hemato

MDPI

Article

Treatment Resistance Risk in Patients with Newly Diagnosed Multiple Myeloma Is Associated with Blood Hypercoagulability: The ROADMAP-MM Study

Grigorios T. Gerotziafas [1,2,*], Despina Fotiou [3], Theodoros N. Sergentanis [3], Loula Papageorgiou [1,2], Jawed Fareed [4], Anna Falanga [5], Michèle Sabbah [2], Laurent Garderet [6], Evangelos Terpos [3], Ismail Elalamy [1,2,7], Patrick Van Dreden [1,8] and Meletios A. Dimopoulos [3]

1 Research Team "Cancer-Hemostasis-Angiogenesis" Research Group "Cancer Biology and Therapeutics", Centre de Recherche Saint Antoine (CRSA), Institut Universitaire de Cancérologie, Faculty of Medicine, Sorbonne University, 75012 Paris, France; loulapap2@gmail.com (L.P.); ismail.elalamy@aphp.fr (I.E.); patrick.vandreden@stago.com (P.V.D.)
2 Department of Thrombosis and Haemostasis, Service d'Hématologie Biologique DMU BioGen, Faculté de Médecine Sorbonne, Hôpital Tenon, Hôpitaux Universitaires de l'Est Parisien, Assistance Publique Hôpitaux de Paris, 75020 Paris, France; michele.sabbah@inserm.fr
3 Department of Clinical Therapeutics, School of Medicine, National and Kapodistrian University of Athens, 11528 Athens, Greece; desfotiou@gmail.com (D.F.); tsergentanis@yahoo.gr (T.N.S.); eterpos@hotmail.com (E.T.); mdimop@med.uoa.gr (M.A.D.)
4 Department of Pathology and Laboratory Medicine, Loyola University Medical Center, Maywood, IL 60153, USA; jfareed@luc.edu
5 Department of Immunohematology and Transfusion Medicine, Hospital Papa Giovanni XXIII, School of Medicine, University of Milan Bicocca, 24127 Bergamo, Italy; annafalanga@yahoo.com
6 Department of Hematology and Cellular Therapy, Hospital Hospital-Pitié Salpêtrière, 75013 Paris, France; laurent.garderet@aphp.fr
7 Department of Obstetrics and Gynecology, I.M. Sechenov First Moscow State Medical University, 119991 Moscow, Russia
8 Clinical Research Department, Diagnostica Stago, 92036 Gennevilliers, France
* Correspondence: grigorios.gerotziafas@inserm.fr

Abstract: Biomarkers of hypercoagulability are potential candidates for the evaluation of risk for primary treatment resistance in patients with newly diagnosed multiple myeloma (NDMM). This study aimed to identify the most clinically relevant biomarkers for the evaluation of treatment-resistance risk. NDMM patients (n = 144) were enrolled prior to treatment initiation. Response to treatment was assessed at 3 months. STA-Procoag-PPL®, factor VIIa factor V, antithrombin, fibrin monomers, soluble thrombomodulin (TM), free TFPI, D-Dimer, P-selectin, heparanase, and thrombin generation (Calibrated Automated Thrombogram® and PPP-Reagent®) were measured. In total, 23% (n = 33) of the patients showed a poor response/resistance to treatment (defined as stable disease, minor response, progressive disease). Poor response/treatment resistance was associated with longer Procoag-PPL® clotting time, higher Peak of thrombin, and higher D-Dimer levels. These biomarkers were included in a prognostic model derived via multivariate analysis. The model had 84% sensitivity and 59% specificity to identify patients at high risk of treatment resistance. The AUC of the ROC analysis for the model was 0.75. In conclusion, Procoag-PPL®, D-Dimer, and Peak of thrombin generation are clinically relevant for the identification of NDMM patients at risk for poor response to antimyeloma treatment. A prospective multicenter study is necessary for the validation of this new approach.

