Nanosized blood microparticles

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CHAPTER 8

Microparticle-associated tissue factor activity and venous thrombosis in Multiple Myeloma

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Abstract

Multiple myeloma (MM) is associated with an increased risk of venous thromboembolic (VTE) complications. Aim of this study was to measure microparticle-associated tissue factor (MP-TF) activity in patients with newly diagnosed MM before and after chemotherapy and to investigate whether MP-TF activity is associated with VTE. MP-TF activity was assessed in 122 newly diagnosed MM patients who were eligible for combination chemotherapy. MP-TF activity levels (17.6 fM Xa/min [8.6-33.2] (median [IQR]) were higher in untreated MM patients compared to normal healthy volunteers (4.1 fM Xa/min [2.3-6.6], p <0.001). MP-TF activity prior to the start of treatment was not different between patients who developed a VTE during follow-up (n=15) and those who did not (n=107). In 75 patients in whom plasma was obtained before and after chemotherapy, MP-TF activity decreased significantly (from 17.4 [10.2-32.8] to 12.0 [7.0-18.5] fM Xa/min, P=0.006). MP-TF activity remained, however, elevated in patients who developed VTE (15.1 [10.3-25.2]), in contrast to patients not developing VTE (11.4 [7.0-25.2], P<0.001). In conclusion, MP-TF activity is increased in patients with MM. Whether MP-TF activity has a pathogenetic role in VTE in MM patients remains to be established in future studies.

Introduction

An increased risk of venous thrombo-embolism (VTE) is observed in multiple myeloma (MM), especially when these patients are treated with combination treatment including high dose dexamethasone, doxorubicin or multi-agent chemotherapy in combination with thalidomide or lenalidomide (1-2). The exact mechanism of the increased risk of VTE is not yet fully understood, but a number of mechanisms have been proposed to contribute to the development of the hypercoagulable state in these patients. These include increased levels of factor VIII or von Willebrand factor and hypofibrinolysis (3-6). Furthermore, an association between acquired APC resistance and the occurrence of VTE in MM has also been reported (5, 7).

Tissue factor is the principal initiator of the coagulation cascade and can be demonstrated on circulating microparticles that are released from cells following activation or during apoptosis (8-9). In addition tissue factor is expressed on the surface of cells in many tumour types and the expression appears to correlate with grade of malignancy and with tumour progression (10-12) TF expression of various tumour types has been associated with the risk of development of VTE complications (13). Tesselaar et al. observed an association of microparticle associated-tissue factor (MP-TF) activity and VTE using a functional assay to measure TF activity in the MP fraction of plasma (MP-TF activity) in patients with disseminated mucinous adenocarcinoma (14). More recent studies have shown similar results for other types of cancer (15-17). Zwicker et al showed that elevated levels of TF-MP were associated with an increased cumulative incidence of VTE (16, 18). Also Khorana et al. found an association between TF antigen levels, TF MP procoagulant activity and development of VTE (19). Some of these studies indicate that at least part of the TF+ MP is derived from tumour cells, but also platelet-derived and monocyte-derived MPs are increased (16, 20-21). Despite the fact that several patient groups with different types of cancer have been studied, no data are available on MP-TF activity in MM patients, who have a very high risk of developing VTE.

Therefore the aim of the current study was to investigate MP-TF activity in MM patients and to assess a possible association with the development of VTE.

