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Full Length Article

Tissue factor-bearing extracellular vesicles, procoagulant phospholipids and D-dimer as potential biomarkers for venous thromboembolism in patients with newly diagnosed multiple myeloma: A comprehensive analysis

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ABSTRACT

Background: Candidate biomarkers to improve venous thromboembolism (VTE) risk prediction in patients with newly diagnosed multiple myeloma (MM) undergoing anti-myeloma therapy include tissue factor-bearing microvesicles (MV-TF), procoagulant phospholipids (procoag-PPL), and D-dimer.
Objective: We aimed to determine the levels of MV-TF, procoag-PPL, and D-dimer at baseline and during initial anti-myeloma therapy and their association with the risk of VTE.
Methods: This prospective, longitudinal, observational study included 71 patients with newly diagnosed MM who were eligible for anti-myeloma therapy. Circulating MV-TF levels were measured using a functional method adapted from the Chapel Hill TF-dependent Factor Xa generation assay, and PPL and D-dimer levels with commercially available assays. The three biomarkers were measured at baseline and throughout treatment.
Results: Baseline and on-treatment MV-TF levels were higher in patients who developed VTE compared to those who did not (4.25 *versus* 2.75 fM at baseline, $p = 0.047$ and 6.5 *versus* 1.5 fM during treatment, $p = 0.001$). Baseline and on-treatment Procoag-PPL clotting times did not differ between the groups. Baseline D-dimer levels tended to be higher in patients who developed VTE than in those who did not (1.38 *versus* 0.7 μg/mL, $p = 0.08$). During treatment, D-dimer levels were significantly higher in the VTE group than in the non-VTE group (1.08 *versus* 0.44 μg/mL, $p = 0.008$).
Conclusion: Our results suggest that MV-TF and D-dimer levels may help to refine VTE risk prediction in nMM patients undergoing anti-myeloma therapy. Adequately sized studies including patients receiving new MM therapies are needed to confirm this hypothesis.

1. Introduction

Multiple myeloma (MM) is associated with the highest risk of venous thromboembolism (VTE) of any hematological malignancy [1] with most events occurring within the first six months after diagnosis [2,3]. The risk of VTE is even higher in MM patients receiving

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immunomodulatory drugs (IMiDs), especially in combination with dexamethasone or multi-agent treatment [4]. In patients with newly diagnosed MM (nMM), early VTE is associated with decreased survival rates [5]. Current guidelines state that all patients with nMM should be assessed for VTE risk and offered thromboprophylaxis with aspirin, low-molecular-weight heparin (LMWH), or direct oral anticoagulants (DOACs) based on VTE risk stratification [6,7]. Clinical risk assessment models (RAMs) such as the SAVED, IMPEDE VTE, and PRISM scores, have been developed to stratify nMM patients according to the risk for VTE, but their performance remains limited in external validation cohorts [3,8,9]. Thus, tailored thromboprophylaxis in MM patients remains an unmet clinical need.

Biomarkers may help to improve VTE risk stratification. Thrombogenesis in MM is multifactorial and patients with MM appear to display a coagulable phenotype with many potential contributory factors including raised von Willebrand factor (VWF) and factor VIII plasma levels and impaired fibrinolysis [10–14]. Whether or not abnormal thrombin generation is present and what are the best condition for its analysis are still debated, also raising the following question: if there is the hypercoagulable state would not be strongly linked to thrombosis in MM, what about an increase in initiators of coagulation [12,15,16]? Extracellular vesicles (EVs), *i.e.* exosomes, microvesicles (MVs), and apoptotic bodies, small and large EVs are cell-derived membranous structures [17,18] that are involved in numerous physiological and pathological processes. The potential role of EVs in various aspects of MM, including metastatic dissemination [19], intercellular communication [20,21], and angiogenesis [22] has recently been highlighted. EVs carry procoagulant activities, supported by tissue factor (TF) and procoagulant phospholipids (PPL), and can promote thrombosis in various conditions such as cardiovascular diseases and cancer [23,24]. TF-bearing microvesicles (MV-TF) activity levels have been reported to be associated with the risk of VTE in cancer patients [25]. TF, which is the main activator of coagulation *in vivo*, is thus thought to be the key determinant of the hypercoagulable state in patients with solid tumors. Nevertheless, studies evaluating MV-TF activity in MM patients are scarce. Nielsen et al. reported that, compared to healthy controls, patients with nMM have increased circulating levels of large EVs exerting procoagulant activities [26]. However, their study could not evaluate the association between MV-TF or PPL and the risk of VTE, as none of the included patients developed VTE. Furthermore, the accuracy of most assays used for circulating TF has been highly debated [27,28].

Beside a recent nested case-control study reported that D-dimer, a fibrin-related marker, measured prior to any antimyeloma therapy, improved VTE prediction in nMM patients. Several teams have reported that an increase in D-dimer is associated with VTE in MM patients [29,30].

In the present study, we analyzed for the first time in the MM setting, a recently well-validated MV-TF assay, along with PPL, and D-dimer levels at baseline and during therapy of nMM and we evaluated the association between those biomarkers and the risk of VTE as well as the impact of antimyeloma therapy.

2. Patients, materials, and methods

2.1. Study population

We used the biobank of the prospective, observational, longitudinal, multicenter METRO study (ClinicalTrials.gov number NCT01508416). Full details of the study have been previously reported [15]. Briefly, consecutive adult patients with nMM according to the International Myeloma Working Group (IMWG) criteria were prospectively enrolled at the time of MM diagnosis prior to initiation of any anti-myeloma treatment. Patients were eligible if they were scheduled to receive first-line treatment with bortezomib, thalidomide and dexamethasone (VTD) or bortezomib, melphalan and prednisone (VMP) or melphalan, prednisone, and thalidomide (MPT) or bortezomib and dexamethasone (VD) or bortezomib, cyclophosphamide and dexamethasone (VCD) or lenalidomide, bortezomib and dexamethasone (LVD). The choice of first-line antimyeloma therapy and thromboprophylaxis regimen was left at the discretion of the treating physician. Participants were excluded if they had severe renal failure requiring hemodialysis, ongoing anticoagulant therapy for any indications other than thromboprophylaxis in the nMM setting, life expectancy of less than six months, or if they withdrew consent. As soon as a VTE event occurred, the patient was removed from the study. The inclusion period spanned from December 2011 to May 2015. All patients were followed-up until the first day of the fourth cycle of antimyeloma therapy.

The METRO study was conducted in accordance with the Declaration of Helsinki and approved by the local ethics committee (EudraCT 2011-A01529–32, IRBN892023/CHUSTE). All patients gave written informed consent to participate in the study.

2.2. Sample collection and MVs isolation

Blood samples were collected at inclusion prior to initiation of any antimyeloma therapy and any anti-thromboprophylaxy therapy and during treatment (*i.e.*, the day before the second or third cycles and at least 24 h after any LMWH injection). All blood samples were obtained by clean venipuncture from a peripheral arm vein that had not been previously catheterized in 0.106 M tri-sodium citrate tube.

Samples were centrifuged twice at 2500 $\times g$ for 15 min at room temperature according to the ISTH and ISEV guidelines [31] to obtain platelet-depleted plasma. Plasma samples were stored at −80 °C at the core laboratory of the SAINBIOSE U1059 INSERM unit (University of Saint-Etienne, France) until analysis (12 years maximum before analysis).