Keywords: D-Dimer; hypercoagulability; multiple myeloma; thrombin generation; treatment resistance

check for updates

Citation: Gerotziafas, G.T.; Fotiou, D.; Sergentanis, T.N.; Papageorgiou, L.; Fareed, J.; Falanga, A.; Sabbah, M.; Garderet, L.; Terpos, E.; Elalamy, I.; et al. Treatment Resistance Risk in Patients with Newly Diagnosed Multiple Myeloma Is Associated with Blood Hypercoagulability: The ROADMAP-MM Study. *Hemato* **2022**, *3*, 188–203. https://doi.org/10.3390/hemato3010016

Academic Editor: Mario Mazzucato

Received: 14 January 2022
Accepted: 13 February 2022
Published: 22 February 2022

1. Introduction

In the era of expanding therapeutic armamentarium and advances in tumor biology research, multiple myeloma outcomes have improved considerably. The disease remains,

however, incurable. Myeloma cells have the ability to develop resistance to treatment, particularly at advanced disease stages [1]. Resistance to antimyeloma treatment is associated with comparatively worse outcomes. Biomarkers that could contribute to identifying the subpopulation of patients refractory to the first line of treatment could be a useful tool for optimizing the therapeutic strategy [2,3].

The reciprocal link between cancer cells and blood coagulation has been well documented. Crosstalk between myeloma plasma cells with platelets and endothelial cells enhances hypercoagulability. Consequently, biomarkers of hypercoagulability are potential candidates worth assessing for their role in predicting treatment outcomes [4,5]. Several experimental and translational research studies support this concept. Cancer cells induce activation of platelets, endothelial cells, and the blood coagulation mechanism, either directly via the expression of procoagulant molecules and the release of procoagulant microparticles or indirectly via enhancement of the inflammatory reaction [5–8]. On the other hand, the activation of platelets and blood coagulation enhances proliferation and metastasis of cancer cells and offers a shield against the immuno-surveillance mechanisms [9] Hypercoagulability is a common laboratory finding in cancer patients [10]. Recent data published from our group established that newly diagnosed, treatment naïve patients with symptomatic multiple myeloma show marked bloodborne hypercoagulability characterized by increased levels of biomarkers of endothelial cell activation, such as tissue factor activity (TFa), soluble thrombomodulin (TM), tissue factor pathway inhibitor (TFPI), and procoagulant phospholipids. This study showed that the increase in TFPI and TM resulted in attenuated thrombin generation [11]. In patients with multiple myeloma, venous thromboembolism (VTE) is a frequent complication which is related to the disease activity and to the anti-myeloma treatments [12,13].

The prospective, longitudinal observational ROADMAP-MM-CAT study (PROspective Risk Assessment anD bioMArkers of hyPercoagulability for the identification of patients with Multiple Myeloma at risk for Cancer Associated Thrombosis) evaluated a large number of biomarkers of hypercoagulability informative for the equilibrium between procoagulant and anticoagulant forces in plasma as well as for the activation state of endothelial cells. Biomarkers were assessed before treatment initiation and showed that the procoagulant phospholipid-dependent clotting time (Procoag-PPL®) and thrombin generation test were predictors of VTE [11].

In the present post-hoc analysis of the data from the ROADMAP-MM-CAT study we investigated the potential role of hypercoagulability in the identification of patients at high risk for primary poor response or resistance to the first line antimyeloma treatment.

2. Materials and Methods

2.1. Study Design and Participants

The ROADMAP-MM-CAT study (NCT03405571) was an investigator-initiated, prospective, non-interventional trial. Newly diagnosed, treatment naïve symptomatic patients with multiple myeloma (based on 2014 IMWG Criteria) [14–16] were diagnosed or referred to the Department of Clinical Therapeutics (Alexandra Hospital, Athens, Greece) from June 2014 to June 2017. Exclusion criteria included age less than 18 years, recent (<6 months) episode of VTE and active anticoagulant treatment (for any indication) prior to enrollment in the study, ongoing pregnancy, recent hospitalization for surgical illness, active malignancy other than multiple myeloma, and surgery in the preceding 3 months.

