Measurement of Microparticle Tissue Factor Activity in Clinical Samples: A Summary of Two Tissue Factor-dependent FXa Generation Assays

Yohei Hisada[1], **Wyeth Alexander**[1], **Raj Kasthuri**[2], **Peter Voorhees**[2], **Fariborz Mobarrez**[3], **Angela Taylor**[4], **Coleen McNamara**[4], **Hakan Wallen**[5], **Marco Witkowski**[6], **Nigel S. Key**[2], **Ursula Rauch**[6], **Nigel Mackman**[1,2,7]

[1]McAllister Heart Institute, Department of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA

[2]Division of Hematology/Oncology, Department of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA

[3]Karolinska Institute, Department of Medicine Solna, Unit of Rheumatology, Stockholm, Sweden

[4]Cardiovascular Research Center, Department of Medicine, University of Virginia, Charlottesville, VA, USA

[5]Karolinska Institute, Division of Cardiovascular Medicine, Department of Clinical Sciences, Danderyd Hospital, Stockholm, Sweden

[6]Department of Cardiology, Charité-Universitätsmedizin Berlin, Germany

Abstract

Thrombosis is a leading cause of morbidity and mortality. Detection of a prothrombotic state using biomarkers would be of great benefit to identify patients at risk of thrombosis that would benefit from thromboprophylaxis. The addition of biomarkers to these scores could improve risk assessment accuracy. Tissue factor (TF) is a highly procoagulant protein that under normal conditions is not present in the blood. However, increased levels of TF in the blood in the form of microparticles (MPs) (also called extracellular vesicles) are observed under various pathological conditions. In this review, we will discuss studies that have measured MP-TF activity using two similar FXa generation assays in a variety of diseases. One of the most robust signals for MP-TF activity (16-26 fold higher than healthy controls) is observed in pancreatic cancer patients with venous thromboembolism. In this case, the TF+ MPs appear to be derived from the cancer cells. Surprisingly, cirrhosis and acute liver injury are associated with 17-fold and 38-fold increases in MP-TF activity, respectively. Based on mouse models, we speculate that the TF+ MPs are derived from hepatocytes. More modest increases are observed in patients with urinary tract infections (6-fold) and in a human endotoxemia model (9-fold) where monocytes are the likely source of the

[7]Address for correspondence: Nigel Mackman, PhD, University of North Carolina at Chapel Hill, 111 Mason Farm Road Campus Box 7126, Chapel Hill, North Carolina, 27599, Telephone: (919) 843 3961, Fax: (919) 966 7639, nigel_mackman@med.unc.edu.
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TF+ MPs. Finally, there is no increase in MP-TF activity in the majority of cardiovascular disease patients. These studies indicate that MP-TF activity may be a useful biomarker to identify patients with particular diseases that have an increased risk of thrombosis.

Keywords

cancer; microparticles; thrombosis; tissue factor

Introduction

Thrombosis is a common pathology associated with a variety of diseases, particularly cardiovascular diseases [1]. Risk assessment scores use clinical information to try to identify patients at risk of thrombosis that would benefit from thromboprophylaxis. The addition of biomarkers to these scores could improve the accuracy of these scores. There are a variety of biomarkers that can be used to monitor the activation of the coagulation cascade. These include initiators, such as tissue factor, proteases that can be detected in complexes, such as thrombin-antithrombin complexes, and finally degradation products of fibrin, such as D-dimer [2]. In addition, global assays that measure levels of thrombin generation have been used to try to identify patients with a prothrombotic state [3]. However, the thrombin generation assay can be quite variable and usually employs an exogenous activator, such as TF, that may mask low levels of endogenous TF. D-dimer can be measured using commercial ELISAs and in our opinion is the most reliable biomarker of a prothombotic state. There are limitations with D-dimer that include an increase with age [4, 5].

In this review, we will focus on studies reporting the measurement of functional TF activity in plasma samples from a variety of different types of patient populations. TF is a transmembrane glycoprotein that functions as the primary initiator of the coagulation cascade [6]. TF can be present in two major forms: a cryptic form that is inactive and a decrypted form that is active [7]. Due to its high procoagulant activity, TF likely triggers many forms of thrombosis, such as formation of occlusive clots after rupture of atherosclerotic plaques and venous thromboembolism (VTE) [8–10]. Microparticles (MPs) (also known as microvesicles or extracellular vesicles) are submicron vesicles that are shed from apoptotic cells, activated cells, and cancer cells [11]. Levels of TF+ MPs in the blood can be measured using activity- and antigen-based assays [12]. We believe that activity assays are more sensitive and reliable than antigen-based assays and the two assays often do not correlate with each other [13, 14]. In addition, antigen-based assays will detect both cryptic and decrypted TF.

In healthy individuals, levels of TF+ MPs are very low or undetectable [12]. However, increased levels of MP-TF activity may contribute to thrombosis associated with certain disease states. This has led to the notion that TF+MP could be used as a biomarker to identify patients who have an increased risk for thrombosis. Table 1 summarizes all of the published MP-TF activity studies. In addition, it contains 5 unpublished studies measuring levels of MP-TF activity in multiple myeloma patients, in cardiovascular disease patients with or without statins and in healthy volunteers receiving endotoxin.