METHODS

Patients

A total of 122 patients with newly diagnosed MM according to the Mayo Clinic criteria who were eligible for chemotherapy followed by autologous stem cell transplantation were included in the study (22). The patients were admitted to the department of Haematology of the Erasmus MC Rotterdam, an academic tertiary referral hospital in the Netherlands. These patients were recruited from the HOVON-50 MM (METC no 01/080) and HOVON-65 MM/GMMG-HD4 (EUDRACT no 2004-000944-26) studies. Only patients who were eligible for intensive chemotherapy with high dose melphalan and autologous stem cell support were included. The Central Medical Ethical Committee of the Erasmus MC approved the study and written informed consent was obtained from all patients before performing the investigation. Patients were randomized to received either 3 courses of VAD (doxorubicin [9 mg/m^2 i.v., day 1-4], dexamethasone [40mg orally, day 1-4, 9-12, 17-20] and vincristine [0.4 mg i.v., day 1-4]), or TAD (thalidomide 200 mg orally daily, doxorubicin [9 mg/m^2 i.v., day 1-4], dexamethasone [40 mg orally, day 1-4, 9-12, 17-20] or PAD (bortezomib [1.3 mg/m^2 i.v., days 1,4,8,11], doxorubicin [9 mg/m^2 i.v., day 1], dexamethasone [40 mg orally day 1-4, 9-12, 17-20]). During thalidomide treatment, patients received a prophylactic dose of LMWH (nadroparin 2850 IE anti-Xa (body weight < 90kg) or 5700 IE anti-Xa (body weight ≥ 90kg) subcutaneously. At entry in the study the patients were staged according to the criteria of Salmon and Durie, as well as to the recently introduced prognostic International Staging System (ISS) (23-24). Plasma levels of MP-TF activity were also determined in twenty healthy volunteers (median age 48 years [range 31-69], 45% males).

In case patients had complaints suggestive of pulmonary embolism, standardized diagnostic strategies were used, including computed tomography of the chest and compression ultrasonography in case of complaints suggestive for deep venous thrombosis of the leg or arm in order to confirm or exclude the VTE. When a VTE was confirmed oral anticoagulant (coumarin) was initiated in therapeutic dose (INR 2.5-3.5).

Materials & methods

Venous blood was collected using a vacutainer system in citrate (0.105 M, Becton-Dickinson, Plymouth, UK). The first five mL of blood were discarded. Platelet-free plasma (PFP) was prepared by double centrifugation (4℃ at 2,000g for 10 minutes [min] followed by 10 min at 4℃ at 21,475g) and sto red in small aliquots at –70℃

until use. The blood samples were collected at time of diagnosis (time point 1) and after three courses of multi-agent chemotherapy (time point 2). Plasma was immediately snap frozen in liquid nitrogen and stored at –70°C until use. The same procedure was used for the preparation of plasma of patients and of healthy volunteers. Plasma was obtained at entry of the study of 122 patients, and plasma was collected at the second time point (after induction chemotherapy) in 75 of the 122 patients. Of the 122 patients 15 developed a VTE during follow-up, of whom plasma samples were available of 11 patients at the second time point.

Dioleoylphosphatidlyserine (DOPS) and Dioleoylphosphatidylcholine (DOPC) were purchased from Avanti Polar Lipids (Alabaster, AL). Hirudin was from Sigma (Germany). The human Factor VII (FVII) and Factor X (FX) were obtained from Kordia Life Sciences (Leiden, NL). Sheep anti-human Tissue Factor IgG1 (TF) was from Affinity Biologicals (Ancaster, Canada). Chromogenic substrate S-2765 was from Instrumentation Laboratory Company (Milano, Italy). Innovin was obtained from Dade Behring (Eschborn, Germany).

Microparticle isolation

The isolation of MP was performed as previously described (25). In order to reduce the contamination with plasma proteins MPs were washed extensively (<0.5% in the final MP preparation). In brief, two vials (each 250 µL) of frozen plasma were thawed at 37°C and then vortexed thoroughly. The vials were centrifuged at 18,890g, 20°C for 30 min. The supernatants were removed carefully except for 25 µL containing the MP pellet. Subsequently, each pellet was washed with 475 µL PBS containing 0.32% citrate (pH 7.45) and centrifuged (18,890g, 20°C, 30 min). This wash and centrifugation step was repeated once with 225 µL PBS-citrate. The supernatants were removed carefully except for the 25 µL containing the MP pellet. Finally, the two MP pellets were resuspended, pooled, and directly assayed for TF activity. The number of platelets in platelet-free plasma (PFP) is 1.72×10^6/L plasma, which is 16-fold lower than in platelet-poor plasma (PPP) prepared by a single centrifugation of 1,550g for 20 min. The MP fraction isolated form frozen/thawed PFP contains 10-fold less MPs when compared to MP fraction isolated from frozen/thawed PPP (1.3×10^8/L and 1.4×10^9/L, respectively). This conclusion is based on the results of flow cytometry measurements (scatter) on MPs isolated from PFP and PPP (Figure 1).

