


ORIGINAL ARTICLE

Thrombin generation in newly diagnosed multiple myeloma during the first three cycles of treatment: An observational cohort study

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Abstract

Background: Multiple myeloma (MM) is associated with a high risk of thrombosis, particularly during the first months of treatment including immunomodulatory drugs (IMiDs). There is no consensus on prevention of thromboembolic risk in patients with de novo MM, and identification of patients requiring anticoagulant thromboprophylaxis remains challenging. Evaluating coagulability by an in vitro thrombin generation (TG) test might be a way of identifying such patients.

Objective: To determine whether TG assessment could reveal an increase in coagulability during the first three chemotherapy cycles.

Methods: This prospective and longitudinal observational study included patients newly diagnosed with MM. TG was determined in platelet-rich and platelet-poor plasma using calibrated automated thrombography with a low tissue factor (TF) concentration.

Results: Seventy-one patients were enrolled, allowing TG analysis during 213 chemotherapy cycles. TG remained unchanged throughout follow-up irrespective of treatment regimen, but values determined before cycles 2 and 3 were significantly higher in patients receiving iMiDs-containing regimens. No association was found between TG and its changes and thrombosis occurrence during follow-up: venous thrombosis in eight patients; no cardiovascular event. A significantly (87%) lower risk of venous thrombosis was observed in patients receiving prophylaxis with a low-molecular-weight heparin (LMWH; OR: 0.13 (95% CI: 0.02-0.76). Neither bortezomib- nor dexamethasone-containing regimens were associated with thrombotic risk. Changes in TG, as studied, were not associated with thrombotic events.

Conclusions: The only factor associated with a reduction in early thrombotic risk was prophylaxis with LMWH. The issue of how to identify patients requiring prophylactic anticoagulation remains unresolved.

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KEYWORDS

blood coagulation tests, heparin, multiple myeloma, thalidomide, thrombosis

Essentials

- Multiple myeloma carries a high risk of thrombosis but there is no consensus on prevention.
- Identification of biomarkers indicating a need for thromboprophylaxis would be helpful.
- In this study, thrombin generation was not predictive of thrombosis risk.
- Heparin prophylaxis was the only factor associated with a reduced risk of thrombosis.

1 | INTRODUCTION

Venous thromboembolic (VTE) events are the second most common cause of death in cancer patients.¹ Hematologic malignancies have been shown to be generally associated with higher rates of thrombosis compared with solid tumors and the risk of thrombosis in patients presenting such cancers has been reported to be 28-fold higher than in people without cancer.² Among hematologic malignancies, multiple myeloma (MM) is associated with the highest risk of thrombosis,² particularly during the first months of first-line treatment,^{3,4} and VTE event is associated with a lower survival rate in this setting.⁵ Immunomodulatory drugs (IMiDs) are known to be associated with an increased VTE risk, but the mechanism underlying this phenomenon is poorly understood.^{6,7} Current guidelines propose aspirin or low-molecular-weight heparin (LMWH) for thromboprophylaxis in MM patients treated with IMiDs, based on VTE risk stratification. However, the risk factors for thromboembolism in these patients are not precisely known. Only a shorter time interval between diagnosis and IMiDs initiation and recombinant erythropoietin (rEPO) treatment have been found to have a significant impact on VTE risk.^{7,8} In a recent study, physicians were asked to assess the VTE risk of each of their patients as low, intermediate or high, based on their own clinical evaluation. A substantial discrepancy between the risk factors recorded and the physicians' assessments was evidenced.⁹ Moreover, LMWH thromboprophylaxis has a substantial impact on health care resource consumption, resulting in a marked cost increase during recent years,¹⁰ and also seems to decrease quality of life.¹⁰ There is no consensus on prevention of VTE risk at present. To help physicians to decide whether or not to initiate VTE prophylaxis in patients with MM, we need to identify the most relevant criteria, construct appropriate algorithms and find useful biomarkers.

Calibrated automated thrombography (CAT) has been proved to be capable of identifying and quantifying hypercoagulability.¹¹ This test evaluates the entire course of thrombin production in adequately stimulated plasma, ie, thrombin generation (TG), and belongs to the class of global coagulation tests assessing the entire coagulation process. Observational studies of patients with cancer have found a higher basal thrombin peak (TP) and/or a higher endogenous thrombin potential (ETP) in patients subsequently manifesting VTE event, compared with patients experiencing no such event.¹²⁻¹⁴ The aim of the present study was to evaluate TG by CAT during the first

three cycles of chemotherapy in patients with newly diagnosed MM (nMM), to determine whether changes in coagulability during initial treatment might be associated with thrombotic risk.