Measurement of MP-TF activity in plasma

There are two slightly different "in-house" assays that have been reported for the measurement of MP-TF activity in plasma using factor Xa (FXa) generation that we will refer to as "kinetic" and "endpoint" assays [15, 16]. The units of the kinetic assay are fM Xa min^{-1} whereas the units for the endpoint assay are pg/mL of TF. Both assays uses commercially available Innovin™ as a standard. Dr. Pabinger's group in Vienna has used both assays and they have adopted the endpoint assay because it is simpler to perform. In one study, both "in-house" assays were used to measure MP-TF activity in 54 pancreatic cancer patients and a robust correlation was found (Spearman correlation coefficient r =0.61, P< 0.001) [17]. We used these data to create an equation that allows a conversion between the assays (y = 0.0009x + 0.0831 where y = pg/mL, x = fM Xa min^{-1}). In addition, a commercial assay has been developed for the measurement of MP-TF activity that captures MPs using a non-inhibitory anti-TF antibody; however, there are no reports of this assay being used for clinical samples [13]. Finally, one study isolated MPs from plasma and measured their procoagulant activity in a one-stage clotting assay with or without an inhibitory anti-TF antibody [18].

Effects of pre-analytical and analytical variables on measurement of MP-TF activity

The level of MP-TF activity can be affected by many pre-analytical variables, but the most important is plasma preparation. This has been discussed extensively in a recent review [12]. For example, there are several centrifugation conditions reported for preparing platelet-poor-plasma (PPP) [15, 16, 19–26]. Other studies used various conditions to prepare platelet-free-plasma (PFP) [17, 27–30]. This makes comparison between studies difficult. Levels of MP-TF activity are significantly lower in PFP compared with PPP since some MPs are lost with the additional centrifugation involved in preparing PFP. For instance, two separate studies reported 52 - 80% reductions in the number of MPs obtained from PFP compared with PPP [14, 31]. We recommend that PFP should be prepared by centrifugation at 2500 x g for 15 minutes without a brake and then repeated as described by the Scientific Collaborative Workshop of the International Society of Thrombosis and Hemostasis [32]. There is a need to standardize the measurement of MP-TF activity in human plasma sample. Indeed, this has been discussed at the Scientific Standardization Committees of the International Society of Thrombosis and Hemostasis. However, in general, people prefer to use their own "in-house" assay and until a reliable commercial assay is developed it will be difficult to compare levels of MP-TF activity in different studies.

For the kinetic assay, healthy controls were reported to have median values of 79 and 127 fM Xa min^{-1} for PPP [19, 33] compared with a median value of 4.1 fM Xa min^{-1} for PFP [27] (Tables 2 and 4). For the endpoint assay, healthy controls were reported to have mean values of 0.21 and 0.25 pg/mL for PPP [16] (unpublished data) compared with 0.01 pg/mL for PFP [28] (Tables 3 and 4). Tables 2–4 indicate the use of either PPP or PFP in the different studies. The source and batch of recombinant TF used to create standard curves also varies between studies and will affect the reported amount of MP-TF activity. For the endpoint assay, we propose four response categories of MP-TF activity for PPP: zero (0.0 – <0.5 pg/ml); weak (0.5 - <1.0 pg/mL), moderate (1.0 – <2.0 pg/mL), and strong (>2.0 pg/mL).

1.   MP-TF activity in cancer patients

Levels of MP-TF in different types of cancer with or without VTE

The incidence of VTE varies between different cancers and can be broadly divided into high risk, such as pancreatic and brain cancer, moderate risk, such as lung, colorectal and hematologic cancers, and low risk, such as breast and prostate cancers [34, 35]. In general, higher levels of MP-TF activity are observed in cancers associated with the highest rates of VTE. For instance, Tesselaar and colleagues found that pancreatic cancer patients with VTE had much higher levels of MP-TF activity than colorectal cancer patients with VTE (Table 2) [19]. Higher levels of MP-TF activity were also observed in pancreatic cancer patients with VTE compared with lung and colorectal cancer patients with VTE (Table 3) [36]. In non-VTE cancer patients, MP-TF activity was highest in those with pancreatic cancer followed by stomach, colorectal and brain cancer (Table 3) [17].

One study reported 4-fold higher levels of MP-TF activity in PFP from 122 multiple myeloma patients compared with healthy controls [27]. In a separate study, we collected serial samples (1-6 samples per patient) from 16 multiple myeloma patients during the course of chemotherapy (cycle 1 and 2) (Supplementary Table 1).. We did not observed an increase in MP-TF activity in multiple myeloma patients and there was no increase in MP-TF activity after chemotherapy 1 and 4 days after treatment (Supplementary Table 2). The reason for these discrepant results between the two studies is unclear but is unlikely to be due to the use of PFP versus PPP as a starting material.

A small study measured MP-TF activity in acute myeloid leukemia (AML), although no healthy controls were included [21]. However, it appears that the 5 AML patients without disseminated intravascular coagulation (DIC) in this study did not have increased MP-TF activity when compared to healthy controls from other studies.