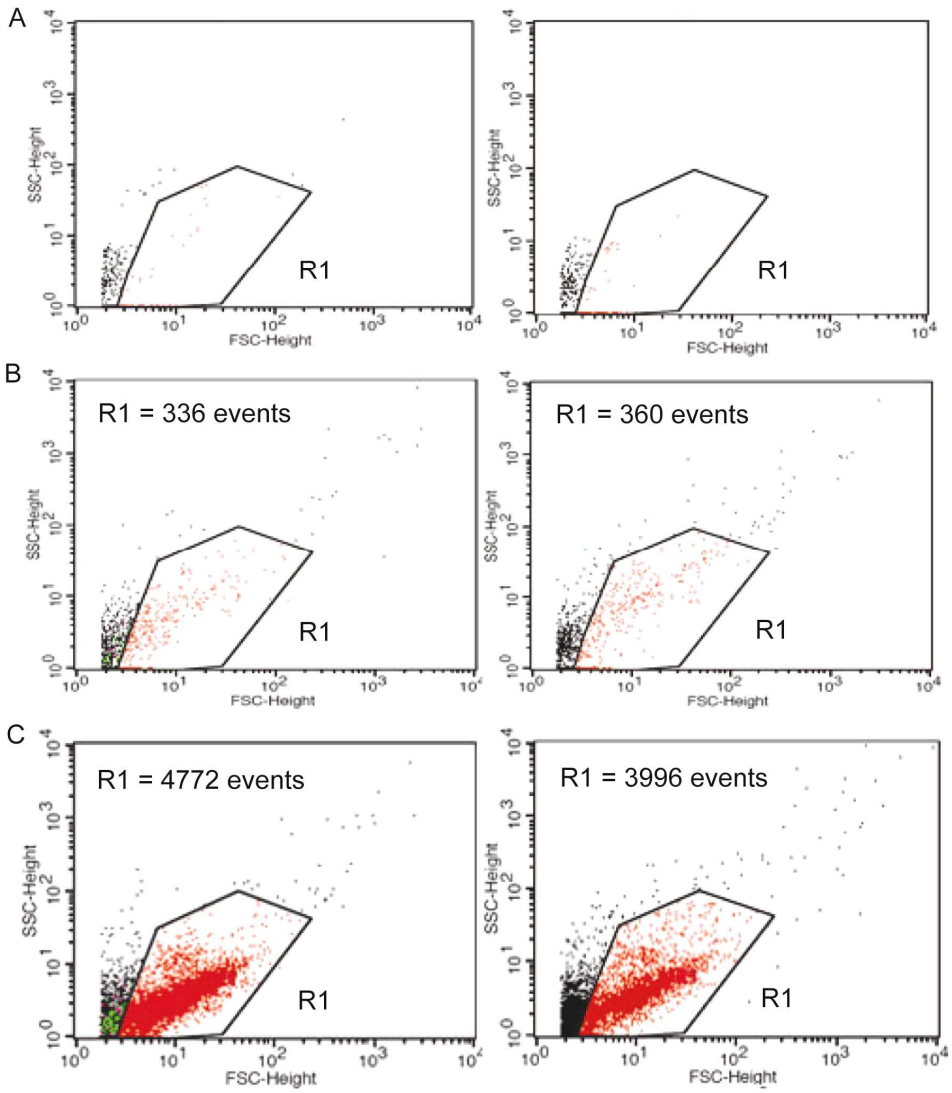

Figure 1: FACS analysis of MPs isolated from PFP (B) and PPP (C). The MP gate (R1) was defined using Hepes buffer (pH 7.45) containing 137 mM NaCl, 4 mM KCl (A.). MPs were isolated from 250 µL plasma and resuspended in 100 µL Hepes buffer; 5 µL of this suspension was diluted to 350 µL for FACS analysis; the Figures show duplicates of the scatter events in 74 µL of the measured suspension.