Plasma samples were first (250 μL) diluted 1:3 with a HEPES solution (500 μL, 150 mM NaCl, 20 mM HEPES and 0.1 % NaN_3, pH 7.4, 0.22 μm filtrated) and centrifuged at 24,000 $\times g$ for 1 h at 20 °C (temperature controlled with a thermostat) without brake (Megafuge 16R, ThermoFisher Scientific, Courtaboeuf, France). The supernatant was then discarded, leaving 50 μL of plasma. For washing, 1 mL of HEPES solution was added. The liquid was withdrawn and resuspended twice using a micropipette before a second centrifugation at 24,000 $\times g$ for 1 h at 20 °C. The supernatant was discarded avoiding disturbing the pellet, and 125 μL of HEPES solution was added. The liquid was again withdrawn and resuspended in 125 μL of HEPES twice.

2.3. Tissue factor bearing-microvesicles - functional assay (MV-TF)

MV-TF was measured with commercial kit (CY-QUANT MV-TF, Stago, Asnières-sur-Seine, France) using a functional method adapted from the Chapel Hill TF-dependent Factor Xa generation assay [32]. MV suspensions prepared as described above were added to individual wells of a 96-well plate and incubated for 30 min at 37 °C with either an inhibitory anti-TF antibody (10 μg/mL final, clone SBTF-1, BioCytex, Marseille, France) or irrelevant antibody (10 μg/mL, clone a-DNP 2H11—2H12, BioCytex, Marseille, France). Subsequently, a mixture of Factor VII (FVII), Factor X (FX), and $CaCl_2$ was added. During a 2-h incubation at 37 °C, FX was cleaved into activated FX (FXa) by the TF/activated FVII (FVIIa) complex. The reaction was stopped by the addition of ethylenediaminetetraacetic acid (EDTA). The FXa generation kinetics was monitored over 15 min by absorbance at 405 nm using a Multiskan Ascent Microplate Reader 354 (ThermoFisher Scientific, ThermoFisher, Courtaboeuf, France).

MV-TF was calculated based on FXa generation, with a calibration using liposome-associated recombinant TF. This calibration range enabled the conversion of values into femtomolar (fM) concentrations of MV-TF. Plasma levels were obtained by dividing the measured level of active MV-TF in the final volume of the resuspending medium with the concentration factor. Calibration curves were performed with very good reproducibility, as exemplified in Supplemental Fig. 1.

2.4. Procoagulant phospholipids (PPL)

The STA®-Procoag-PPL assay (Stago, Asnières-sur-Seine, France) was used on a STA-R Max coagulation analyzer (Stago, Asnières, France) according to the manufacturer's instructions to measure the PPL activity in plasma samples. Briefly, the assay is based on the clotting time of a plasma sample diluted with PPL-depleted normal plasma after addition of factor Xa and $CaCl_2$. All coagulation factors are supplied at physiological levels by the reagent PPL-depleted plasma except PPL, which is supplied by the plasma sample being tested. The clotting time (in seconds) is inversely proportional to PPL activity, a shorter clotting time indicating increased PPL. Standard pool plasma (SPP) with buffer only (blank) was measured several times to establish a reference range for the PPL clotting time.

2.5. D-dimer

D-dimer levels were measured using the quantitative latex STA®-Liatest® D-Di assay (Stago, Asnières, France) according to the manufacturer's instructions on an STA-R Max coagulation analyzer (Stago, Asnières, France).

2.6. Statistical analysis

All statistical analyses were performed using GraphPad Prism software version 8.0 (GraphPad Software, San Diego, CA, USA). Continuous variables were expressed as medians and interquartile ranges (IQR), and categorical variables were expressed as numbers and percentages. Continuous variables were compared using the nonparametric Mann–Whitney *U* test or the Wilcoxon test, as appropriate. Correlations between continuous variables were analyzed by calculating Spearman's rank correlation coefficient. All tests were 2-sided, and a *p*-value <0.05 was considered statistically significant.

3. Results

The characteristics of the included 71 patients with nMM scheduled to receive first-line antimyeloma therapy are summarized in Table 1. The median age was 67 years (IQR, 59–73) and 32 (45 %) patients were male. Thirty-one patients received VTD, 17 VMP, 14 MPT, 5 VD, 2 VCD and 2 LVD. Thromboprophylaxis with aspirin, LMWH, unfractionated heparin (UFH) or fondaparinux was administered in 54 (76 %) of 71 patients (Table 1). During a mean follow-up of 133 ± 46 days, eight patients developed symptomatic VTE (deep vein thrombosis: 6; pulmonary embolism: 2). The median time to VTE was 47 days (range, 1–122 days). At the time of VTE, six of eight patients were receiving thromboprophylaxis (aspirin: 4; and LMWH: 2). VTE occurred before the time of the second blood collection in three of eight patients.

MV-TF, PPL and D-dimer were analyzed in 58, 58 and 56 patients with available plasma samples, respectively (see Fig. 1 for more details).

3.1. MV-TF

Compared to baseline, circulating MV-TF significantly decreased during treatment (median, 3 fM; IQR, 1.5–4.5 *versus* 2 fM; IQR, 1–3.5; $p = 0.02$; Fig. 2A). Patients who developed VTE had significantly higher baseline MV-TF compared to those who did not (median, 4.25 fM; IQR, 2.75–6.125 *versus* 2.75 fM; IQR, 1.5–4.50; $p = 0.047$; Fig. 2B). Those who developed VTE during treatment had significantly higher circulating MV-TF on-treatment compared to those who did not (median, 6.5 fM; IQR, 3.5–11.5 *versus* 1.5 fM; IQR, 1.0–3.0; $p = 0.001$; Fig. 2C).

3.2. Plasma procoagulant phospholipids (Procoag-PPL)

Compared to baseline, Procoag-PPL significantly decreased during treatment (median clotting times, 58.5 s; IQR, 49.00–71.25 *versus* 75.5 s;

Table 1
Baseline demographic and clinical characteristics of the whole METRO study population.

No. of patients $n = 71$ (%)		
General characteristics		
Age (years)	Median	67
	IQR	59–73
Males (N)		32
Body mass index MI (kg/m^2)	Median	26
	IQR	22–29
	BMI ≥ 30 kg/m^2	7
Creatinine (μmol/L)	Median	77
	IQR	66–100
Platelets (10^9/L)	Median	235
	IQR	194–283
Prothrombin time (activity expressed as %)	Median	92
	IQR	83–98
Medical history	Venous thromboembolism	4
	- Including pulmonary embolism	2
	Cardiovascular	45
	- hypertension	32
	Including: diabetes	
	coronary artery disease	3
Familial medical history	Venous thromboembolism	6
Multiple myeloma characteristics		
International staging system	I	16
	II	24
	III	22
Serum monoclonal component	IgG	40
	IgA	19
	IgD	3
	Light chain	9
Treatments		
MM therapy regimens	Bortezomib + dexamethasone + thalidomide (VTD)	31
	Bortezomib + melphalan + prednisone (VMP)	17
	Melphalan + prednisone + thalidomide (MPT)	14
	Bortezomib + dexamethasone (VD)	5
	Bortezomib + dexamethasone + cyclophosphamide (VCD)	2
	Bortezomib + lenalidomide + dexamethasone (LVD)	2
IMiDs containing regimens		47
Steroids	Dexamethasone	40
	Prednisone	31
Hematopoietic growth factors	Erythropoietin stimulating agents	7
Pharmacological thromboprophylaxis	Prophylaxis (all indication including)	54
	Prophylaxis because of multiple myeloma	50
	Type (possible sequential or concomitant use):	
	Low molecular weight heparin	32
	Aspirin	24
	Unfractionated heparin	5
	Fondaparinux	2