Levels of MP-TF activity with stage of cancer in pancreatic cancer

Previous studies have found that TF expression correlates with histological grade in cancer [37–39]. Circulating TF+ MPs present in cancer patients appear to be derived from the tumor since levels are dramatically reduced after resection [40]. Therefore, one would expect levels of MP-TF activity to increase with cancer stage. Thaler and colleagues measured MP-TF activity in pancreatic cancer patients with localized (stage II-III) and metastatic tumors (stage IV) [20]. MP-TF activity was significantly higher in the cancer patients compared to healthy controls irrespective of the stage. However, levels of MP-TF activity were highest in those patients with metastatic, non-resectable disease. In a separate study [41], MP-TF activity also increased with stage of pancreatic cancer (Figure 1). In summary, levels of MP-TF activity increase with stage of cancer, which is most likely due to increased TF expression in the tumor. Accordingly, the highest MP-TF activity levels reported to date are in patients with stage IV pancreatic cancer. We would anticipate a similar relationship between levels of MP-TF activity and stage for other types of cancer.

Association of MP-TF activity with VTE in cancer patients

A number of retrospective studies have shown that cancer patients with VTE have higher levels of MP-TF activity than cancer patients without VTE (Table 2 and 3) [15, 19, 36]. One study found that levels of MP-TF activity were higher in 51 cancer patients with VTE compared to 49 cancer patients without VTE [19]. The two groups were matched for age, sex, type of cancer, stage of disease and mode of treatment. The study analyzed many different types of cancer, including gastro-intestinal, genito-urinary, breast and ovarian. Importantly, pancreatic cancer patients with VTE showed 15-fold higher levels of MP-TF activity than those without VTE, whereas colorectal cancer patients with VTE showed only a 2-fold increase in MP-TF activity compared with those without VTE. Of note, 10 out of 32 patients with elevated MP-TF activity were pancreatic cancer patients. However, a potential problem with retrospective studies is that MP-TF activity may be increased as a consequence of the VTE rather than MP-TF activity contributing to thrombosis.

Prospective studies are more informative because they measure MP-TF activity as patients are being monitored for VTE over a defined follow-up period. Three prospective studies have shown a correlation between elevated levels of MP-TF activity and VTE in cancer patients (Table 3) [16, 41, 42]. Khorana and colleagues measured MP-TF activity in serial samples from 10 pancreatic cancer patients and found that MP-TF activity increased in the two patients that developed VTE [16]. A second study found a correlation between MP-TF activity and VTE in 43 cancer patients, 5 of which developed VTE in the 6 month follow-up period [42]. Of note, 3 of the 5 patients that developed VTE had pancreatic cancer. MP-TF activity was also correlated with VTE in 117 pancreaticobiliary cancer patients that were followed for 6 months [41]. Thaler and colleagues found "borderline non-significant associations between MP-TF activity and increased risk of VTE in Cox proportional hazard models" in pancreatic cancer patients using the endpoint assay [17]. No associations were observed between MP-TF activity and VTE in brain, stomach or colorectal cancer patients [17]. This study had a 2 year follow-up period that may explain the weak association between MP-TF activity and VTE. Finally, there was no correlation between MP-TF activity and VTE in multiple myeloma patients [27]. In summary, several studies have found the highest levels of MP-TF activity in pancreatic cancer patients and a relationship between MP-TF activity and VTE in these patients but not in other types of cancer. At present, it is unclear why this association seems to be confined to pancreatic cancer.

MP-TF activity and survival of cancer patients

Four studies have reported a relationship between the level of MP-TF activity and survival in cancer patients [15, 17, 19, 41]. In one study, the median survival of 32 cancer patients (10 of whom had pancreatic cancer) with elevated MP-TF activity was 3.5 months compared to 13 months for 68 cancer patients with low MP-TF activity [19]. Thaler and colleagues also found an association between elevated levels of MP-TF activity and reduced survival in pancreatic cancer patients [17]. Finally, Bharthuar and colleagues found that median survival of pancreaticobiliary cancer patients with elevated levels of MP-TF activity was 98.5 days compared with 231 days for those with low levels of MP-TF activity [41]. These studies indicate that MP-TF activity is a biomarker for survival in pancreatic cancer patients.

MP-TF and chemotherapy

Chemotherapy is designed to induce cytoreduction of cancer cells and therefore would be expected to increase levels of MP-TF activity in plasma. A number of studies have measured MP-TF activity in cancer patients before and after chemotherapy [26, 27, 43]. Mukherjee and colleagues found that chemotherapy did not increase MP-TF activity in patients with early stage breast cancer [43]. A second study reported no change in the levels of MP-TF activity in patients with metastatic testicular cancer following two courses of chemotherapy [26]. As described above, we did not see an increase in the levels of MP-TF activity after chemotherapy in patients with multiple myeloma (Supplementary Table 2). Only one study reported an association between MP-TF activity and chemotherapy; it was shown that chemotherapy significantly decreased the level of MP-TF activity in patients with multiple myeloma [27]. These studies suggest that chemotherapy does not increase MP-TF activity in cancer patients.

Levels of MP-TF activity in malignancy induced DIC

DIC is a condition characterized by systemic activation of coagulation. One study found that 5 patients with DIC and a variety of cancers had elevated levels of TF+ MPs measured using a one-stage clotting assay [18]. A second study observed very high levels of MP-TF activity in 2 AML patients with DIC compared with 5 AML patients without DIC [21]. Importantly, the levels of MP-TF activity fell dramatically after the DIC was resolved. These studies suggest that TF+MPs may contribute to DIC associated with malignancy but larger studies are needed to confirm these results. Additional prospective studies are needed to show that MP-TF activity is increased prior to the DIC and is not caused by the DIC.