Tissue factor activity assay

MP-associated TF activity was measured at room temperature by determining the FVII-dependent factor Xa (FXa) generation in the presence of excess negatively charged phospholipids. MP-TF activity is expressed as fM FXa/min. This relates to the activity of the TF-VIIa complex when all TF has been saturated with FVIIa. In all samples, FXa generation was measured both in the presence and absence of FVII and in the presence and absence of sheep anti-human TF IgG_1 to confirm the FVII- and TF-dependence of the FXa generation. Twenty five µM DOPS: DOPC (10:90) were incubated for 15 min in 10 mM Hepes buffer (pH 7.45) containing 137 mM NaCl, 4 mM KCl, 5 mg/mL Ovalbumine, 6 mM $CaCl_2$, and 50 nM Hirudin. In 100 µL of this solution, 20 µL of MP-suspension was added and incubated for 15 min before 40 µL of 1.1 nM FVII (or buffer) was added. After 10 min, 25 µL S2765 was added and the reaction was started by adding 40 µL of 250 nM FX. The rate of p-Nitroaniline (pNA) formation as a product of the reaction was measured at 405 nm (expressed in mAbs) for 90 min, plotted as a function of time (t) and, after correction for the absorbance in the absence of FVII, as a function of t^2. The slope of the latter curve is a measure for the rate of FXa generation and expressed as $mAbs/min^2$ or as fM FXa/min. The MP-associated TF activity was calculated from the difference in the rate of Xa generation in the absence and presence of 11.1 µg/mL anti-TF antibody and reported as fM Xa/min in the assay. Dilutions of a recombinant full length TF (Innovin, 750 pg/mL and 150 pg/mL) were used as positive controls and to monitor interassay variation of the TF activity assay. Plasma of a patient with elevated MP-TF activity was used for the measurement of the interassay variation of the complete procedure, including the isolation of MP from plasma and the measurement of TF activity. Both CVs were <10% (N=9). This variation includes the MP isolation from plasma and the MP-TF activity measurement. The lower detection limit of the assay is about 2 fM Xa/min

Statistical analysis

Since the levels of MP-TF activity are not normally distributed, also not after transformation, the basic descriptive statistics are presented as median and interquartile range for continuous variables and as count (percentages) for categorical variables. Differences between two groups were tested with the Mann-Whitney test. Comparisons over time were evaluated with a paired analysis using the Wilcoxon signed rank test. The association between VTE risk and MP-TF activity was assessed by logistic regression analysis. All statistical analyses were performed using SPSS for windows, version 14 (SPSS Inc, Chicago, USA).

RESULTS

Patient characteristics

A total of 122 patients with MM were included in this prospective study, of whom 39 (32%) had stage I, 66 (54%) stage II and 16 (13%) had stage III disease according to the ISS criteria (23). The median age was 55 years (range 29-71) and 58% were males. Seventy patients received VAD, thirty-six TAD and fifteen PAD induction chemotherapy. The baseline patient characteristics are summarized in Table 1. Of the 122 included patients a post-treatment blood sample was obtained of 75 patients. The baseline characteristics were not different between these 75 patients and the 47 of whom only a pre-treatment plasma sample was obtained, with the exception of a slightly lower hemoglobin level at diagnosis (7.1 [6.4-7.9] vs 6.5 [5.7-7.2], p=0.01 (Table 2).

Table 1. MM patient characteristics

Table I. Multiple Myeloma patient characteristics

		Total group	No VTE	VTE
Number of patients		122	107	15
Age yrs (median, range)		55 (29-71)	56 (29-71)	52 (38-65)
Male gender		58 %	59 %	53 %
β2 microglobulin (mg/l)*		3.08 (2.02-4.63)	3.18 (2.12-4.79)	2.40 (1.37-3.25)
Platelets ($x10^9$/l)*		242 (182-287)	236 (178-283)	268 (179-291)
Albumin (g/l)*		37 (32-42)	37 (32-42)	39 (33-44)
Calcium (mmol/l)*		2.33 (2.21-2.42)	2.33 (2.20-2.41)	2.334 (2.24-2.50)
Hemoglobin (mmol/l)*		6.9 (5.9-7.7)	6.8 (6.0-7.7)	7.8 (6.1-8.3)
Monoclonal protein	kappa	82 (66%)	71 (67%)	11 (73%)
(IgG and IgA)	lambda	40 (34%)	36 (33%)	4 (27%)
ISS stage	I	39 (33%)	30 (28%)	9 (60%)
	II	67 (54%)	62 (58%)	5 (33%)
	III	16 (13%)	15 (14%)	1 (7%)
Treatment	VAD	71 (58%)	64 (59%)	7 (47%)
	TAD	36 (30%)	30 (28%)	6 (40%)
	PAD	15 (12%)	13 (12%)	2 (13%)
Durie & Salmon	II a & b	23 (19%)	16 (15%)	7 (47%)
	III a & b	99 (81%)	91 (85%)	8 (53%)