Blood samples were collected in newly diagnosed treatment naïve patients with symptomatic multiple myeloma at baseline. Poor response or resistance to the anti-myeloma treatment was evaluated 3 months post treatment initiation. All patients provided written informed consent and patients received treatment according to institutional practice. The protocol of the study was in accordance with the commitment of the Helsinki declaration and was approved by the institutional ethics committee.

2.2. Control Group

The control group consisted of 30 healthy individuals, without any known hereditary or acquired thrombophilia or personal history of thrombotic or bleeding disorders. Controls had the same ages as patients.

Procedures: A standardized case report form (CRF) was completed for all patients enrolled in the study at baseline and at 3 months post treatment initiation which assessed disease parameters, status of disease, ongoing treatments. Hemogram, renal and liver blood function tests were recorded. Renal function was assessed using the Cockcroft–Gault formula. Performance status (PS) was evaluated according to the ECOG classification. At diagnosis, patients were classified according to the international staging system (ISS) for multiple myeloma as stages I, II, or III. Patients were classified as having high risk cytogenetics at diagnosis if bone marrow fluorescence in situ hybridization analysis was positive for t(4;14), t(14;16), or del17q cytogenetic abnormalities. The presence of lytic bone disease was assessed with whole body computerized tomography (WBCT) scans. Magnetic resonance imaging (MRI) of the thoracic and lumbar spine was used to assess the pattern of bone marrow infiltration (normal, focal pattern, salt-and-pepper, or diffuse).

Molecular and functional blood analysis: Blood samples were routinely taken between 9:00 a.m. and 12:00 a.m. Blood was obtained by atraumatic antecubital venipuncture at baseline and collected in Vacutainer® tubes (5 mL tubes, containing 0.109 mol/L trisodium citrate; 1 volume trisodium citrate to 9 volumes blood). They were used to extract platelet-poor plasma (PPP) by double centrifugation at 2000× *g* for 20 min at room temperature and plasma aliquots were stored at −80 °C until assayed. Plasma samples were centralized to the core laboratory at the INSERM UMRS 938 (Centre de Recherche Saint Antoine) and the tests were performed blindly from clinical data. Procoagulant phospholipid-dependent clotting time (*Procoag-PPL*®) was measured with STA®Procoag-PPL, according to the manufacturer's instructions [17,18]. The levels of factor VIIa (Staclot® VIIa-rTF), factor V (FV), antithrombin (AT), fibrin monomers (FM), soluble thrombomodulin (TM), free TFPI, and D-Dimer were measured with commercially available assays according to the manufacturer's instructions, on a STA-R® analyzer. The assays and the analyzer were purchased from Diagnostica Stago, Asnières France. Tissue Factor activity (TFa) in PPP was measured as previously described [19,20]. Plasma levels of P-Selectin and heparanase were measured with ELISA Kits from Cusabio Biotech (CliniSciencies, Nanterre, France) and R&D Systems (Lille, France), respectively. Thrombin generation was assessed in samples of PPP with the PPP-Reagent® (5 pM TF and 4 µM procoagulant phospholipids) on a Calibrated Automated Thrombogram (CAT®, from Stago, Asniers, France). The following parameters of the thrombogram were analyzed: (a) lag-time, that indicates the initiation phase of thrombin generation, (b) time to reach maximum concentration of thrombin (ttPeak), (c) maximum concentration of thrombin (Peak), (d) mean rate index (MRI) of the propagation phase of thrombin generation calculated by the formula: Peak/(ttPeak–lag-time) and expressed in nM/min, and (e) endogenous thrombin potential (ETP) that shows the integral enzymatic activity of thrombin as described elsewhere [21–23]. The inter- and intra-assay coefficients of variation of the assays ranged from 3% to 7%.