2 | METHODS**2.1 | Study design**

This prospective observational study (NCT01508416) was conducted at four centers in France from December 2011 to May 2015 and enrolled patients with nMM (ie, before any treatment). The respective institutional review boards approved the study. All patients gave their written informed consent before entering the study, which was performed in accordance with the Declaration of Helsinki. The investigators designed the study and were responsible for data collection and analysis.

2.2 | Patients

Previously untreated patients were assessed for eligibility for the study and were enrolled if eligible. Inclusion criteria were medical insurance coverage, written consent, and nMM requiring chemotherapy, in a broad sense, according to current standards. Exclusion criteria were renal failure necessitating hemodialysis, ongoing anti-coagulant therapy for any reason other than thromboprophylaxis in the nMM setting (see below), impossibility of a 3-month follow-up, and life expectancy <6 months. All nMM treatments were allowed, including: bortezomib (1.3 mg/m² on days 1, 4, 8, and 11), thalidomide (100 mg/d), and dexamethasone (320 mg) (VTD); bortezomib (1.3 mg/m² on days 1, 4, 8, 11, and 22 for all cycles, and 25, 29, and 32 for the first cycle), melphalan (9 mg/m² on days 1-4) and prednisone (60 mg on days 1-4) (VMP); melphalan (0.25 mg/kg/d on days 1-4), prednisone (2 mg/kg/d on days 1-4), and thalidomide (100 mg/d) (MPT); bortezomib (1.3 mg/m² on days 1, 4, 8, and 11) and dexamethasone (160 mg) (VD); bortezomib (1.3 mg/m² on days 1, 4, 8, and 11), cyclophosphamide (750 mg/m² on day 1) and dexamethasone (160 mg) (VCD); and lenalidomide (25 mg on days 1-4), bortezomib (1.3 mg/m² on days 1, 4, 8, and 11) and dexamethasone (140 mg) (LVD). The treatment choice and the use of antithrombotic prophylaxis with aspirin, heparin or fondaparinux was left to the discretion of the treating physician.

2.3 | Sample collection

All blood samples were analyzed in a core laboratory at the U1059 INSERM unit (University of Saint-Etienne, France). Blood was collected before the start of treatment (baseline) and just the day before the second, third and fourth cycles of chemotherapy. Blood samples were always obtained by clean venipuncture from a peripheral arm vein not previously catheterized. For patients on heparin or fondaparinux, no injection was performed within at least 24 hours before blood sampling. In the case of heparin prophylaxis, anti-Xa activities were checked.

2.4 | Preparation of platelet-rich plasma and platelet-poor plasma

Laboratory tests were performed on platelet-rich plasma (PRP) for patients from centers 1 and 2. Blood was drawn into S-Monovette tubes, mixed therein with a 1:10 volume of 0.106 mol/L tri-sodium citrate (Sarstedt, Mamay, France), and centrifuged at 140 g for 10 minutes at 20°C. Platelet count was not adjusted. PRP was used within 2 hours after blood collection. To obtain platelet-poor plasma (PPP), blood was centrifuged twice at 2500 g for 15 minutes. PPP was stored at -80°C and thawed for 5 minutes in a water bath at 37°C before TG assay.

2.5 | Thrombin generation study

TG was measured at 37°C using CAT and a Fluoroscan Ascent Fluorometer equipped with a dispenser (Thermolab Systems, Helsinki, Finland). To initiate TG, 20 µL of PPP reagent LOW (Diagnostica Stago, Asnières sur Seine, France), comprising recombinant tissue factor (TF;

final concentration 1 pmol/L) and phospholipids (final concentration 4 µmol/L), were added to 80 µL of PPP in each well. For PRP, 20 µL of a solution of recombinant human TF (Dade Innovin, final concentration 1 pmol/L) were added to 80 µL of PRP in each well. TG was then triggered by dispensing 20 µL of the FluCa reagent containing CaCl₂ and a thrombin-specific fluorogenic substrate (GGR-AMC) in HEPES buffer. All samples were analyzed in duplicate. Parameters of interest were derived from each TG curve using Thrombinoscope version 5.0 software (Biodis, Signes, France).

2.6 | Clinical follow-up

All patients included were examined at baseline and before each treatment cycle until the first day of the fourth cycle. Depending on the type of treatment, the total follow-up period varied from 84 days (eg, with bortezomib, dexamethasone and thalidomide treatment) to 168 days (eg, with melphalan, prednisone, and thalidomide treatment). All proven episodes of bleeding and arterial or venous thromboembolism were recorded at each visit with the corresponding documentation, and patients were instructed to contact the investigators in the case of any suspected event. Any patient requiring anticoagulant treatment at therapeutic doses was withdrawn from the study.