2. MP-TF activity in non-cancer patients

Levels of MP-TF activity in patients with cardiovascular disease

Hypercholesterolemia is associated with atherosclerosis and atherothrombosis [10]. Various studies have reported elevated levels of TF antigen in the circulation of cardiovascular disease patients [44]. Levels of MP-TF activity were elevated in familial hypercholesterolemia patients compared with controls [24]. We measured MP-TF activity in 3 different studies with cardiovascular disease patients treated with statins. In the first study, we measured MP-TF activity in 19 patients with peripheral arterial disease who received high-dose atorvastatin (80 mg/day) or placebo for 8 weeks in a cross-over fashion (Supplementary Table 3) [45]. There was no difference in the mean values of MP-TF activity at baseline, during atorvastatin or during placebo (Figure 2A). Analysis of the levels in individual patients did not reveal any consistent changes. Atorvastatin treatment was associated with decreased MP-TF activity in the three patients with the highest basal levels of MP-TF activity, but was also associated with increases in MP-TF activity in 4 other patients (Figure 2A). In a second study, coronary artery disease patients were given either a moderately high-dose of atorvastatin (40 mg/day) (n=19) or a low-dose of atorvastatin (10 mg/day) plus ezetimibe (10 mg/day) (n=14) for 4 weeks [46]. There was no difference in the mean value of MP-TF activity in either group before or after treatment (Figure 2B). In the atorvastatin alone group, MP-TF activity was decreased in 2 patients and increased in another patient, whereas in the atorvastatin and ezetimibe group, MP-TF activity was

decreased in 2 patients and increased in 3 other patients (Figure 2B). Finally, we compared levels of MP-TF activity in a small study consisting of 11 matched pairs of patients with or without statins (Supplementary Table 4). Surprisingly, the mean level of MP-TF activity in the statin group was higher than the level observed in the non-statin group (Supplementary Figure 1). Five patients in the statin group and 3 patients in the non-statin group had levels of MP-TF activity that were above the cut-off of healthy individuals (Supplementary Figure 1). It is unclear why some of the patients in this study had elevated levels of MP-TF activity. Combining baseline data from the 3 studies indicates that 10/63 (16%) of cardiovascular disease patients had moderately elevated levels of MP-TF activity and that statin treatment did not consistently reduce these levels.

Levels of MP-TF activity in patients with cirrhosis and liver injury

Cirrhosis is associated with various forms of thrombosis, particularly portal vein thrombosis. A recent study measured levels of MP-TF activity in patients with varying degrees of cirrhosis as determined by the Child-Pugh score [28]. MP-TF activity increased in a step-wise manner with disease severity with the 14 patients with the most severe cirrhosis having 17-fold higher levels of MP-TF activity compared with healthy controls (Table 4). Similarly, Stravitz and colleagues reported a 38-fold increase in MP-TF activity in 34 subjects with acute liver injury (ALI) compared with healthy controls [47]. In a separate study, patients with hepatitis C virus had detectable levels of MP-TF activity, although no healthy controls were included in the study [30]. Although the liver expresses low levels of TF, it is likely that TF+ MPs are released from the liver after acute and chronic injury. Indeed, in a mouse model of chronic liver injury we found that deletion of the TF gene in hepatocytes but not myeloid cells abolished the activation of coagulation [48].

Levels of MP-TF activity in a human endotoxemia model and infection

An early study reported increased levels of MP-TF activity in human volunteers injected with *Escherichia coli* lipopolysaccharide (LPS) using a functional assay that involved capture of the TF+ MPs [49]. A more recent study demonstrated a 6.6-fold increase in the mean level of MP-TF activity using the kinetic assay in 7 volunteers receiving LPS [50]. Similarly, we found that the mean level of MP-TF activity was increased 8.5-fold 3 hours after LPS administration using the endpoint assay (Table 4) (Mooberry M, manuscript in revision).

MP-TF activity was also measured in patients with urinary tract infections where the APACHE II score was used to measure disease severity [33]. Infections induce TF expression and activation of coagulation. Again, MP-TF activity increased in a step-wise manner with severity in urinary tract infection with a 6-fold increase in patients with the highest APACHE II score [33]. Similarly, patients with severe influenza A infection had increased levels of MP-TF activity compared with healthy controls (Table 4), and the level of MP-TF activity correlated with mortality [22].

Levels of MP-TF activity in patients with HIV

Baker and colleagues measured levels of MP-TF activity in HIV patients with or without antiretroviral therapy and found a 39% decrease in the treated patients (Table 4) [51]. In the

untreated patients, those with hepatitis B/C coinfection (n=14) had higher levels of MP-TF activity compared to those without hepatitis (n=40). In a separate study, higher levels of MP-TF activity were found in patients with hepatitis C virus compared to HIV patients [30]. However, neither of these studies included healthy controls which makes it difficult to conclude if the MP-TF activity is really increased in HIV patients.