Biomarkers measured in the cohort of patients were compared against values in the control group. The duration of the storage was not significantly different between samples obtained from patients and controls. All measurements in patients and controls were performed at the same time.

The normal values of the studied biomarkers were defined in the control group and were compared to the corresponding normal reference range used by the core laboratory. These normal ranges were established according to the requirements for the good quality of laboratory practice by performing the tests in healthy individuals representative of the general population regarding age, sex, ethnicity, and BMI. The Upper Normal Limit (UNL) and the Lower Normal Limit (LNL) for each biomarker were defined in the control group as follows: UNL= mean + 2 SD, and LNL: = mean − 2 SD. The UNL and LNL of the

studied biomarkers were compared to the corresponding normal reference range used by our laboratory.

Outcomes: A landmark analysis at 3 months after treatment initiation was performed. The primary end-point of this study was the response to treatment at 3 months from treatment initiation (following 3 cycles of treatment), according the International Myeloma Working Group 2016 response criteria [24]. Three months from treatment initiation is a usual interval for routine assessment of the response to the antimyeloma treatment. The group of patients with "poor response or resistance" to first line anti-myeloma treatment included those with Progressive Disease (PD), Stable Disease (SD), or Minor Response (MR). The group "responders" to the treatment included patients with Partial Response (PR), Very Good Partial Response (VGPR), or Complete Response (CR). No patients were lost in the follow up.

Role of the funding source: The study was supported by an unrestricted grant from Leo Pharma. Protocol development, construction of the database, data collection, statistical analysis, data interpretation, and manuscript writing were all completed by the investigators with no involvement from the funding sources.

Statistical Methods: Data were checked for normal distribution and continuous variables are described as mean ± standard deviation and categorical variables as frequency and percentage. The modeling of the predicted values for the biomarkers was completed by defining "poor response or resistance" to antimyeloma treatment as the dependent variable. In view of the deviation from normality (as evidenced by the Shapiro–Wilk test) the comparison of biomarker levels between patients and healthy individuals was performed using the Mann–Whitney–Wilcoxon test for independent samples. The inter-correlations between biomarkers using Spearman's rank correlation coefficients were estimated. Univariate logistic regression analysis examined the associations between biomarkers and treatment poor response or resistance. At the univariate analysis, the level of statistical significance was set at 0.05.

Regarding the associations between the clinical outcome and the biomarkers, the latter were converted to binary variables through Receiver Operating Characteristic (ROC) curve analysis; the selection of cut-off levels was based on the maximization of Youden's index. Subsequently, multivariate logistic regression analysis was performed with the clinical outcome as the dependent variable; biomarker variables proven significant using the univariate logistic regression analysis were examined as possible independent variables. Using a stepwise procedure, at the final multivariate logistic model all variables with p-values less than 0.10 were retained; the area under the ROC curve (AUC) was estimated to describe the fit of the multivariate model. All statistical analyses of the studied biomarkers were adjusted for clinical predictors, including dialysis, renal function, BMI, age, and cardiovascular disease.

To prevent erroneous inclusion of predictors into the model, the rule of thumb (1–10 event per variable) was applied: one candidate predictor per 10 outcome events were included in the data set [25,26]. The number of enrolled patients was defined according to this rule. Calibration of the model was controlled with the Hosmer–Lemeshow test. Data were analyzed using the STATA/SE version 13 statistical software (Stata Corp., College Station, TX, USA).

Ethics: As described elsewhere, the protocol of the study was in accordance with the commitment of the Helsinki declaration and was approved by the institutional ethics committee (Hospital Ethics and Scientific Committee). All patients provided informed written consent before enrollment in the study. The study has been registered in clinicaltrials.gov (accessed on 29 December 2021) (NCT03405571).