2.7 | Outcome measures

The primary endpoint was a change in TG from baseline determined the day before each treatment cycle. The TG parameters evaluated were: ETP, corresponding to the area under the curve, lag-time (LT), TP, time to peak (ttP), and velocity, corresponding to the propagation

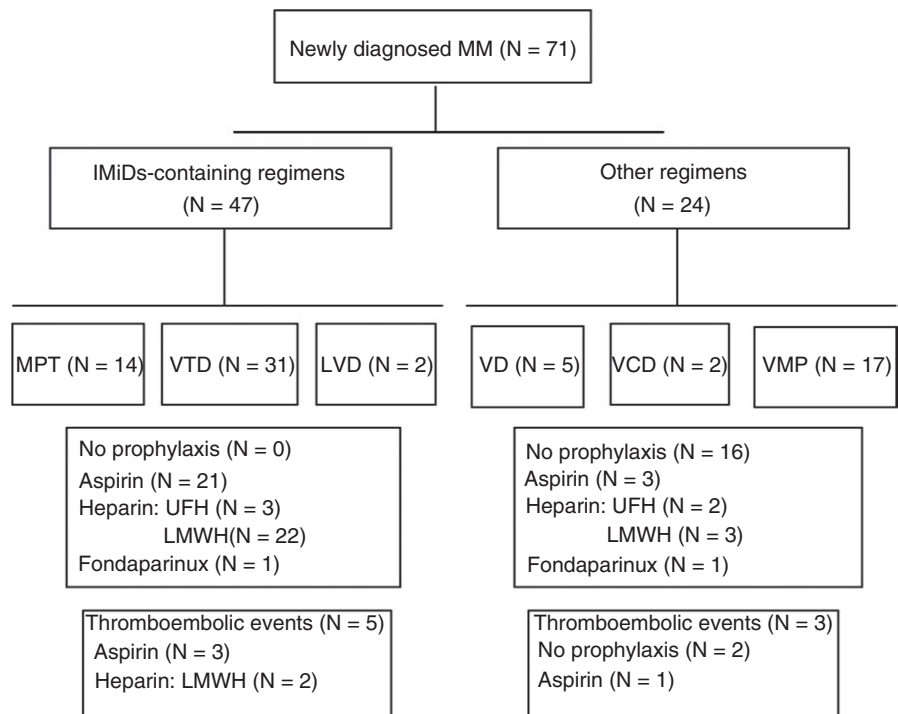


FIGURE 1 Treatment of patients included in the trial. MPT, melphalan, prednisone, and thalidomide; VTD, bortezomib, thalidomide, and dexamethasone; LVD, lenalidomide, bortezomib, and dexamethasone; VD, bortezomib and dexamethasone; VCD, bortezomib, cyclophosphamide, and dexamethasone; VMP, bortezomib, melphalan, and prednisone; UFH, unfractionated heparin; LMWH, low-molecular-weight heparin

TABLE 1 Baseline demographic and clinical characteristics of the study population

	No. of patients, n = 71 (%)
General characteristics	
Age (y)	
Median	67
IQR	59-73
Male	32 (45%)
Body mass index (kg/m ²)	
Median	25.5
IQR	22.1-28.5
BMI ≥ 30 kg/m ²	7 (10%)
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	
Thromboembolism	
Including pulmonary embolism	4 (6%)
Cardiovascular	2 (3%)
Including: hypertension	45 (63%)
Diabetes	32 (45%)
Coronary artery disease	7 (10%)
Family medical history	3 (4%)
Thromboembolism	6 (9%)
Multiple myeloma characteristics	
International Staging System stage	
I	16 (26%)
II	24 (39%)
III	22 (36%)
Immunoglobulin	
IgG	40 (56%)
IgA	19 (27%)
IgD	3 (4%)
Light chain	9 (13%)
Treatments	
Chemotherapy regimens	
Bortezomib + dexamethasone + thalidomide	31 (44%)
Bortezomib + melphalan + prednisone	17 (24%)
Melphalan + prednisone + thalidomide	14 (20%)
Bortezomib + dexamethasone	5 (7%)

(Continues)

TABLE 1 (Continued)

	No. of patients, n = 71 (%)
Bortezomib + dexamethasone + cyclophosphamide	2 (3%)
Bortezomib + lenalidomide + dexamethasone	2 (3%)
IMiDs containing regimens	47 (66%)
Steroids	
Dexamethasone	40 (56%)
Prednisone	31 (44%)
Erythropoietin	7 (10%)
Thromboprophylaxis	
Prophylaxis (including all indications)	54 (77%)
Prophylaxis because of multiple myeloma	50 (71%)
Type (possible sequential or concomitant use)	
Low-molecular-weight heparin	32 (46%)
Aspirin	24 (34%)
Unfractionated heparin	5 (7%)
Fondaparinux	2 (3%)

Ig, immunoglobulin; iMiDs, immunomodulatory drugs; IQR, interquartile range.

phase of thrombin generation and calculated according to the formula TP/(ttP-LT).