IMPEDE VTE score			
Score	VTE risk within 6 months	Without VTE (n = 63)	With VTE (n = 8)
	Median	5	3
	IQR	1–8	1–8
≤3	Low	30	4
>4 and <7	Intermediate	15	2
≥8	High	18	2

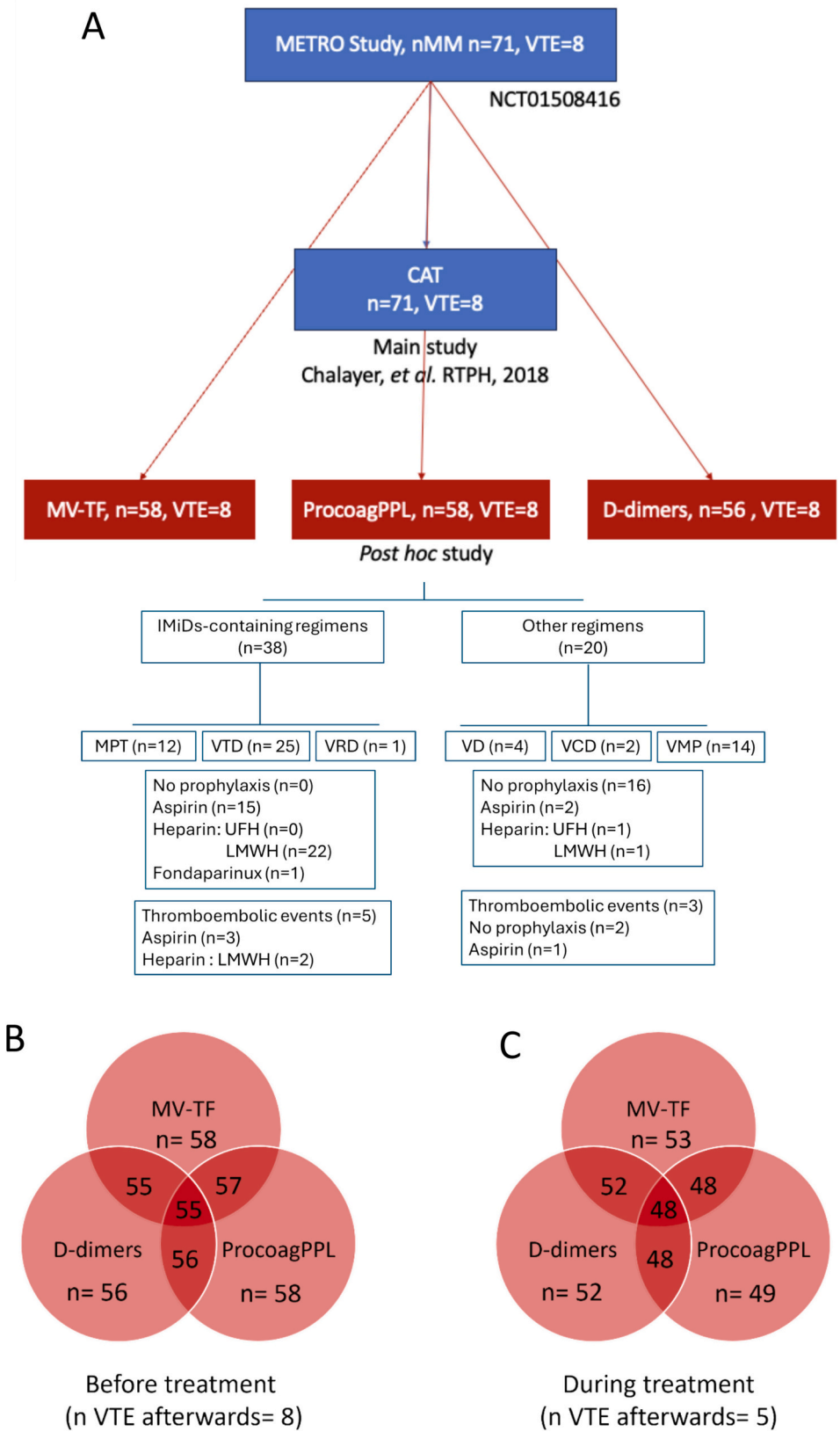

Fig. 1. Plasma samples collected within the framework of the METRO study that could be assayed for the biomarkers.
VTD: Bortezomib, thalidomide, dexamethasone, VMP: Bertezomib, melphalan, prednisone; MPT: Melphalan, prednisone, thalidomide; VD: Bortezomib, dexamethasone; VCD: Bortezomib, dexamethasone cyclophosphamide; VRD: Bortezomib, Revlimid (lenalidomide), dexamethasone.