3. Results

A total of 144 eligible patients with symptomatic newly diagnosed multiple myeloma were enrolled in the study from June 2014 to June 2017. No patients were lost to follow-up or excluded from analysis due to missing data. A summary of the clinical and demographic

characteristics of all patients at inclusion are summarized in Table 1. At inclusion no patient had received any anti-myeloma treatment or thromboprophylaxis. Median age was 66.0 ± 11.6 (36–86) years and 53% of the population was male. Regarding ISS disease stage: 32% were ISS-I, 23% ISS-II, and 45% ISS-III. High risk cytogenetics were detected in 27% of patients. In 32% of patients the treatment was immunomodulatory drug (IMiD) (90% lenalidomide and 10% thalidomide) and in 64% proteasome inhibitor (PI) based, whereas 4% of patients received other regimens.

Table 1. Baseline demographic, clinical, and routine biological data of multiple myeloma patients.

Patient Clinical Characteristics		
	Responders (*n* = 111)	**Non-Responders (*n* = 33)**
Age (years)	66.0 ± 12.0 (37–86)	65.0 ± 11.0 (36–82)
Male/female	59/52 (53%/47%)	17/16 (51%/49%)
BSA (m^2)	1.84 ± 0.19 (1.41–2.50)	1.87 ± 0.20 (1.46–2.30)
BMI (kg/m^2)	25.9 ± 5.3 (17.2–44.8)	24.6 ± 4.9 (17.9–43.4)
ISS stage—*n* (%)		
I	35 (31%)	11 (33%)
II	26 (24%)	7 (21%)
III	50 (45%)	15 (46%)
Anti-myeloma Treatment—*n*(%)		
PI-based	73 (65%) VCD (65), VMP (7), ICD (1)	19 (58%) VCD (15), VD (2), VMP (1), ICD (1)
IMiD-based	35 (32%) RAD (23), CTD (5), Rd (4), VRD (2), VTD (1)	11 (33%) RAD (5), CTD (1), Rd (5)
Other	3 (3%) MDex (3)	3 (9%) CD (2), MDex (1)
ECOG Performance Status—*n*(%)		
0 **1** **2** **3** **4**	50 (45) 45 (41) 12 (11) 3 (2) 1 (1)	7 (21) 14 (43) 11 (33) 1 (3) 0 (0)
Dialysis at diagnosis—*n* (%)	12 (11%)	2 (6%)
Bone disease present—*n* (%)	79 (71%)	23 (70%)
High risk cytogenetics—*n* (%)	21 (19%)	6 (18%)
Thromboprophylaxis after Enrolment in the Study—*n* (%)		
None	30 (27)	17 (51)
Aspirin	61 (55)	13 (39)
LMWH (tinzaparin)	20 (18)	3 (10)
Patients' Biological Data(mean ± SD; range)		
β2-microglobulin (mg/dl)	7.6 ± 8.0 (0.06–39)	8.5 ± 8.7 (2.5–48.5)
M-peak (g/dl)	2.7 ± 2.6 (0–7.6)	3.4 ± 2.8 (0–9)
U-peak (mg/24 h)	313 ± 880 (0–5987)	413 ± 980 (0–6667)
Bone marrow infiltration (%)	61.0 ± 26.4 (0–100)	64 ± 23.0 (0–100)