VAD; vincristine, adriamycin and dexamethasone, TAD; thalidomide, adriamycin and dexamethasone, PAD; bortezomib, adriamycin and dexamethasone. *Median and 25-75% range.

MP-TF activity at baseline and after induction chemotherapy

The results of the MP-associated tissue factor activity levels are summarized in Table 3. The median MP-TF activity of the total group of patients with MM was 17.6 fM Xa/min (IQR 8.6-33.2), which was significantly higher than that in healthy volunteers (4.1 fM Xa/min (IQR 2.3-6.6); P<0.001, range 0-11.8) (Figure 2).

Table 2. Baseline characteristics of MM patients

Multiple Myeloma patient baseline characteristics

		Total group	Patients with only pretreatment plasma sample available	Patients with pre-and post treatment sample available	P value
Number of patients		122	47	75	
Age yrs (median, range)		55 (29-71)	57 (50-62)	58 (50-61)	0.46
Male gender		58 %	51%	62%	0.24
β2 microglobulin (mg/l)*		3.08 (2.02-4.63)	2.90 (2.06-4.44)	3.08 (1.80-4.67)	0.73
Platelets (x 10^9/l)*		242 (182-287)	236 (187-279)	245 (178-287)	0.61
Albumin (g/l)*		37 (32-42)	37 (32-40)	38 (32-42)	0.39
Calcium (mmol/l)*		2.33 (2.21-2.42)	2.30 (2.21-2.40)	2.36 (2.21-2.46)	0.22
Hemoglobin (mmol/l)*		6.9 (5.9-7.7)	6.5 (5.7-7.2)	7.1 (6.4-7.9)	0.01
Monoclonal protein (IgG and IgA)	kappa	82 (66%)	26 (58%)	56 (74%)	0.07
	lambda	40 (34%)	19 (42%)	20 (26%)	
ISS stage	I	39 (33%)	14 (30%)	25(34%)	0.71
	II	67 (54%)	27 (59%)	39 (51%)	
	III	16 (13%)	5 (11%)	11 (14%)	
Treatment	VAD	71 (58%)	30 (65%)	41 (54%)	0.47
	TAD	36 (30%)	11 (24%)	25 (33%)	
	PAD	15 (12%)	5 (11%)	10 (13%)	
Durie & Salmon	II a & b	23 (19%)	5 (9%)	18 (24%)	0.09
	III a & b	99 (81%)	40 (91%)	58 (76%)	

VAD; vincristine, adriamycin and dexamethasone, TAD; thalidomide, adriamycin and dexamethasone, PAD; bortezomib, adriamycin and dexamethasone. *Median and 25-75% range