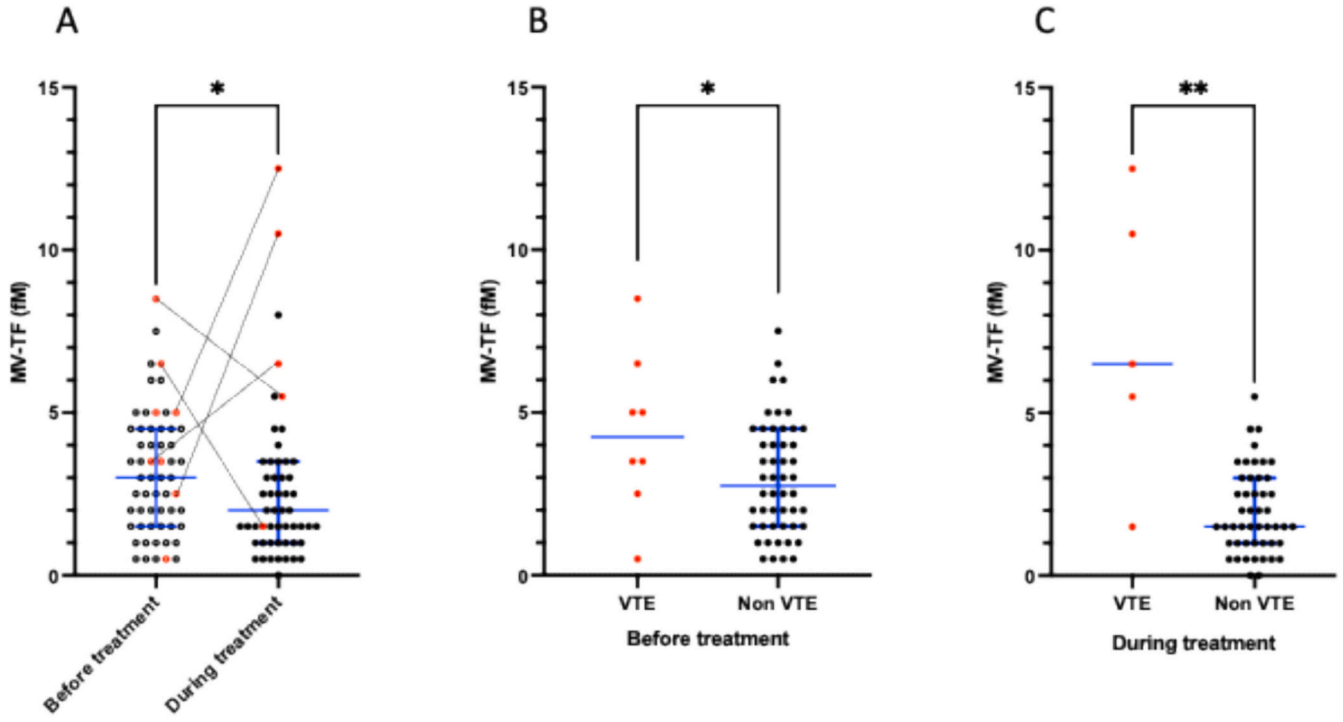

Fig. 2. Circulating tissue factor-bearing microvesicles levels (MV-TF) of patients with MM before and during treatment.
Distribution of microvesicle-associated tissue factor (MV-TF; functional test) levels before and during treatment. A: MV-TF levels before ($n = 58$) and during treatment ($n = 54$; three VTE during treatment inception). B: comparison of the results before treatment of the VTE group ($n = 8$) and the non-VTE group ($n = 50$), C: same comparison, results during treatment inception VTE (VTE group n = 5, non-VTE group $n = 49$). Red dots represent samples from patients who experienced VTE during MM treatment. As soon as a VTE event occurred, the patient was removed from the study. Links were drawn between patients (n = 5) who experienced a VTE event after T2. Changes in MV-TF were assessed with Wilcoxon test and levels were compared between the two patients' groups (VTE or not): Mann-Whitney test. Medians are shown as middle horizontal bar and interquartile ranges (IQR) as upper and lower horizontal bars. $p > 0.05$; *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$.and ****: $p < 0.001$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

IQR, 64.9–81.45; $p < 0.001$; Fig. 3A). Patients who developed VTE had similar baseline Procoag-PPL compared to those who did not (median clotting time, 52.5 s; IQR, 40.8–64.5 *versus* 60 s; IQR, 50.0–72.0; p = ns; Fig. 3B). Similarly, during treatment, no difference was observed in Procoag-PPL between those who further developed VTE and those who did not (median clotting time, 69.2 s; IQR: 60.15–73.45 *versus* 76.05 s; IQR: 65.38–82.05; p = ns; Fig. 3C).

3.3. D-dimer levels

Compared to baseline, D-dimer levels significantly decreased during treatment (median, 0.74 μg/mL; IQR, 0.3–1.6 *versus* 0.51 μg/mL; IQR, 0.24–0.7; p < 0.001; Fig. 4). The median time between sampling and VTE for the measure before treatment was 46 days (9–100, $n = 8$) and 75 days (10–79, $n = 5$) for the measure during treatment. Patients who developed VTE had higher baseline D-dimer levels than those who did not (median, 1.38 μg/mL; IQR, 0.57–3.46 *versus* 0.7 μg/mL; IQR, 0.29–1.49; Fig. 4B) but the difference was not significant ($p = 0.08$).

In univariate analysis, the adjusted odds ratio (OR) for VTE was 9.5 (95 % CI 1.0–93.1) for those with baseline D-dimer >2.1 μg/mL (specificity 86 %, sensibility 50 %). During treatment, those who further developed VTE had significantly higher D-dimer levels than those who did not (median, 1.08 μg/mL; IQR, 0.46–1.41 *versus* 0.44 μg/mL; IQR, 0.22–0.63; $p = 0.008$; Fig. 4C).

3.4. Associations with antimyeloma therapy modalities

We evaluated the associations between IMiDs and dexamethasone as part of the regimen and biomarker levels by comparing patients who received the specific agent to those who did not (Supplemental Fig. 2). MV-TF (median, 2 fM; IQR, 1.25–3.75 *versus* 2 fM; IQR, 1.125–3.375; $p = 0.345$) and Procoag-PPL (median clotting time, 70.30 s; IQR, 64.70–81.60 *versus* 76.8 s; IQR, 66.35–82.03; $p = 0.29$) did not differ between the IMIDs and the non-IMiDs groups. D-dimer levels were significantly lower in the IMiDs group (median, 0.38 μg/mL; IQR, 0.21–0.62) compared to the non-IMiDs group (median, 0.61 μg/mL; IQR, 0.39–0.73; $p = 0.046$). MV-TF (median, 2 fM: IQR, 1–3.5 *versus* 1.75 fM; IQR, 0.625–3.375; $p = 0.5001$), Procoag-PPL (median clotting times, 73.40 s; IQR, 66.95–81.63 *versus* 76.20 s; IQR, 60.15–81.45;$p = 0.37$) and D-dimer levels (median, 0.48 μg/mL; IQR, 0.17–0.68 *versus* 0.51 μg/mL; IQR, 0.36–0.65; $p = 0.31$) did not differ between the dexamethasone and the non-dexamethasone groups.

3.5. Correlations between biomarkers

Before therapy initiation, no correlation was found between MV-TF and PPL levels ($r = -0.09$, $p = 0.245$) or D-dimer levels ($r = 0.07$, $p = 0.315$). Similarly, we observed no correlation between PPL and D-dimer levels ($r = -0.03$, $p = 0.422$) (Fig. 5A, B, and C). During treatment, we found no correlation between MV-TF and D-dimer levels ($r = 0.09$, $p = 0.26$) or between PPL and D-dimer levels ($r = 0.10$, p = 0.24) (Fig. 5D and F). Nonetheless, we observed a weak but statistically significant correlation between MV-TF and PPL levels ($r = -0.35$, $p = 0.007$) (Fig. 5E).

4. Discussion

The present study investigated the potential value of three biomarkers, namely MV-TF, PPL, and D-dimer levels, for predicting VTE in patients with nMM during the first 3 cycles of therapy, as well as their