Table 1. *Cont.*

Patient Clinical Characteristics		
Total protein (g/dl)	8.2 ± 2.4 (5.1–12.0)	8.7 ± 1.9 (5.4–14.3)
Creatinine (mg/dl)	1.77 ± 3.2 (0.47–15.0)	1.98 ± 2.9 (0.9–28.0)
Urea (mg/dl)	56.1 ± 40.0 (5–180)	60.4 ± 43.9 (5–276)
GFR (mL/min)	73.0 ± 43.0 (4.2–230.0)	73.0 ± 43.0 (4.2–230.0)
LDH (U/L)	190 ± 96 (70–690)	211 ± 93 (99–789)
ALT (U/L)	24.1 ± 21.2 (7–162)	24.6 ± 19.9 (6–159)
AST (U/L)	23.8 ± 24.0 (7–169)	24.3 ± 23.0 (6–178)
Albumin (g/dl)	3.9 ± 0.84 (2.4–6.7)	3.6 ± 0.9 (2.1–6.8)
Calcium (mg/dl)	9.5 ± 1.3 (6.7–13.0)	9.8 ± 1.7 (6.9–13.4)
Hb (g/dl)	10.4 ± 2.1 (7.0–16.0)	10.6 ± 2.0 (7.3–17.5)
White blood cell count ($\times 10^6/\mu L$)	7.1 ± 4.0 (0.58–18.0)	6.0 ± 2.9 (0.48–18.8)
Neutrophils ($\times 10^6/\mu L$)	4.4 ± 2.4 (0.2–10.0)	4.0 ± 2.3 (0.3–12.6)
Platelets ($\times 10^3/\mu L$)	260 ± 120 (34–879)	250 ± 119 (26–760)

ISS: International Staging System per ISS; LMWH: low molecular weight heparin; PI: proteasome inhibitor based; VCD: bortezomib, cyclophosphamide, dexamethasone; VD: bortezomib, dexamethasone; VMP: bortezomib, melphalan, dexamethasone; ICD: Ixazomib, cyclophosphamide, dexamethasone; IMiD: immunomodulatory drug based; RAD: Lenalidomide, Adriamycin, Dexamethasone; CTD: cyclophosphamide, thalidomide, dexamethasone; Rd: Lenalidomide, dexamethasone; VRD: bortezomib, lenalidomide, dexamethasone; VTD: bortezomib, thalidomide, dexamethasone; CD: cyclophosphamide, dexamethasone; MDex: melphalan, dexamethasone; MM: multiple myeloma; BMI: body mass index; BSA: body surface area; M-peak: serum monoclonal protein; U-peak: urine monoclonal protein; Hb: hemoglobin; LDH: lactate dehydrogenase; GFR: glomerular filtration rate; ALT: alanine aminotransferase; AST: aspartate aminotransferase; ECOG: Eastern Cooperative Oncology Group.

After enrollment in the study and based on current thromboprophylaxis guidelines, current risk stratification, and standard clinical care provided by the institution, 33% of patients did not receive any thromboprophylaxis, 51% received aspirin 100 mg o.d., and 16% received tinzaparin 4500 anti-Xa IU s.c. o.d. Median follow up time was 15.5 months (27 days–35 months).

The overall rate of symptomatic VTE during follow up was 10.4% (n = 15 out of 144 patients). Nine out of 15 events (60%) occurred within 3 months from treatment initiation. Six of these patients did not receive any thromboprophylaxis; 6 patients were on aspirin at the time of the event and 3 were on LMWH. Among the patients with VTE 11 were responders to treatment.

At 3 months from treatment initiation, 23% (n = 33) of the patients showed resistance to the antimyeloma treatment.

Among the patients with resistance to any antimyeloma treatment, 7.6% (n = 11) had progressive disease, 9.7% (n = 14) had stable disease, and 5.7% (n = 8) had minor response (MR). Among responders (n = 111), 42.3 % (n = 61) achieved PR and 34.7% (n = 50) had VGPR.

The risk of resistance to treatment was slightly, but not significantly, higher in patients who received any kind of thromboprophylaxis (aspirin or LMWH) (OR = 1.10; 95%CI: 0.47–2.56, $p > 0.05$). The risk of resistance to the treatment was higher but again not significant in patients who received aspirin (OR = 1.46; 95%CI: 0.55–3.88, $p > 0.05$). Due to the low number of patients on LMWH the analysis of the odds ratio for resistance to the treatment was underpowered.