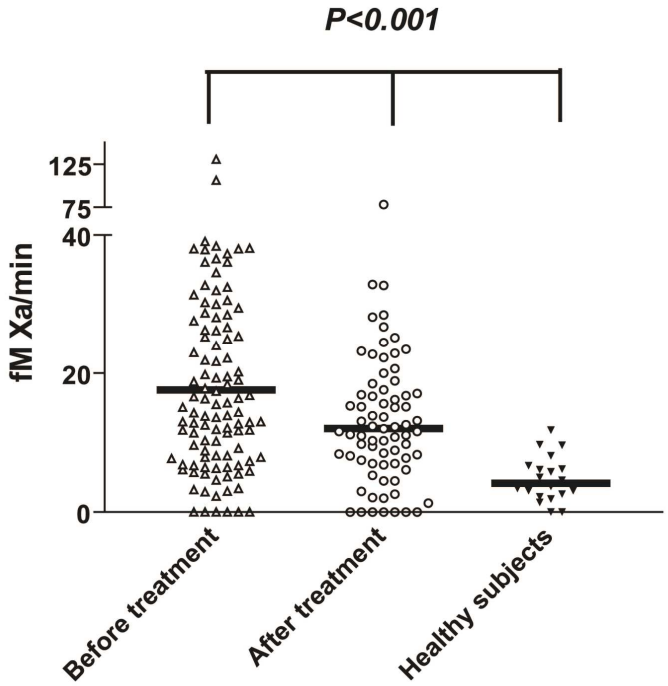

Figure 2: MP-Tissue factor activity in fM Xa/min in MM patients, before (n=122) and after induction chemotherapy (n=75) and in healthy subjects (n=20). The horizontal line indicates the median level. P-value denotes the difference between both before and after treatment versus healthy subjects. The p-values of paired analysis between before and after treatment are given in Table 3.

We observed significantly lower MP-TF activity levels in MM patients with light chain lambda (12.1 fM Xa/min [IQR 5.7-18.4] than in MM patients with light chain kappa (24.0 fM Xa/min [11.8-37.9]; $p<0.05$). None of the clinical patient characteristics (sex, beta-2 microglobulin, albumin, calcium, hemoglobin, platelets or disease stage) correlated with the MP-TF activity levels. MP-TF levels were not associated with age (r^2 =0.102). There was no significant difference in TF-MP activity at baseline between the 47 patients of whom no second blood sample was obtained (17.8 fM Xa/min [6.7-36.1]) and the 75 patients of whom also blood was drawn after chemotherapy (17.4 fM Xa/min [10.2-32.8], p=0.45).

Table 3. MP-TF activity in fM Xa/min

MP-TF activity in fM Xa/min						
	Total group at baseline		MM patients with pre- and post treatment plasma sample			p-value
	Number of patient	Before treatment	Number of patients	Before treatment	After treatment	
Total group	122	17.6 (8.6-33.2)	75	17.4 (10.2-32.8)	12.0 (7.0-18.5)	0.006
ISS stage						
1	39	14.4 (5.7-28.5)	25	14.4(6.6-27.4)	13.2 (6.5-19.2)	0.47
2	67	19.4 (11.9-32.6)	39	19.8 (11.8-36.1)	11.0 (7.0-18.9)	0.02
3	16	15.9 (6.7-40.3)	11	16.3(6.8-38.0)	12.6 (9.4-16.2)	0.11
		P=0.37			P=0.96	

ISS; International Staging System, VTE; venous thrombo-embolism. Data of MP-TF activity are given in median (25-75%).
P-value is on the effect of treatment using a paired analysis, thus only including patients that were measured at both time points, before and after chemotherapy.

Paired analysis in 75 patients of whom plasma was obtained at two time points, before and after chemotherapy, of MP-TF activity revealed a significant reduction of MP-TF activity following induction treatment (from 17.4 fM Xa/min [10.2-32.8] to 12.0 fM Xa/min [7.0-18.5], P=0.006, Table 3). This reduction was observed in all ISS stages of disease. Subgroup analysis for the different induction treatments revealed that VAD, TAD and PAD induced similar decrease of MP-TF activity levels (data not shown). When comparing MP-TF activity at the moment of response evaluation after the induction chemotherapy, no relationship between the change in MP-TF activity and the remission status (CR versus PR versus SD/PD) could be observed (data not shown). In addition the MP-TF activity levels were independent from the absolute or relative M-protein levels before and after induction therapy.

MP-TF activity and venous thrombosis

Fifteen of the 122 patients (12%) developed a VTE complication during the induction treatment (n=13) or high dose melphalan treatment and autologous stem cell transplantation (n=2). The baseline characteristics were similar for patients who did and did not develop VTE (Table 1). The incidence of VTE was 10% in the VAD treated patients, 16.7% in patients treated with thalidomide (TAD) and 13% in