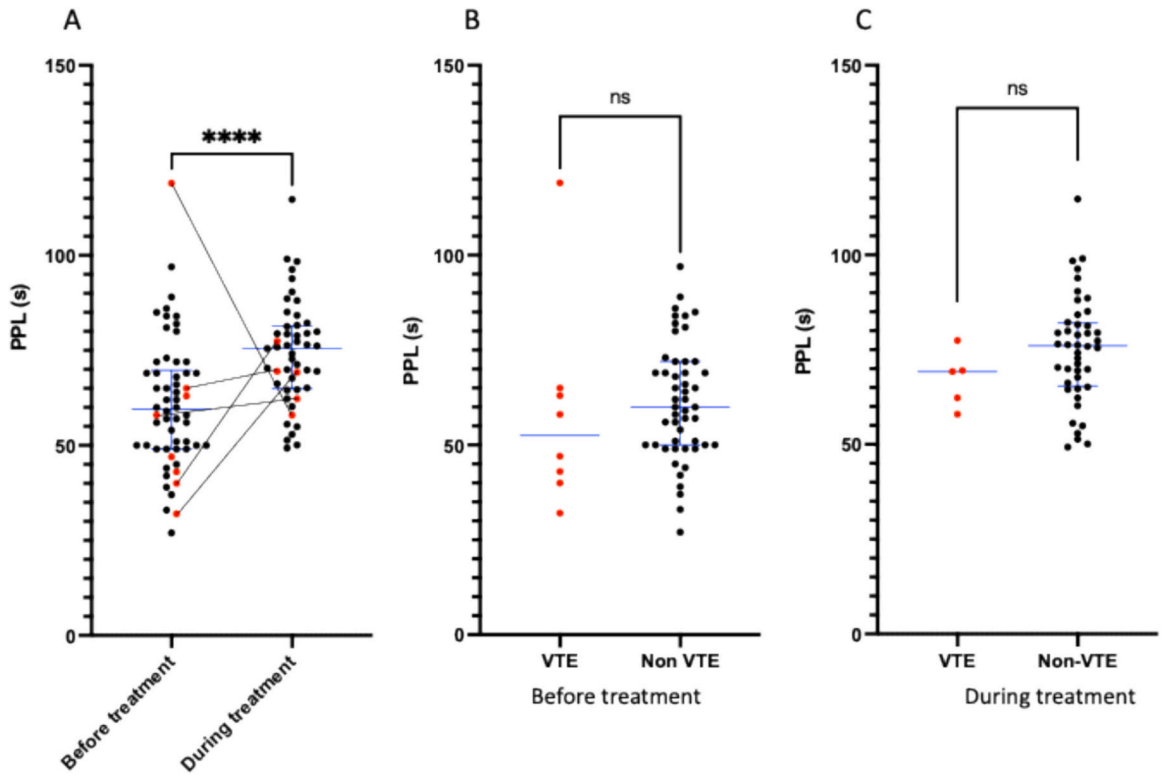

Fig. 3. Clotting times of the Procoag PPL assay of patients with MM before and during treatment.
A: Distribution of PPL coagulation times in MM patients before ($n = 58$) and during treatment ($n = 52$). B: Comparing the VTE group (n = 8) and the non-VTE group ($n = 50$) before any MM treatment. C: Comparison between the VTE (n = 5) and non-VTE ($n = 47$) groups during treatment. Red dots represent samples from patients who experienced VTE event during treatment. As soon as a VTE event occurred, the patient was removed from the study. Links were drawn between patients (n = 5) who experienced a VTE event after T2. Changes were assessed using a Wilcoxon test and levels were compared between the two patients' groups: Mann-Whitney test. Medians are shown as middle horizontal bar and interquartile ranges (IQR) as upper and lower horizontal bars. $p > 0.05$; *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$.and ****: $p < 0.001$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

changes during initial antimyeloma therapy. This is the first study demonstrating a significant association between MV-TF levels and the risk of VTE in patients with nMM. Indeed, the baseline MV-TF levels were significantly higher in patients who experienced VTE than in those who did not. MV-TF levels decreased during the initial therapy in both groups, but the difference in MV-TF levels between patients who experienced VTE and those who did not remain statistically significant. The decrease in MV-TF levels during initial antimyeloma therapy does not appear to be directly related to neoplastic plasma cells decrease. TF expression has been reported to be absent on plasma cells in MM patients [33]. In MM patients, MV-TF may originate from endothelial cells and monocytes-derived EVs and promote coagulation [34,35]. The decrease in MV-TF levels during initial antimyeloma therapy may rather be due to the decreased inflammatory environment resulting from MM treatment, which subsequently reduces endothelial cell activation.

Few studies have evaluated MV-TF in MM patients. Auwerda et al. previously reported increased baseline MV-TF levels in a cohort of 122 nMM patients who were eligible for antimyeloma therapy compared to healthy controls [36] with a 12 % incidence of VTE during the follow-up. However, in contrast to our results, baseline MV-TF activity levels did not differ between nMM patients who experienced VTE and those who did, but they found a significant difference during treatment as in our study. This difference may be due to cohort differences (median age 55 *versus* 67 years in ours, – in Auwerda's study patients on thalidomide received also received LMWH but not patients on doxorubicin). Nielsen et al. reported elevated baseline MV-TF levels in 38 nMM patients compared to healthy controls, but none of the included patients developed VTE [37]. In our study, MV-TF activities were low compared to other cancers [38,39]. Nevertheless, our results suggest that circulating MV-TF levels could potentially guide the choice of thromboprophylaxis agent or the decision to continue or discontinue thromboprophylaxis after initial treatment cycles. Nevertheless, further studies with large sample sizes are warranted to confirm our findings.

VTE patients tended to have shorter baseline PPL clotting times compared to non-VTE patients, but this trend was not statistically significant. The small number of events may have limited statistical power.

Consistent with previous studies, we found a significant association between D-dimer levels and VTE risk. Sanfilippo et al. recently proposed the addition of D-dimer to the IMPEDE VTE score to improve its performance [40].

Finally, we found no correlation between the levels of MV-TF, PPL, and D-dimer level before antimyeloma treatment initiation. During antimyeloma treatment, we observed a weak but significant correlation between TF and PPL. PPL are involved in the MV-TF functional assay, but only a part of them are required for TF and FVIIa being assembled and active on FX. Conversely, the presence of active TF could shorten the clotting time of the Procoag-PPL assay, but the addition of exogenous FXa in the assay bypasses the first step of the coagulation cascade making the PPL measurement almost independent from the TF content. In addition, MV-TF measurements involve concentrated EVs, while PPL measurements are performed in diluted plasma. Levels of D-dimer, the final product of the coagulation process, are modulated by the fibrinolytic system status, which has been reported to be altered in MM patients [41]. This highlights the complexity of coagulation imbalances in MM patients and suggests that a single test may not fully capture their coagulability status.

While IMiDs and dexamethasone are known to increase the risk of VTE in MM patients, our results indicate that such treatment modalities