Levels of MP-TF activity in patients with other diseases

A study comparing levels of MP-TF activity in 74 morbidly obese patients before and after bariatric surgery reported significantly decreased MP-TF activity following surgery [52]. This finding is consistent with the fact that obesity is associated with activation of coagulation [53, 54]. In contrast, several studies have reported no increases in MP-TF activity. A study of antiphospholipid syndrome (APS) revealed APS patients expressed MP-TF activity levels below assay sensitivity limits [29]. Similarly, MP-TF activity was not increased in patients with lupus anticoagulant with a history of thrombosis compared with healthy controls [55]. Another study reported no difference in MP-TF activity in paroxysmal nocturnal hemoglobinuria patients before and after treatment with eculizumab, a monoclonal antibody to complement protein C5 [56].

Summary

The majority of the studies of MP-TF activity have focused on cancer patients because cancer cells express high levels of TF and because certain types of cancer are associated with high rates of VTE. Indeed, MP-TF activity was increased 16 to 26-fold over basal levels for pancreatic cancer patients with VTE. However, the mechanism by which TF+ MPs contribute to VTE is not well understood [57]. In contrast, lower levels of MP-TF activity are observed with other types of cancer. Liver injury is also associated with a robust increase in MP-TF activity. As expected, MP-TF activity increases in a human model of endotoxemia (7 to 9-fold) and in patients with urinary tract infection (6-fold) but these increases are more modest compared with pancreatic cancer. Surprisingly, significant increases in MP-TF activity were not observed in the majority of cardiovascular disease patients and statins did not generally decrease the signal. Efforts should be made to standardize pre-analytic variables in plasma preparation and the assay itself so that different studies can be directly compared. In addition, it would be helpful to identify the cellular origin of the TF+ MPs in different diseases and to increase the sensitivity of the detection of TF+ MPs in plasma.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements:

Reference

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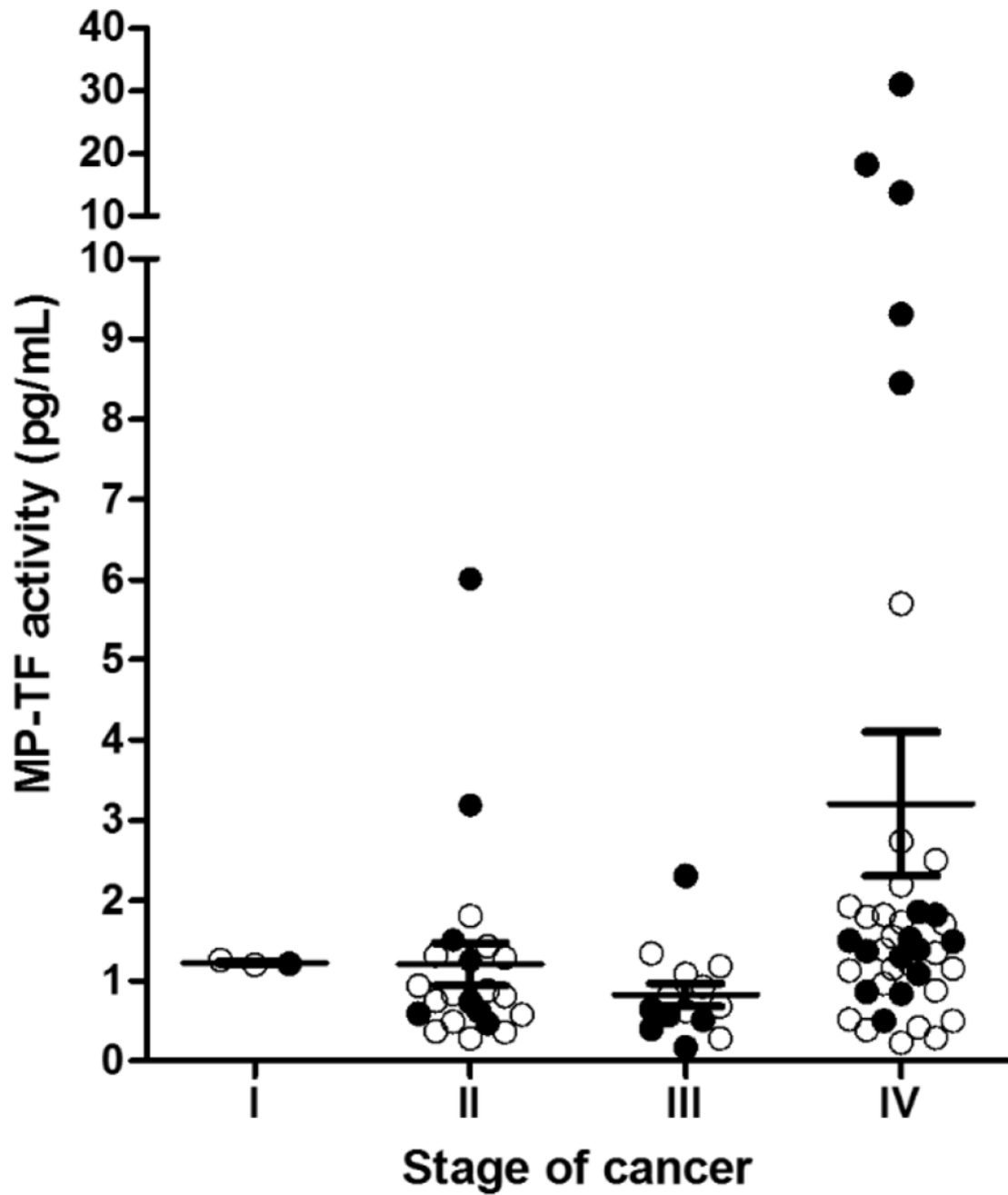
Figure 1. Levels of MP-TF activity in pancreatic cancer patients.