The composite secondary endpoint was defined as the proportion of patients developing a first episode of image-confirmed deep-vein thrombosis, pulmonary embolism, or any acute cardiovascular event (acute myocardial infarction, ischemic stroke, or peripheral arterial thrombosis), or sudden, otherwise unexplained death (presumed to be caused by pulmonary embolism, acute myocardial infarction, or stroke) during the first three treatment cycles.

Major bleeding was defined as fatal bleeding, symptomatic bleeding in a crucial area or organ, or bleeding causing a reduction in hemoglobin concentration of 2 g/dL or necessitating transfusion of two or more units of whole blood or red blood cells (RBC). Clinically relevant bleeding was defined as macroscopic hematuria or epistaxis, or repeated hemoptysis requiring a change in medical management, or unusual menometrorrhagia, or intra-articular hematoma, or any other bleeding event sufficiently relevant to require a change in medical management. Minor bleeding was defined as any other bleeding episode not meeting the criteria for major bleeding or clinically relevant bleeding.

2.8 | Statistical analysis

We used the secondary endpoint as the basis for calculating the sample size as no data had been published concerning our primary endpoint. Based on a VTE events rate of 4.1 (95% CI, 2.8-5.9) per 100 patient-cycles⁶ and an expected 5%-8% rate of VTE events in patients with nMM treated with IMiD-containing regimens,⁴ 70

patients (210 cycles) would be needed to observe 5-10 cases of VTE events for assessment of the secondary endpoint. Continuous variables were expressed as medians with interquartile ranges (IQR). TG parameters were presented as box-plots, a repeated measures analysis of variance was used to assess changes in TG over time. Thromboembolism event incidence throughout follow-up was expressed as a percentage with 95% CIs. For each comparison, the threshold for statistical significance was set at 0.05 (two-tailed). A subgroup analysis of the primary endpoint was planned for patients receiving treatment regimens containing and not containing IMiDs, respectively. The post hoc analysis of the association between thromboembolism and potential prognostic factors other than TG (such as age, comorbidities, erythropoietin use, type of steroids, and use of bortezomib-based regimens) was performed using logistic regression. Interactions with thromboprophylaxis were also assessed in the model. In view of the results obtained in the univariate analysis, no multivariate analysis was performed. $P < 0.05$ were considered to be statistically significant. Missing data were not imputed. All analyses were performed on the entire patient population studied.

3 | RESULTS

A total of 71 patients were enrolled in the study, of whom 47 (66%) received IMiDs containing regimens (Figure 1). Patient characteristics are described in Table 1. The average follow-up \pm SD was 133 ± 46 days. TG values determined just before each chemotherapy cycle did not differ significantly from baseline values either in PPP or in PRP, whatever the parameter considered. Regarding ETP, for

instance, a repeated measures analysis of variance revealed no change either in PPP ($P = 0.37$; basal median value: 1217 nmol/L/min; Table 2, Figure 2), or in PRP ($P = 0.94$; basal median value: 1472 nmol/L/min; Figure 3). Thrombograms recorded just before the second and third chemotherapy cycles for patients receiving IMiDs-containing regimens showed significantly higher ETP and TP values than those recorded for patients receiving IMiD-free regimens (Table 3), this difference being no longer detectable at the last time-point, just before the fourth treatment cycle. In contrast, the use of thromboprophylaxis, including the use of aspirin, was not associated with any TG parameter either in PPP or in PRP (see Table S1). Blood was withdrawn at least 24 hours after the last anticoagulant injection and in the case of heparin prophylaxis, anti-Xa activities were checked (see Table S1).

During the study period, objectively confirmed VTE events, symptomatic in all cases except one, occurred in eight patients (11.3%, 95% CI, 5-21): four patients under aspirin prophylaxis, two under LMWH, and two receiving no prophylaxis (Figure 1, Table 4). The median time to VTE events occurrence was 47 days (range 1-122 days). No acute cardiovascular event or sudden death was reported. Bleeding occurred in one patient (1.4%) receiving prophylactic LMWH (Table 4). A significantly (87%) lower risk of venous thrombosis was observed in patients receiving prophylaxis with a LMWH (OR = 0.13 [95% CI, 0.02-0.76], $P = 0.02$) (Table 5). Bortezomib-containing regimens and dexamethasone-containing regimens had no impact on thromboembolism risk.