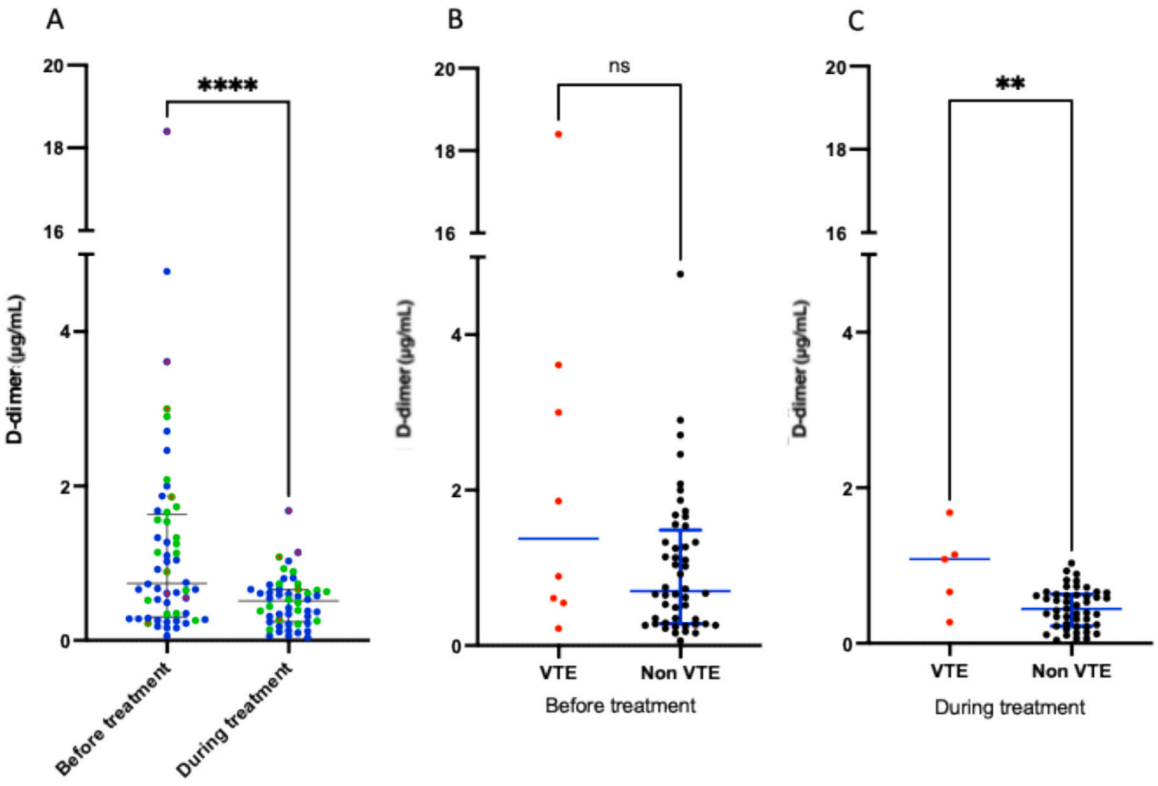

Fig. 4. D-dimer levels before and during treatment.
A: Distribution of D-dimers levels in MM patients before ($n = 56$) and during treatment initiation ($n = 52$). B: Comparison between the VTE (n = 8) and non-VTE ($n = 48$) groups. C: Comparison between the VTE (n = 5) and non-VTE ($n = 46$) groups during treatment (of note, scaling is different). The red dots in B and C represent samples from patients who experienced VTE during treatment. The patient was removed from the study as soon as a VTE event occurred. Links were drawn between the patients (n = 5) who experienced a VTE event after T2. Blue and green dots in A represent IMiDs and not contagining IMiDs regimens of MM treatment, respectively. Changes were assessed using the Wilcoxon test, and levels were compared between the two patient groups using the Mann-Whitney test. Medians are shown as middle horizontal bar and interquartile ranges (IQR) as upper and lower horizontal bars. $p > 0.05$; *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$.and ****: $p < 0.001$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

did not significantly affect MV-TF, PPL, or D-dimer levels. This suggests that the increased VTE risk associated with IMiDs and dexamethasone is not reflected by those biomarkers. In a previous study, we showed that IMiDs treatment increased thrombin generation [15]. Nielsen et al. showed a different trend under VCD treatment regarding MV-TF. In our study, only two patients received VCD, which is no longer a first-line treatment in nMM [26].

Our study has several limitations. First, the sample size and the small number of VTE events may have limited the statistical power. Due to the study design as a *post hoc* analysis, a sample size was not calculated. However, obtaining statistically significant results with such a population size, small but quite similar to those of other MM studies, seems very encouraging. A future prospective study will be conducted with a sample size calculation based on the herein reported study, and will specifically address the prediction potential of D-dimers at baseline. Expanding the cohort size but also including current treatment protocols will also improve the generalizability and applicability of our findings. Moreover, as a multivariate analysis required at least 10 events per variable, we were not able to conduct this analysis [42]. Second, we focused only on active TF of large EVs (mainly MVs and apoptotic bodies are harvested by the centrifugation preparative protocol) and we did not evaluate TF of small EVs (exosomes). However, EVs are thought to be the main source of procoagulant activity due to their phosphatidylserine exposure [26]. Third, we did not differentiate EVs based on their cellular origin. The implementation of an immunocapture method could overcome this limitation in future studies [43]. Fourth, we found significant variability in EVs preparation by high-speed centrifugation and pellet drying (~20 % variation), which should be improved for routine use. Finally, the study design does not allow comparison with normal values, which are difficult to establish in age-matched non-MM controls, with similar comorbidities. Nevertheless, our results provide valuable insights into the pathogenesis of VTE in patients with nMM. D-dimer levels remain a useful biomarker in routine practice and could potentially improve prediction. Furthermore, our study suggests that MV-TF and D-dimer levels could be used in future randomized trials to determine whether thromboprophylaxis should be continued. Moreover, future studies aiming at isolating and characterizing EVs from different cellular origins, especially from tumor plasma cells, using advanced immunocapture and separation techniques are warranted.

In conclusion, baseline and on-treatment MV-TF and D-dimer levels are elevated in patients with nMM who develop VTE. Inclusion of these biomarkers in current RAMs for predicting the risk of VTE may improve their performance.

CRediT authorship contribution statement

S. Charles: Writing – original draft, Visualization, Validation, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **T. Fatrara:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation. **T. Bouriche:** Writing – review & editing, Resources, Methodology. **A. Bonifay:** Writing – review & editing, Methodology. **T. Lecompte:** Writing – review & editing, Writing – original draft, Validation, Supervision, Methodology, Formal analysis, Conceptualization. **F. Dignat-George:** Writing – review & editing. **B. Tardy:** Writing – review & editing, Conceptualization. **C. Frere:** Writing – review & editing,