MP-TF activity in patients with stage I (n=3), II (n=22), III (n=14), and IV (n=41) pancreatic cancer. Data are shown by mean ± SEM. The filled circles represent patients with VTE.
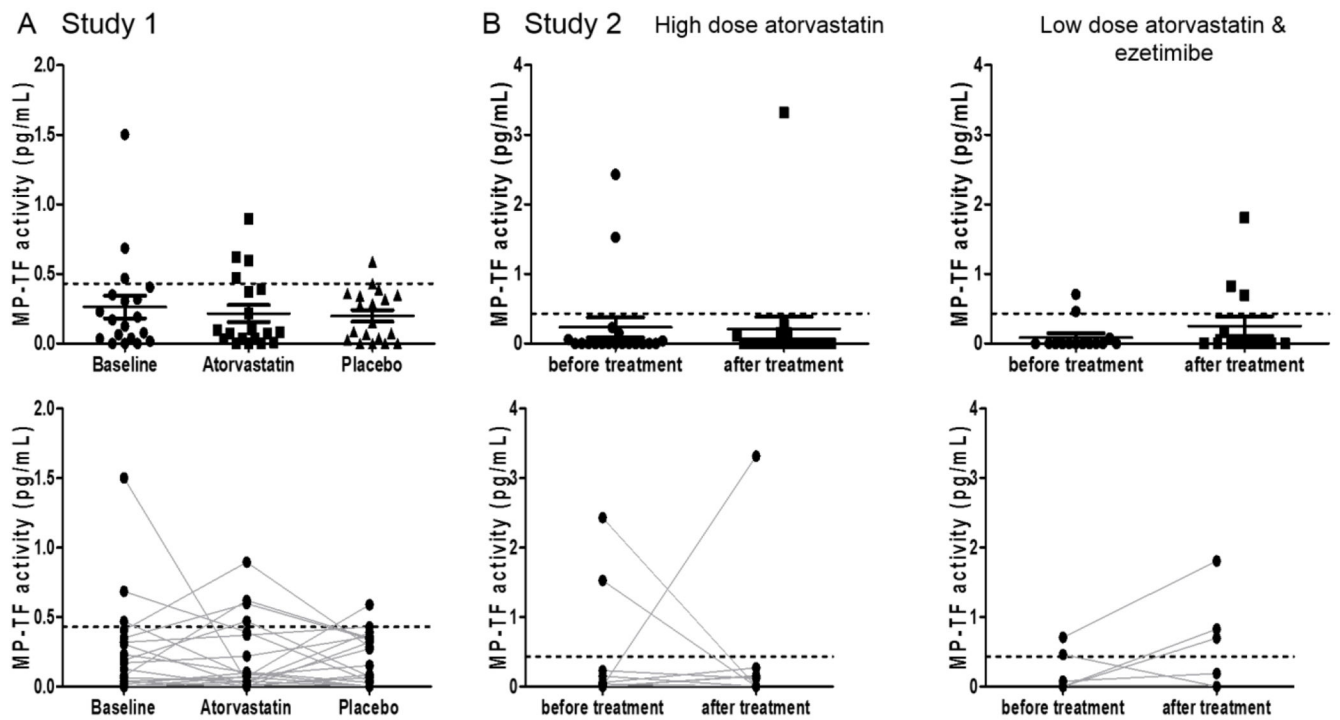
Figure 2. Effect of statins on MP-TF activity.

(A) Effect of atorvastatin on MP-TF activity in patients with peripheral arterial occlusive disease randomized to 8 weeks of treatment with atorvastatin or placebo in a cross-over study. Each sample was collected after 8 weeks of treatment with atorvastatin or after 8 weeks of treatment with placebo. (Top panel) MP-TF activity (mean ± SEM). (Bottom panel) Changes in the levels of MP-TF activity in each patient during atorvastatin treatment. (B) Effect of atorvastatin on MP-TF activity in patients with coronary artery disease randomized to either high dose atorvastatin (40 mg/day) only (left panels) or low dose atorvastatin (10 mg/day) plus ezetimibe (10 mg/day) (right panels). (Top panel) MP-TF activity (mean ± SEM). (Bottom panel) Changes in the levels of MP-TF activity in each patient during treatment. Data are shown by mean ± SEM. The dotted line represents the upper limit of the normal MP-TF activity range (0.43 pg/mL, mean plus 2 standard deviations).

Table 1.

Clinical studies measuring MP-TF activity.