4 | DISCUSSION

In this study involving 71 patients, TG parameters measured in PPP and PRP did not change significantly during the first three cycles

TABLE 2 Endogenous thrombin potential and thrombin peak in platelet-poor plasma (PPP)

	Baseline (N = 71)	Before cycle no. 2 (N = 68)	Before cycle no. 3 (N = 66)	Before cycle no. 4 (N = 63)
Missing data				
Blood sampling not performed	2	1	8	13
Analyses not performed because antiXa >0.05	11	18	16	8
Endogenous thrombin potential (nmol/L \times min)				
Mean (SD)	1193 (323)	1262 (302)	1238 (268)	1163 (281)
Median	1217	1251	1206	1171
Min.-Max.	311-1860	314-1995	744-1722	394-1689
IQR	1035-1415	1090-1481	1005-1459	1015-1382
P-value	$P = 0.37$			
Thrombin peak (nmol/L)				
Mean (SD)	151 (51)	157 (48)	146 (43)	137 (46)
Median	150	164	145	143
Min.-Max.	29-248	24-245	58-248	21-223
IQR	120-188	12-196	109-182	109-167
P-value	$P = 0.24$			

IQR, interquartile range (Q1-Q3); SD, standard deviation.

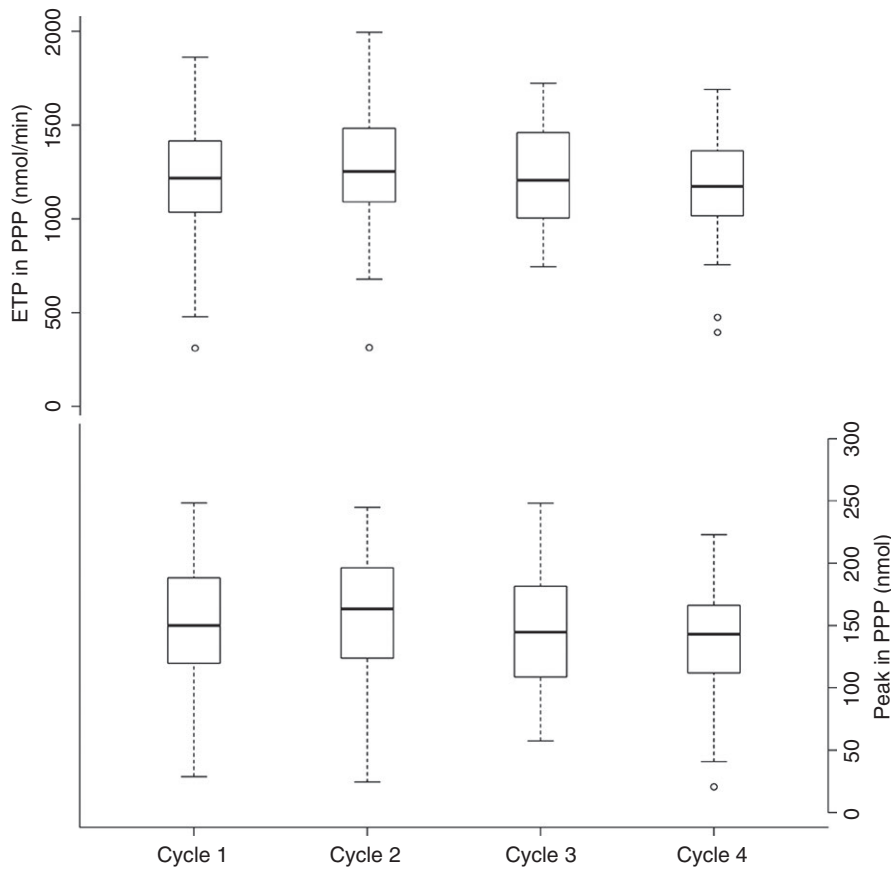


FIGURE 2 Endogenous thrombin potential and thrombin peak in platelet-poor plasma during the first three cycles of treatment. ETP, endogenous thrombin potential; PPP, platelet-poor plasma