patients who received Bortezomib (PAD). The MP-TF activity prior to the start of treatment was not different between patients who developed VTE (16.8 [11.4-36.1]; n= 15), and those who did not (17.8 [8.1-32.5]; n=107). In the 75 patients of whom plasma was obtained before and after chemotherapy we found that in the patients who did not develop VTE (n=64) during the course of treatment, the MP-TF activity levels decreased significantly (from 18.3 [IQR 9.8-32.6]) to 11.4 [IQR 7.0-25.2]; P<0.001). In contrast, in patients who did develop VTE (n=11) the MP-TF activity levels remained unchanged after induction chemotherapy (from 16.8 [IQR 11.4-34.6]) to 15.1 [IQR 10.3-25.2]; P=0.71, Figure 3). We calculated the risk of VTE in MM patients based on MP-TF activity levels below (n=40) and above (n=82) the upper limit in the healthy volunteers (11.8 fM Xa/min). The calculated risk for VTE in individuals with high MP-TF levels was 1.4 (0.4-4.7) [OR (95% CI)], for TF levels measured before start of induction and 3.0 (0.7-12.4) for MP-TF levels measured after induction therapy.

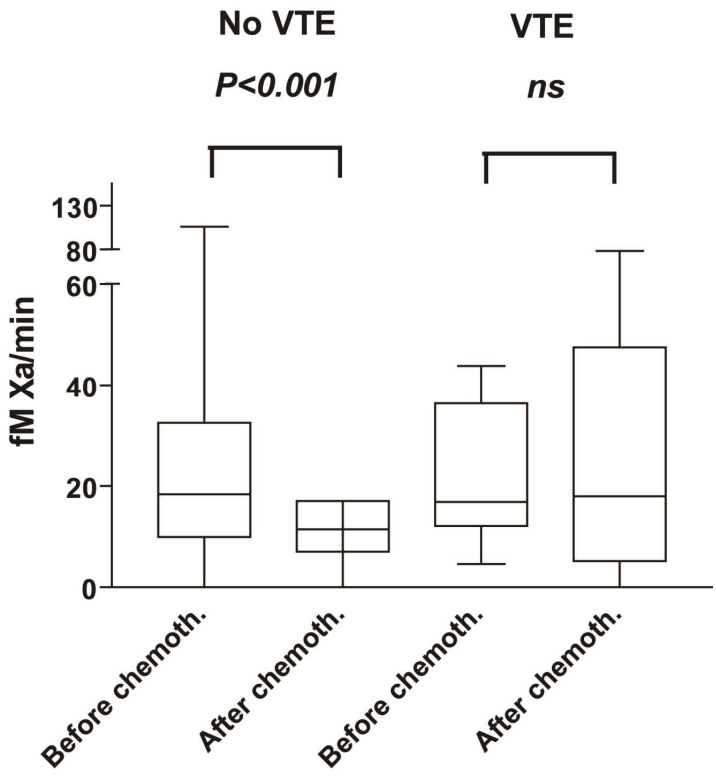

Figure 3: MP-Tissue factor activity in fM/Xa/min before and after induction chemotherapy in 75 MM patients, of whom plasma was available for two time points who did (VTE, n=11) and who did not (no VTE, n=64) develop VTE.

DISCUSSION

In this study we have shown that MP-TF activity levels are increased in patients with MM. The MP-TF activity levels in these patients decrease after induction chemotherapy. In patients experiencing VTE during or following treatment of MM, MP-TF activity levels did not decrease in contrast to the non-VTE patients in whom MP-TF activity decreased significantly compared to baseline.