Disease	Conclusion	Ref
1. Cancer & VTE	MP-TF activity is increased in metastatic breast and pancreatic cancer patients	15
2. Cancer & VTE	Increased MP-TF activity precedes VTE in pancreatic cancer patients	16
3. Cancer & DIC	MP-TF activity is associated with DIC in cancer patients	18
4. Cancer & VTE	Cancer patients with VTE have higher levels of MP-TF activity than those without VTE	19
5. Cancer & VTE	Cancer patients with VTE have higher levels of MP-TF activity than those without VTE	36
6. PE	MP-TF activity is not associated with PE	25
7. Breast cancer	MP-TF activity is not increased after chemotherapy	43
8. MM & chemo	MP-TF activity is increased in MM patients and decreased after chemotherapy	27
9. FH	Familial hypercholesterolemia patients have elevated MP-TF activity	24
10. Cancer & VTE	MP-TF activity is associated with mortality in pancreatic cancer patients	17
11. Cancer & VTE	MP-TF activity is associated with VTE in cancer patients	42
12. PNH	MP-TF activity is not increased in PNH patients	56
13. Endotoxemia	MP-TF activity is increased in human endotoxemia model	50
14. Cancer & chemo	Chemotherapy does not increase MP-TF activity in testicular cancer patients	26
15. Cancer & VTE	MP-TF activity is associated with VTE and mortality in pancreaticobiliary cancer	41
16. Cancer	MP-TF activity is associated with stage and grade of pancreatic cancer	20
17. Liver injury	MP-TF activity is increased in acute liver injury patients	47
18. HIV	Antiretroviral therapy is not associated with decreased MP-TF activity in HIV patients	51
19. Leukemia & DIC	Acute myelocytic leukemia patients with DIC have elevated MP-TF activity	21
20. Cirrhosis	MP-TF activity is increased in cirrhosis patients	28
21. APS	MP-TF activity is not increased in antiphospholipid syndrome patients	29
22. UTI	MP-TF activity is increased in urinary tract infection patients	33
23. HCV/HIV	HCV patients have higher levels of MP-TF activity than HIV patients	30
24. LA	MP-TF activity is not increased in LA patients	55
25. Obesity	Bariatric surgery reduces MP-TF activity in morbidly obese patients	52
26. Influenza A	MP-TF activity is increased in influenza A patients	22
27. MM	MP-TF activity is not increased in MM patients	NP
28. CVD & atorvastatin	Atorvastatin does not reduce MP-TF activity in CVD patients	NP
29. CVD & atorvastatin	Atorvastatin does not reduce MP-TF activity in CVD patients	NP
30. CVD & Statin	Statins do not alter MP-TF activity in paired patients	NP
31. Endotoxemia	LPS increases MP-TF activity in human volunteers	NP

APS: antiphospholipid syndrome, FH: familial hypercholesterolemia, HCV: hepatitis C virus, HIV: human immunodeficiency virus, LA: lupus anticoagulant, MM: multiple myeloma, NP: not published, PE: pulmonary embolism, PNH: paroxysmal nocturnal hemoglobinuria, UTI: urinary tract infection

Table 2.

MP-TF activity in cancer – kinetic assay.

Group	MP-TF activity (fM Xa min^{-1})		PPP/PFP	Ref
	Mean±SD (range)	Median (range)		
Healthy controls (n=37)	132±47		PPP	15
VTE, non-cancer (n=6)		148 (135–331)		
Cancer no VTE (n=33)	209	170 (59–665)		
Cancer VTE (n=7)	3643	2620 (410–14180)[*]		
Healthy controls (n=37)	132	127 (44–247)	PPP	19
Cancer no VTE (n=49)	162	150 (23–535)		
Cancer VTE (n=51)	1125	355 (19–12333)[*]		
Colorectal no VTE (n=14)		119 (23–535)		
Colorectal VTE (n=14)		246 (55–1578)		
Pancreatic no VTE (n=10)		138 (80–228)		
Pancreatic VTE (n=10)		2080 (510–12333)		
Healthy controls (n=20)		4.1 (2.3–6.6)[‡]	PFP	27
Multiple myeloma (n=122)		17.6 (8.6–33.2)[*‡]		
Before chemo (n=75)		17.4 (10.2–32.8)[‡]		
After chemo (n=75)		12.0 (7.0–18.5)[*‡]		
No VTE (n=107)		17.8 (8.1–32.5)[‡]		
VTE (n=15)		16.8 (11.4–36.1)[‡]		
Pancreatic cancer (n=72)		45.9 (12.3–193.9)[‡]	PFP	17
Brain cancer (n=148)		16.4 (5.5–30.1)[‡]		
Healthy controls (n=19)	(8–113)		PPP	26
Testicular cancer (n=13)	16±8 (10–30)			

[*] significant difference compared with controls

[‡] 25th–75th percentile

Table 3.

MP-TF activity in cancer – endpoint assay.