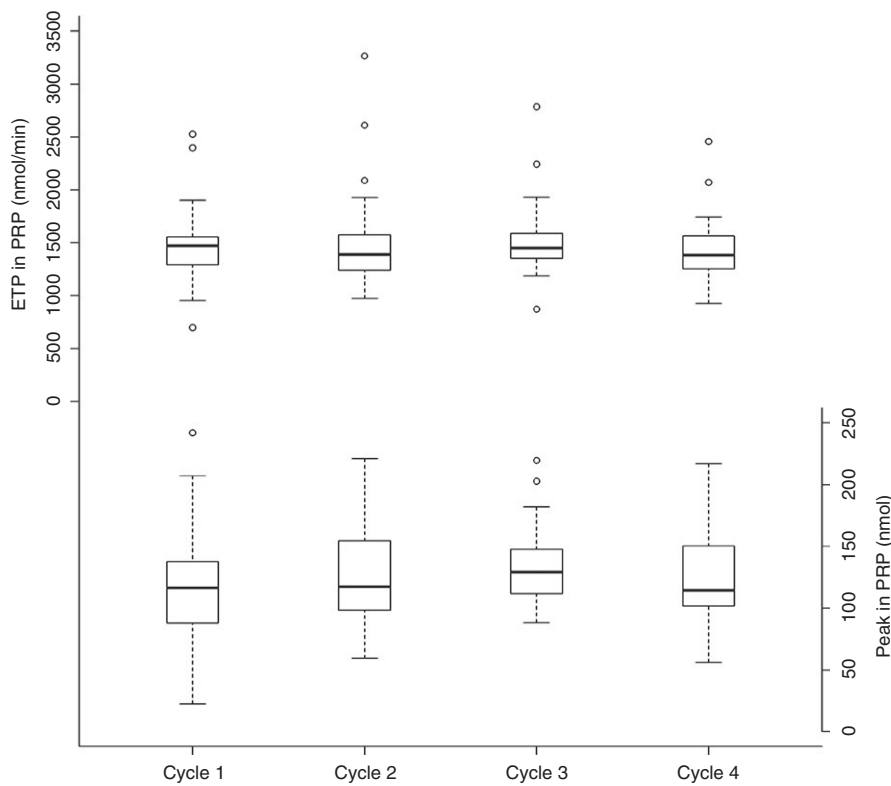


FIGURE 3 Endogenous thrombin potential and thrombin peak in platelet-rich plasma during the first three cycles of treatment. ETP, endogenous thrombin potential; PRP, platelet-rich plasma

of chemotherapy in nMM patients, irrespective of the use or type of thromboprophylaxis. These parameters were not associated with the onset of early thromboembolism events, which occurred at a

rate of 11% as expected. Even though this result could be challenged by studying a larger number of patients, the ability of the TG test to predict VTE events in such a population is unlikely to be useful,

TABLE 3 Endogenous thrombin potential (ETP) and thrombin peak (TP) in platelet-poor plasma (PPP) according to the immunomodulatory drugs (IMiDs) use

	IMiDs containing regimens (N = 47)	IMiDs-free regimens (N = 24)	Mean difference (95% CI)
ETP at baseline (nmol/L × min)			
Mean (SD)	1218 (336)	1146 (300)	72 (-99; 242)
Median (IQR)	1281 (1053-1437)	1138 (978-1280)	
ETP before cycle 2			
Mean (SD)	1343 (334)	1164 (231)	178 (20; 337)
Median (IQR)	1353 (1185-1518)	1162 (981-1291)	
ETP before cycle 3			
Mean (SD)	1320 (232)	1126 (280)	194 (26; 362)
Median (IQR)	1338 (1149-1493)	1057 (917-1326)	
ETP before cycle 4			
Mean (SD)	1160 (315)	1168 (213)	-9 (-168; 150)
Median (IQR)	1227 (1004-1361)	1140 (1017-1400)	
TP at baseline (nmol/L)			
Mean (SD)	155 (49)	145 (54)	10 (-19; 38)
Median (IQR)	158 (125-188)	143 (111-183)	
TP before cycle 2			
Mean (SD)	169 (48)	143 (46)	26 (0; 52)
Median (IQR)	172 (146-202)	139 (116-169)	
TP before cycle 3			
Mean (SD)	158 (36)	129 (47)	29 (2; 57)
Median (IQR)	160 (125-188)	117 (101-165)	
TP before cycle 4			
Mean (SD)	132 (48)	148 (43)	-17 (-45; 11)
Median (IQR)	143 (108-164)	143 (119-188)	

ETP, endogenous thrombin potential; IQR, interquartile range; PPP, platelet-poor plasma; SD, standard deviation; TP, thrombin peak.

according to our results, at least under the experimental conditions we used for CAT. Some patients had a previous history of thrombosis (Table 1). This could have influenced both the laboratory results and the risk of thrombosis during chemotherapy, but we decided to not exclude these patients as in the Palumbo study.⁴ In addition, the medical history of thromboembolism could have influenced the MM treatment choice.