Tissue factor is commonly regarded as the main determinant of a hypercoagulable state in patients with cancer (26). We measured tissue factor activity on circulating microparticles, small membrane vesicles derived from different cell types, including monocytes, platelets and tumour cells (14). In patients with untreated MM we observed increased MP-TF activity levels compared to healthy controls. From previous studies it is well known that several tumour types express high levels of tissue factor on their surface (13, 27). In addition, Kakkar et al. have shown that plasma tissue factor levels are increased in patients with malignancy (28). Also in patients with haematological disease, including acute myeloid leukaemia and malignant lymphoma, high plasma tissue factor antigen levels have been observed (29). Tissue factor messenger RNA levels in leukocytes from patients with hematopoietic tumours are increased compared to healthy controls (30). More recently plasma levels of tissue factor antigen were not found to be elevated in a small number of MM patients and also tissue factor mRNA levels measured in peripheral blood mononuclear cells were not elevated in these patients (31). In a recent study Shimizu described tissue factor production by clonal plasma cells in a patient with MM. In these plasma cells the cytoplasm stained positive for TF, which disappeared after chemotherapy (32). Since membrane-bound tissue factor exists both in an encrypted, inactive form as in an active form, tissue factor plasma antigen levels may not be representative of tissue factor activity in plasma, and therefore measurement of plasma tissue factor activity levels is preferred. We measured increased MP-TF levels in myeloma patients compared to healthy individuals. The levels of MP-TF activity in our study are lower in patients and control subjects than those reported previously by Tesselaar et al. (14). Centrifugation speed during plasma preparation has an effect on the number MPs in the final preparation. In the present study we used platelet-free plasma for the MP isolation (10 min at 2,000g followed by 10 min at 21,475g), while Tesselaar et al. used platelet-poor plasma (single centrifugation, at 1,550g for 20 min). This double centrifugation resulted in an about tenfold reduction of MP numbers in the isolated MP preparation as measured by FACS analysis (see methods section and Figure 1).

At present we do not know the cellular source of the MPs carrying active TF. Previously it has been shown that TF bearing MPs may be derived form cancer cells, platelets and leucocytes (14, 21, 33). Information on the cellular source of the MPs that carry TF activity would be extremely helpful to determine the pathogenetic role of these MPs in the development of thrombosis.

The stage of disease according to Salmon-Durie or the prognostic ISS stages were not correlated with the levels of MP-TF activity, suggesting that the tumour load is not associated with MP-TF activity levels. Remarkably, patients with a lambda type M-component exhibited significantly lower MP-TF activity compared to the kappa type M-component. The clinical significance of this observation is not yet clear since there are no data in the literature, which indicate a different incidence of thrombo-embolic complications.

Thrombo-embolic complications were observed in 15 patients (12%) and occurred with a similar frequency during all three chemotherapeutic regimens. It should be noted that patients, who were treated with chemotherapy in combination with the anti-angiogenic drug thalidomide, also received thromboprophylaxis with LMWH. Despite this thromboprophylaxis, 17% of patients treated with TAD experienced a thrombo-embolic complication. Interestingly, in patients who developed VTE, MP-TF activity levels remained elevated after induction treatment, while in patients who did not develop VTE the levels decreased significantly.

The high levels of MP-TF activity measured after induction treatment may suggest a pathogenetic role of MP-TF in the development of VTE in patients with MM. However the persistent high MP-TF levels may also be a result of thrombus formation. Because we measured MP-TF activity only twice it is difficult to establish that the highest levels of MP TF activity were observed at a time associated with thrombosis. It is known from previous studies that VTE in MM patients is most frequently observed during the first months of treatment (3, 34-35). This coincides with the highest MP-TF activity levels in plasma, as found in our study. It is also possible that MP-TF activity levels remained high as a consequence of the development of VTE. A causative role of MP-TF activity in the development of VTE can therefore not be established from our study. This should be assessed in larger studies, in which MP-TF activity is measured more frequently during induction treatment of MM.

The results of our study may have implications for prophylactic antithrombotic treatment of MM patients to prevent VTE during chemotherapy. So far no consensus on the optimal prophylactic antithrombotic regimen has been reached (2). Nowadays MM patients receive no prophylaxis, LMWH, fixed low-dose warfarin

or aspirin as prophylaxis (2, 35-36). The observation that MM patients have increased levels of MP-TF activity may suggest that antithrombotic agents targeted at inhibition of TF-initiated secondary hemostasis by LMWH or vitamin K antagonists is preferable to aspirin.

Our patients were treated with multi-agent chemotherapy and after three courses the MP-TF activity levels decreased irrespective of the therapeutic regimen (VAD or TAD or PAD). Chemotherapy in combination with anti-angiogenic drugs in MM is associated with the highest risk of thrombo-embolic complications. Our results indicate that this high risk in the TAD treated patients cannot be attributed to an increase of MP-TF activity (37).

In conclusion, MP-TF activity levels are increased in patients with MM and decrease significantly after induction chemotherapy. MP-TF activity levels after chemotherapy remained high in patients developing VTE. Whether MP-TF activity levels have a pathogenetic role in VTE in MM patients remains to be established.

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