulating MP.[69]

Another unclarified issue involves the synthesis and clearance of TFMP. Since MP may be rapidly cleared from the systemic circulation, counterbalance by increased MP production is obligatory in order for MP to have long-lasting effects. However, neither the specific triggers for TFMP release nor the exact clearance mechanisms are currently known. Finally, the assumed higher density of TF on the surface of MP compared with parent cells suggests that the incorporation of TF in MP is a selective process,[69] but the exact mechanisms underlying MP and TFMP assembly have not yet been elucidated.

From a clinical perspective, the application of TFMP as a biomarker is best studied in the field of cancer-associated VTE. Current evidence for a predictive value of TFMP for the development of VTE is conflicting, which may be due to differences between studies with respect to patient selection, blood sample handling, and type of assay used. If future studies are able to demonstrate the predictive ability of TFMP for cancer-associated VTE, its use in daily clinical practice will probably still be limited due to the current labor intensive assays. Nevertheless, identification of cancer patients at high risk of VTE is urgently needed due to the high morbidity and mortality associated with this condition,

with TFMP remaining as promising targets. Whether it is possible to selectively target TFMP by blocking antibodies to prevent cancer-associated VTE or infection-related coagulopathies without abrogating the physiological hemostatic properties of TF, it remains to be seen.

Conclusion

The constitutive presence of coagulant, cell-derived TFMP in body fluids other than blood is likely to provide an additional hemostatic effect to counteract external damage, thereby reducing blood loss. In contrast, levels of circulating intravascular TFMP are low to maintain blood fluidity. Increased release of TFMP is associated with various pathological conditions, including cancer and infectious disease, and these blood-borne TFMP are likely to contribute to development of thrombotic complications such as VTE and DIC. Hence, TFMP could be a useful biomarker to identify patients at high risk for thrombosis who would benefit most from thromboprophylaxis. Although much progress has been made in measuring both TFMP levels and TFMP PCA, most assays still have substantial shortcomings. Improvements and standardization of detection methods are needed to help bring this field forward and establish a role of TFMP in thrombotic risk prediction.

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