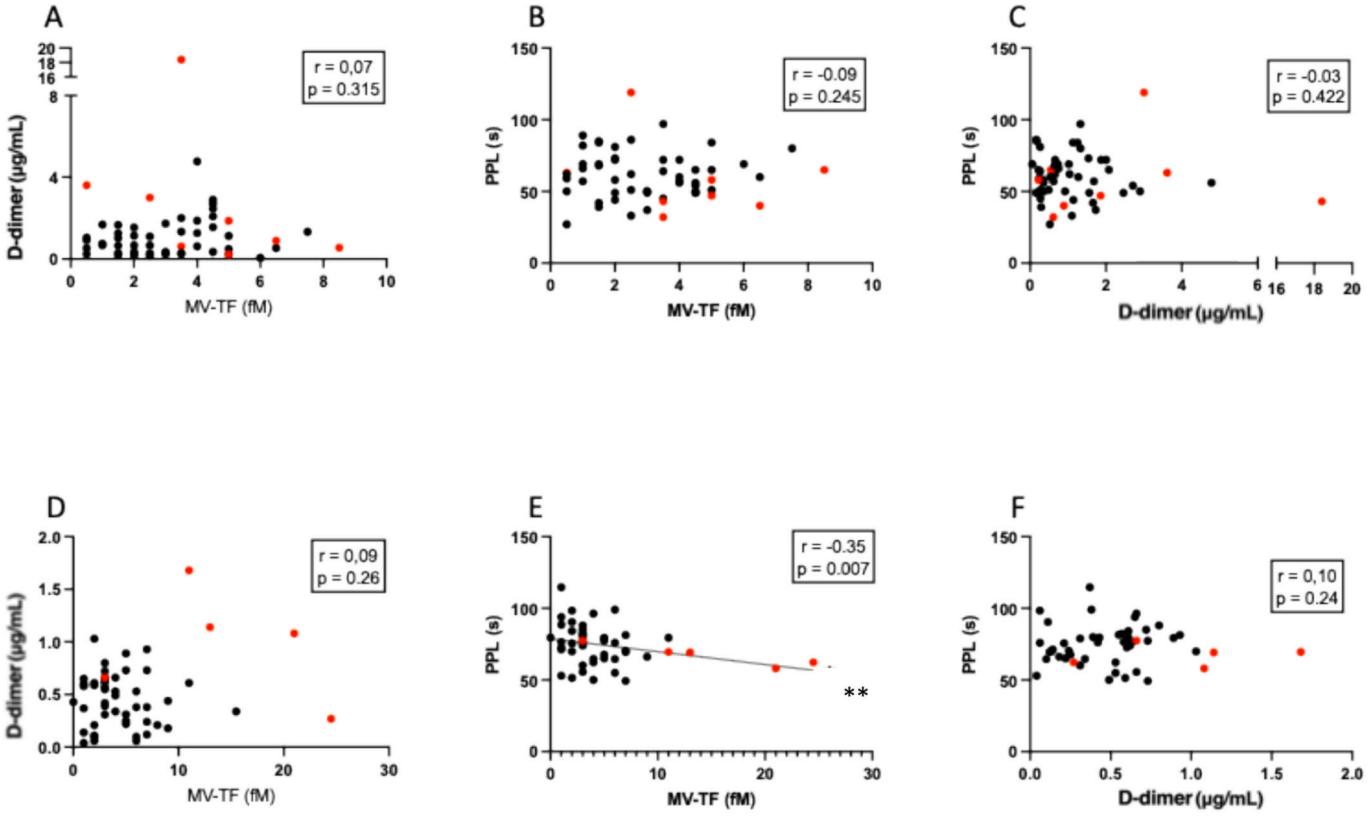

Fig. 5. Spearman correlations between MV-TF, D-dimer and Procoag PPL.
Before MM treatment: A: Between MV-TF and D-dimer ($n = 55$); B: Between MV-TF and PPL ($n = 57$); C: Between D-dimer and PPL (n = 56). During MM treatment: D: Between MV-TF activity and D-dimer ($n = 51$); E: Between MV-TF activity and PPL (n = 52); F: Between D-dimer and PPL (n = 51). The red dots represent samples from patients who experienced VTE during treatment. **: $p < 0.01$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Validation, Methodology, Investigation. **R. Lacroix:** Writing – review & editing, Validation, Methodology, Formal analysis. **E. Chalayer:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used Paperpal [NAME TOOL / SERVICE] in order to improve language and readability. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

Declaration of competing interest

We disclose as a conflict of interest that BioCytex provided CY-QUANT MV-TF kits and T.Bouriche is full-time employee of BioCytex. Other authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.thromres.2025.109256.

References

[1] F.I. Mulder, E. Horváth-Puhó, N. van Es, H.W.M. van Laarhoven, L. Pedersen, F. Moik, et al., Venous thromboembolism in cancer patients: a population-based cohort study, Blood 137 (14) (8 avr 2021) 1959–1969.
[2] M. Carrier, G.L. Gal, J. Tay, C. Wu, A.Y. Lee, Rates of venous thromboembolism in multiple myeloma patients undergoing immunomodulatory therapy with thalidomide or lenalidomide: a systematic review and meta-analysis, J. Thromb. Haemost. 9 (4) (2011) 653–663.
[3] E. Chalayer, A. Talbot, L. Frenzel, L. Karlin, P. Collet, D. Guyotat, et al., Prediction of venous thromboembolism in patients with multiple myeloma treated with lenalidomide, bortezomib, dexamethasone, and transplantation: lessons from the substudy of IFM/DFCI 2009 cohort, J. Thromb. Haemost. 20 (8) (2022) 1859–1867.
[4] W. Li, D. Garcia, R.F. Cornell, D. Gailani, J. Laubach, M.E. Maglio, et al., Cardiovascular and thrombotic complications of novel multiple myeloma therapies: a review, JAMA Oncol. 3 (7) (1 juill 2017) 980–988.
[5] M.W. Schoen, K.R. Carson, S. Luo, B.F. Gage, A. Li, A. Afzal, et al., Venous thromboembolism in multiple myeloma is associated with increased mortality, Res. Pract. Thromb. Haemost. 4 (7) (2020) 1203–1210.
[6] S.K. Kumar, N.S. Callander, K. Adekola, L. Anderson, M. Baljevic, E. Campagnaro, et al., Multiple myeloma, version 3.2021, NCCN Clinical Practice Guidelines in Oncology, J. Natl. Compr. Cancer Netw. 18 (12) (2 déc 2020) 1685–1717.
[7] L. Frenzel, O. Decaux, M. Macro, K. Belhadj-Merzoug, S. Manier, C. Touzeau, et al., Venous thromboembolism prophylaxis and multiple myeloma patients in real-life: results of a large survey and clinical guidance recommendations from the IFM group, Thromb. Res. 233 (2 déc 2023) 153–164.