We found no significant changes in TG over time. These results contrast with those of two recent studies, in which certain TG parameters (ETP and peak height) were found either to be higher for patients with MM experiencing thromboembolic events compared with those manifesting no events,¹⁴ or to show an increase during 3 months of MM therapy.¹⁵ In our opinion, these results warrant cautious appraisal in view of several methodological issues. First, the number of patients was low in both studies: 36 and 24, respectively. Furthermore, the study populations included both patients with nMM (13 and 17, respectively) and relapsing MM (23 and 7, respectively), yet the thromboembolic risk differs between these two groups of patients.⁶ In addition, the timing of blood sampling for the assessment of TG during MM treatment was not defined in one study¹⁴ and was not related to the number of chemotherapy cycles

in the other.¹⁵ Finally, in one study, most thromboembolic events (7 out of a total of 11) were observed more than 6 months after the start of MM treatment (at 48 months in the case of two events), rendering interpretation of the clinical relevance of laboratory findings debatable¹⁴; in the other study, TG values observed in the three patients experiencing thromboembolic events did not differ from those of patients manifesting no thromboembolic events.¹⁵

It is worth noting that we found a substantial variation in ETP results, with some patients having a surprisingly low ETP, presumably associated with a hypocoagulable state. This can probably be explained by certain abnormalities known to be responsible for hemorrhagic diathesis in patients with MM.¹⁶

In addition, we did not consistently find higher baseline values of either ETP or peak thrombin in patients with nMM compared to those reported in patients with monoclonal gammopathy of undetermined significance and healthy controls.^{15,17-19} The coagulability associated with nMM therefore remains an enigma.

We found that ETP values determined in PPP were significantly higher during the first two treatment cycles in patients receiving IMiDs-containing regimens compared with those receiving IMiDs-free regimens. This result is consistent with the hypothesis that

TABLE 4 Incidence of thromboembolic events, acute cardiovascular events and bleeding

Type of event	Total (N = 71)
Thromboembolic events	8 (11%)
95% CI	11% (5%-21%)
Time of thromboembolic event (d)	
Median	47
IQR	10-106
Venous thromboembolic events	8 (11%)
Deep venous thrombosis	6 (9%)
Clinical symptoms	
Present	5 ^a (7%)
Localization	
Catheter related	0 (0%)
Superior vena cava	0 (0%)
Lower limb	5 (7%)
Proximal	0
Distal	5
Upper limb	2 (3%)
Proximal	1
Distal	1
Pulmonary embolism (PE)	2 (3%)
Fatal PE	0 (0%)
Acute cardiovascular events	0 (0%)
Bleeding	
Minor bleeding	0 (0%)
Clinically relevant	1 (1%)
Major bleeding	0 (0%)

CI, confidence interval; PE, pulmonary embolism.

^aOne asymptomatic thrombosis was detected incidentally during an examination performed for another reason.

patients treated with IMiDs might present transient acquired hypercoagulability, as suspected in light of the results of meta-analyses and observational studies. However, we did not find any association between IMiD treatment and a VTE higher risk, and surprisingly, the difference between the two patient groups was no longer evident beyond the first two treatment cycles. Moreover, our results suggest that the thromboembolic risk related to IMiDs might also be over-evaluated.²⁰ A protective effect of bortezomib against VTE, when added to an IMiDs, has also been reported in some studies.²¹ Our data do not confirm these findings, in agreement with the results of a meta-analysis.²² In our study, thromboembolic events occurred in seven of eight patients under bortezomib and in the univariate analysis, bortezomib use was not associated with protection against thromboembolic events. However, we noted that IMiD-containing regimens were predominantly used in younger patients (63.7 vs 71.8 years), whereas bortezomib was used in patients of all ages.

Finally, we observed that the only factor associated with a reduction in early thromboembolic risk was the use of heparin prophylaxis from baseline, whereas no significant difference was observed in two randomized studies, respectively comparing the effect of LMWH, aspirin and low-dose warfarin, and the effect of LMWH and aspirin, in patients with nMM.^{4,23} The fact that older patients with more thromboembolic risk factors were included in our study compared with the patients included in these two randomized studies (median age: 66.9 vs 61 and 58, respectively; more than two risk factors: 25% vs 5% and, at least two risk factors: 55% vs 2%) could explain, at least in part, the different effects of LMWH observed in these two studies and in ours. This hypothesis deserves to be tested in a randomized study.