Group	MP-TF activity (pg/mL)		PPP/PFP	Ref
	Mean±SD (range)	Median (range)		
Healthy controls (n=15)	0.21±0.11		PPP	16
Pancreatic cancer (n=10)	0.95 (0–3.1)			
No VTE (n=8)	0.46±1.26[*]			
VTE patient No.5	4.4			
VTE patient No.10	5.5			
Cancer no VTE (n=13)	0.5±0.5		PPP	36
Cancer VTE (n=53)	1.7±3.8[*]			
Pancreatic (n=5)	6.6±10.8			
Lung (n=10)	2.4±2.5			
Colorectal (n=14)	0.7±0.8			
Pancreatic (n=60)		0.10 (0.04–0.19)[‡]	PFP	17
Stomach (n=43)		0.07 (0.0–0.17)[‡]		
Colorectal (n=126)		0.05 (0.01–0.15)[‡]		
Brain (n=119)		0.04 (0.0–0.08)[‡]		
Healthy controls (n=22)		0.26 (0.06–0.41)[‡]	PPP	42
Cancer no VTE (n=38)		0.21 (0.04–0.35)[‡]		
Cancer VTE (n=5)[†]		0.82 (0.25–6.9)[‡]		
Pancreaticobiliary (n=117)	2.15±3.8	1.20 (0.17–31.01)	PPP	41
No VTE (n=65)	1.40±1.5	1.13 (0.23–10.65)		
VTE (n=36)	3.08±5.3	1.20 (0.17–31.01)[*]		
Healthy controls (n=22)		0.05 (0.00–0.76)	PPP	20
Pancreatic cancer (n=73)		0.37 (0.00–11.91)		
Localized resected (n=18)		0.18 (0.00–1.39)		
Localized unresected (Stage II–III) (n=13)		0.29 (0.00–0.88)		
Metastatic recurrent (Stage IV) (n=13)		0.28 (0.04–0.74)		
Metastatic non-resectable (Stage IV) (n=29)		0.88 (0.19–11.91)[*]		
AML patient (n=7)	1.26±1.77		PPP	21
No VTE, No DIC (n=2)	0.17±0.08			
VTE (n=3)	0.30±0.14			
DIC patient No.1	4.43			
DIC patient No.2	3.16			
Healthy controls (n=5)	0.25±0.08		PPP	NP
Multiple myeloma (n=11)	0.25±0.20			

Group	MP-TF activity (pg/mL)		PPP/PFP	Ref
	Mean±SD (range)	Median (range)		
Before chemo (n=9)	0.24±0.19			
After chemo (n=9)	0.28±0.29			

AML: acute myelocytic leukemia, NP: not published

[*] significant difference compared with controls

[†] including 3 pancreatic cancer patients

[‡] 25th – 75th percentile

Table 4.

MP-TF activity in different diseases.

Disease	Group	MP-TF activity			PPP/PFP	Ref
		Endpoint		**Kinetics**		
		Mean±SD (range)	Median (range)	Median±SD (range)		
Hypercholesterolemia	Healthy controls (n=17)	0.01			PPP	24
	FH patients (n=25)	0.66				
Endotoxemia	Healthy controls (n=7)			47±41		50
	After LPS treatment (n=7)			309±267		
Acute liver injury	Healthy controls (n=13)	0.24±0.14			PPP	47
	ALI patients (n=34)	9.05±8.82[*]				
HIV	Untreated (n=54)		0.35 (0.24–0.54)[‡]		PPP	51
	Antiretroviral (n=109)		0.22 (0.14–0.34)[*‡]			
Cirrhosis	Healthy controls (n=9)	0.01±0.01			PFP	28
	Child–Pugh A (n=10)	0.05±0.05				
	Child–Pugh B (n=9)	0.10±0.07				
	Child–Pugh C (n=14)	0.22±0.15[*]				
APS	Asymptomatic (n=72)		0.09 (0.05–0.14)[‡]		PFP	29
	Symptomatic (n=30)		0.13 (0.10–0.17)[‡]			
UTI	Healthy controls (n=19)			79 (57–126)[‡]	PPP	33
	UTI (n=215)			197 (113–398)[*‡]		
	Non bacteremic (n=167)			186 (110–324)[‡]		
	Bacteremic (n=48)			325 (166–641)[‡]		
	APACHE II score 0–4 (n=78)			152 (98–280)[‡]		
	APACHE II score 5–9 (n=93)			227 (123–501)[‡]		
	APACHE II score 10–14 (n=34)			259 (144–599)[‡]		
	APACHE II score 15–17 (n=10)			471 (180–848)[‡]		
HIV/HCV	HIV (n=15)	0.31±0.32			PFP	30
	HCV (n=15)	0.15±0.21				
	HIV/HCV (n=15)	0.14±0.05				
Lupus anticoagulant	Healthy controls (n=30)	0.06 (0.00–0.10)[‡]			PPP	55
	Patients (n=113)	0.04 (0.00–0.11)[‡]				

Disease	Group	MP-TF activity			PPP/PFP	Ref
		Endpoint		**Kinetics**		
		Mean±SD (range)	**Median (range)**	**Median±SD (range)**		
Obesity	Before surgery (n=74)	0.32±0.43			PFP	52
	After surgery (n=74)	0.18±0.41 [*]				
Influenza A	Healthy controls (n=27)	0.05±0.02			PPP	22
	Influenza A patients	3.80±0.90 [*]				
CVD	Baseline (n=19)	0.26±0.35			PPP	NP
	Placebo (n=19)	0.22±0.26				
	Atorvastatin (n=19)	0.20±0.18				
CVD	Before high dose atorvastatin (n=19)	0.23±0.64			PPP	NP
	After high dose atorvastatin (n=19)	0.21±0.76				
	Before low dose atorvastatin plus ezetimibe (n=14)	0.09±0.22				
	After low dose atorvastatin plus ezetimibe (n=14)	0.25±0.53				
Endotoxemia	Endotoxemia 0 hour (n=13)	0.06±0.05			PFP	NP
	Endotoxemia 3 hours (n=13)	0.51±0.18 [*]				
	Endotoxemia 6 hours (n=13)	0.47±0.23 [*]				

APS: antiphospholipid syndrome, CVD: cardiovascular disease, HCV: hepatitis C virus, HIV: human immunodeficiency virus, NP: not published, UTI: urinary tract infection

[*] significant difference compared with controls

[†] 25th–75th percentile