[8] K.M. Sanfilippo, S. Luo, T.F. Wang, M. Fiala, M. Schoen, T.M. Wildes, et al., Predicting venous thromboembolism in multiple myeloma: development and validation of the IMPEDE VTE score, Am. J. Hematol. 94 (11) (nov 2019) 1176–1184.
[9] A. Li, Q. Wu, S. Luo, G.S. Warnick, N.A. Zakai, E.N. Libby, et al., Derivation and validation of a risk assessment model for immunomodulatory drug–associated thrombosis among patients with multiple myeloma, J. Natl. Compr. Cancer Netw. 17 (7) (1 juill 2019) 840–847.
[10] S. Nomura, T. Ito, H. Yoshimura, M. Hotta, T. Nakanishi, S. Fujita, et al., Evaluation of thrombosis-related biomarkers before and after therapy in patients with multiple myeloma, J. Blood Med. 9 (janv 2018) 1–7.
[11] L. Papageorgiou, P. Van Dreden, M.A. Dimopoulos, A. Larsen, M. Mohty, I. Elalamy, et al., Procoagulant microparticles derived from myeloma plasma cells have a determinant role in the hypercoagulable state associated with multiple myeloma. A modelization in vitro study, Blood 134 (Supplement_1) (13 nov 2019) 2425.
[12] C. Comerford, S. Glavey, J. Quinn, J.M. O'Sullivan, The role of VWF/FVIII in thrombosis and cancer progression in multiple myeloma and other hematological malignancies, J. Thromb. Haemost. JTH. 20 (8) (août 2022) 1766–1777.
[13] O. Jarchowsky, O. Avnery, M.H. Ellis, Thrombosis in multiple myeloma: mechanisms, risk assessment and management, Leuk. Lymphoma 64 (12) (déc 2023) 1905–1913.
[14] I. Sánchez Prieto, I. Gutiérrez Jomarrón, C. Martínez Vázquez, P. Rodríguez Barquero, P. Gili Herreros, J. García-Suárez, Comprehensive evaluation of genetic and acquired thrombophilia markers for an individualized prediction of clinical thrombosis in patients with lymphoma and multiple myeloma, J. Thromb. Thrombolysis 57 (6) (27 avr 2024) 984–995.
[15] E. Chalayer, B. Tardy-Poncet, L. Karlin, C. Chapelle, A. Montmartin, M. Piot, et al., Thrombin generation in newly diagnosed multiple myeloma during the first three cycles of treatment: an observational cohort study, Res Pract Thromb Haemost. 3 (1) (13 déc 2018) 89–98.
[16] L. Li, M. Roest, Y. Sang, J.A. Remijn, R. Fijnheer, K. Smit, et al., Patients with multiple myeloma have a disbalanced whole blood thrombin generation profile, Front. Cardiovasc. Med. 9 (27 juin 2022) 919495.
[17] J.C. Akers, D. Gonda, R. Kim, B.S. Carter, C.C. Chen, Biogenesis of extracellular vesicles (EV): exosomes, microvesicles, retrovirus-like vesicles, and apoptotic bodies, J. Neuro-Oncol. 113 (1) (mai 2013) 1–11.
[18] N. Arraud, R. Linares, S. Tan, C. Gounou, J.M. Pasquet, S. Mornet, et al., Extracellular vesicles from blood plasma: determination of their morphology, size, phenotype and concentration, J. Thromb. Haemost. 12 (5) (2014) 614–627.
[19] M. Colombo, D. Giannandrea, E. Lesma, A. Basile, R. Chiaramonte, Extracellular vesicles enhance multiple myeloma metastatic dissemination, Int. J. Mol. Sci. [Internet] 20 (13) (1 juill 2019) [cité 18 janv 2021]. Disponible sur, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6650870/.
[20] I. Saltarella, A. Lamanuzzi, B. Apollonio, V. Desantis, G. Bartoli, A. Vacca, et al., Role of extracellular vesicle-based cell-to-cell communication in multiple myeloma progression, Cells 10 (11) (16 nov 2021) 3185.
[21] D. Giannandrea, N. Platonova, M. Colombo, M. Mazzola, V. Citro, R. Adami, et al., Extracellular vesicles mediate the communication between multiple myeloma and bone marrow microenvironment in a NOTCH dependent way, Haematologica 107 (9) (1 sept 2022) 2183–2194.
[22] M. Zarfati, I. Avivi, B. Brenner, T. Katz, A. Aharon, Extracellular vesicles of multiple myeloma cells utilize the proteasome inhibitor mechanism to moderate endothelial angiogenesis, Angiogenesis 22 (1) (févr 2019) 185–196.
[23] R. Zhou, E. Bozbas, K. Allen-Redpath, P. Yaqoob, Circulating extracellular vesicles are strongly associated with cardiovascular risk markers, Front. Cardiovasc. Med. 9 (2022) 907457.
[24] M. Zarà, G.F. Guidetti, M. Camera, I. Canobbio, P. Amadio, M. Torti, et al., Biology and role of extracellular vesicles (EVs) in the pathogenesis of thrombosis, Int. J. Mol. Sci. 20 (11) (11 juin 2019) 2840.
[25] C.J. Cui, G.J. Wang, S. Yang, S.K. Huang, R. Qiao, W. Cui, Tissue factor-bearing MPs and the risk of venous thrombosis in cancer patients: a meta-analysis, Sci. Rep. 8 (1) (26 janv 2018) 1675.
[26] T. Nielsen, S.R. Kristensen, H. Gregersen, E.M. Teodorescu, G. Christiansen, S. Pedersen, Extracellular vesicle-associated procoagulant phospholipid and tissue factor activity in multiple myeloma, PLoS One 14 (1) (14 janv 2019) e0210835.
[27] A. Bonifay, N. Mackman, Y. Hisada, A.T.A. Sachetto, C. Hau, E. Gray, et al., Comparison of assays measuring extracellular vesicle-tissue factor in plasma samples: communication from the ISTH SSC Subcommittee on Vascular Biology, J. Thromb. Haemost. JTH. 22 (10) (October 2024) 2910–2921.
[28] N. Mackman, A.T.A Sachetto, Challenges with measuring tissue factor antigen and activity in human plasma, Blood Vessels Thromb. Hemost. 1 (4) (December 2024) 100022.
[29] F.W.G. Leebeek, Update of thrombosis in multiple myeloma, Thromb. Res. 140 (1 avr 2016) S76–S80.
[30] K.M. Sanfilippo, M.A. Fiala, H. Tathireddy, D. Feinberg, R. Vij, B.F. Gage, D-dimer improves risk prediction of venous thromboembolism in patients with multiple myeloma, Blood 136 (5 nov 2020) 26–27.
[31] J.A. Welsh, D.C.I. Goberdhan, L. O'Driscoll, E.I. Buzas, C. Blenkiron, B. Bussolati, et al., Minimal information for studies of extracellular vesicles (MISEV2023): from basic to advanced approaches, J. Extracell. Vesicles 13 (2) (févr 2024) e12404.
[32] A.A. Khorana, C.W. Francis, K.E. Menzies, J.G. Wang, O. Hyrien, J. Hathcock, et al., Plasma tissue factor may be predictive of venous thromboembolism in pancreatic cancer, J Thromb Haemost JTH. 6 (11) (nov 2008) 1983–1985.
[33] G. Cesarman-Maus, E. Braggio, H. Maldonado, R. Fonseca, Absence of tissue factor expression by neoplastic plasma cells in multiple myeloma, Leukemia 26 (7) (juill 2012) 1671–1674.
[34] A. Aharon, T. Tamari, B. Brenner, Monocyte-derived microparticles and exosomes induce procoagulant and apoptotic effects on endothelial cells, Thromb. Haemost. 100 (05) (2008) 878–885.
[35] W. Holnthoner, C. Bonstingl, C. Hromada, S. Muehleder, J. Zipperle, S. Stojkovic, et al., Endothelial cell-derived extracellular vesicles size-dependently exert procoagulant activity detected by thromboelastometry, Sci. Rep. 7 (1) (16 juin 2017) 3707.
[36] J.J.A. Auwerda, Y. Yuana, S. Osanto, M.P.M. de Maat, P. Sonneveld, R.M. Bertina, et al., Microparticle-associated tissue factor activity and venous thrombosis in multiple myeloma, Thromb Haemost. 105 (1) (janv 2011) 14–20.
[37] T. Nielsen, S.R. Kristensen, H. Gregersen, E.M. Teodorescu, S. Pedersen, Prothrombotic abnormalities in patients with multiple myeloma and monoclonal gammopathy of undetermined significance, Thromb Res 202 (2021) 108–118.
[38] D. Faille, M.C. Bourrienne, E. de Raucourt, L. de Chaisemartin, V. Granger, R. Lacroix, et al., Biomarkers for the risk of thrombosis in pancreatic adenocarcinoma are related to cancer process, Oncotarget 9 (41) (29 mai 2018) 26453–26465.
[39] N. van Es, Y. Hisada, M.D. Nisio, G. Cesarman, A. Kleinjan, I. Mahé, et al., Extracellular vesicles exposing tissue factor for the prediction of venous thromboembolism in patients with cancer: a prospective cohort study, Thromb. Res. 166 (1 juin 2018) 54–59.
[40] K.M. Sanfilippo, M.A. Fiala, D. Feinberg, H. Tathireddy, T. Girard, R. Vij, et al., D-dimer predicts venous thromboembolism in multiple myeloma: a nested case-control study, Res Pract Thromb Haemost. 7 (8) (1 nov 2023) 102235.
[41] H. Ghansah, R. Orbán-Kálmándi, I.B. Debreceni, É. Katona, L. Rejtő, L. Váróczy, et al., Low factor XIII levels and altered fibrinolysis in patients with multiple myeloma, Thromb. Res. 234 (févr 2024) 12–20.
[42] P. Peduzzi, J. Concato, E. Kemper, T.R. Holford, A.R. Feinstein, A simulation study of the number of events per variable in logistic regression analysis, J. Clin. Epidemiol. 49 (12) (déc 1996) 1373–1379.
[43] L. Vallier, T. Bouriche, A. Bonifay, C. Judicone, J. Bez, C. Franco, et al., Increasing the sensitivity of the human microvesicle tissue factor activity assay, Thromb. Res. (182) (1 oct 2019) 64–74.