Even though our study population included patients with several cardiovascular risk factors, we recorded no cardiovascular event, challenging the results of former studies, which reported an increase in the risk of arterial thrombosis.²⁴ In those studies, a 5.6% rate of arterial thrombosis was reported with regimens including vincristine and doxorubicin, drugs that are no longer used in the context of MM. The reported arterial risk therefore seems to be no longer relevant.

The main limitation of our study is the close to systematic use of thromboprophylaxis, but no study can be proposed and ethically accepted with a placebo comparator.

Like some other investigations, our study also has the possible limitation that we did not take into account disease-related, time-limited exposures to other risk factors that may have had an acute impact on thrombosis risk. As such exposures are potentially detectable and even modifiable, such as infection and all-cause hospitalization, identifying these high-risk periods may lead to better prediction of thrombotic events, result in enhanced surveillance or prophylactic measures, and highlight periods where increased surveillance or prophylactic interventions may have the greatest impact. The second limitation is that our study included elderly patients with high levels of thromboembolic risk factors and patients receiving several different types of MM therapy. Our results might possibly have been different in a younger population with fewer thromboembolic risk factors and in a population receiving more highly selected MM therapies, even though to date no specific thromboembolic risk factors have been clearly identified. The third limitation concerns the way in which we evaluated TG. In particular, TG assessment under conditions sensitive to the protein C inhibitor system (with addition of exogenously activated protein^{18,25,26} or thrombomodulin¹⁵) and/or with the use of different concentrations of TF, might have given different results. Notably, we did not find any differences in TG determined in the presence of platelets (PRP) as opposed to their absence (PPP). A low concentration of TF was used to maximize the possibility of evidencing hypercoagulability while taking into account factor VIII levels, which are known to be high and even very high in patients with MM.^{27,28} The predisposition of patients with MM to thrombosis could be due to changes in endothelium, leukocytes, fibrin structure and lysis,^{29,30} which are not captured by TG studies.

TABLE 5 Univariate analysis: risk factors for venous thromboembolic events

	TE events (N = 8)	No event (N = 63)	Univariate OR (CI 95%) P-value
Increase in ETP in PPP between baseline and cycle 4	5 (100%)	33 (66%)	
Or before TE events	0 (0%)	17 (34%)	NA P = 0.95
Thrombin peak in PPP at baseline (nmol/L)			
Median	186	149	P = 0.22
IQR	120-218	114-181	
Time to peak in PPP at baseline (min)			
Median	10.8	9	P = 0.82
IQR	7.3-11.3	8.4-10.2	
Medical history of TE events			
No	8 (100%)	59 (94%)	NA P = 0.98
Yes	0 (0%)	4 (6%)	
rEPO started during the first 2 cycles			
No	7 (88%)	48 (77%)	0.49 (0.06; 4.33)
Yes	1 (13%)	14 (23%)	
Treatment including an IMiD			
No	3 (38%)	21 (33%)	0.83 (0.18; 3.83)
Yes	5 (63%)	42 (67%)	
Treatment including dexamethasone			
No	4 (50%)	27 (43%)	0.75 (0.17; 3.27)
Yes	4 (50%)	36 (57%)	
Treatment including bortezomib			
No	1 (13%)	13 (21%)	1.82 (0.21; 16.1)
Yes	7 (88%)	50 (79%)	
LMWH			
No	5 (71%)	12 (25%)	0.13 (0.02; 0.76)
Yes	2 (29%)	37 (76%)	
Aspirin			
No	4 (50%)	26 (53%)	1.13 (0.25; 5.04)
Yes	4 (50%)	23 (47%)	

CI, confidence interval; ETP, endogenous thrombin potential; IMiD, immunomodulatory drug; IQR, interquartile range; LMWH, low-molecular-weight heparin; OR, odds ratio (the odds ratio of 1 is the reference category); PPP, platelet-poor plasma; rEPO, recombinant erythropoietin; TE events, thromboembolic events.

Bold value indicates the significant values.

To conclude, under the conditions we chose for TG assessment, TG remained unchanged in patients with nMM during the first three treatment cycles and in particular, did not differ according to whether or not these patients subsequently presented a thrombotic event. The issue of how to select nMM patients requiring heparin prophylaxis therefore remains unresolved.

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RELATIONSHIP DISCLOSURE

The authors state that they have no conflict of interest.

AUTHOR CONTRIBUTIONS

EC, BT, and BTP designed research; EC, BTP, LK, AM, MP, DG, PC, and BT performed research; CC undertook the statistical analysis; EC supervised blood samples collection and were responsible of the clinical aspects; and EC, BT, BTP, and TL analyzed data and wrote the paper. All authors approved the final version of the paper.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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