3.1. Hypercoagulability at Diagnosis of Multiple Myeloma Prior to Treatment Initiation

At inclusion, prior to treatment administration, patients showed significantly increased levels of TFa, FVIIa, D-Dimer, and FM and significantly shorter Procoag-PPL® as compared to the group of healthy individuals. Levels of P-selectin and TM were significantly lower in

patients as compared to healthy individuals. The levels of heparanase were not significantly different in the group of patients as compared to the healthy individuals. Overall, thrombin generation was attenuated in patients compared to healthy individuals. Lag-time and ttPeak were significantly increased and Peak, MRI, and ETP were significantly lower (Table 2).

Table 2. Profile of hypercoagulability in NDMM patients prior to treatment initiation.

	Normal Reference Range	Healthy Subjects (*n* = 30)	MM (*n* = 144)	*p*	Non-Responders (*n* = 33)	Responders (*n* = 111)
Cellular-derived Hypercoagulability						
Procoag-PPL® (s)	42–85	62.8 ± 8.6	45.6 ± 0.22.6	<0.0001	51.9 ± 24.1	43.8 ± 21.9
TFa (ng/mL)	0.02–0.45	0.26 ± 0.13	3.9 ± 13.1	<0.0001	2.4 ± 3.8	4.4 ± 14.8
Heparanase (ng/mL)	0.08–0.16	0.13 ± 0.03	0.3 ± 0.5	0.476	0.4 ± 0.8	0.4 ± 0.5
TM (ng/mL)	70–120	90.1 ± 18.1	73.2 ± 68.1	<0.05	68.2 ± 88.1	74.7 ± 77.0
P-selectin (µg/mL)	82–42	62.6 ± 103.9	38.1 ± 31.8	<0.0001	31.6 ± 32.7	40.1 ± 31.5
TFPI (ng/mL)	15–26	18.2 ± 4.0	31 ± 18.5	0.02	37.1 ± 18.6	34.4 ± 16.3
Blood Coagulation Factors and Natural Inhibitors						
FVIIa (U/mL)	73–29	50.9 ± 10.6	74.1 ± 147.6	0.022	51.6 ± 47.7	75.1 ± 161.3
FV (%)	70–120	90.0 ± 12.0	78.0 ± 11.0	0.23	80.5 ± 35.4	91.7 ± 34.6
AT (%)	70–120	92.0 ± 12.0	95.4 ± 17.7	<0.005	95.3 ± 15.3	95.4 ± 18.4
In vivo Fibrin Formation/lysis						
D-Dimer (µg/mL)	<0.50	0.31 ± 0.08	1.80 ± 3.41	<0.0001	2.5 ± 3.8	1.6 ± 3.3
FM (µg/mL)	0.5–5.50	2.5 ± 0.5	14.29 ± 31.8	<0.0001	20.4 ± 40.7	19.6 ± 34.8
Thrombogram Parameters						
Lag-time (min)	2.1–3.8	2.5 ± 0.4	4.2 ± 2.2	<0.0001	3.9 ± 1.2	4.3 ± 2.4
ttPeak (min)	4.0–6.6	5.3 ± 0.7	7.3 ± 2.8	<0.0001	6.9 ± 1.7	7.5 ± 3.1
Peak (nM)	222–330	287.8 ± 35.7	214.4 ± 80.1	<0.0001	218.1 ± 58.2	213.4 ± 85.9
MRI (nM/min)	60–120	109.9 ± 24.5	80.2 ± 45.7	<0.0001	80.8 ± 34.6	80.1 ± 48.7
ETP (nMxmin)	1600–1178	1496.8 ± 191.4	1181.8 ± 398	<0.0001	1150.6 ± 247.1	1191.4 ± 434.7

Procoag-PPL®: procoagulant phospholipid dependent clotting time; TFa: tissue factor activity; TM: thrombomodulin activity; TFPI: tissue factor pathway inhibitor; FVIIa: activated factor VII; FV: factor V; ATIII: antithrombin; FM: fibrin monomers; ttPeak: time to Peak of thrombin; MRI: mean rate index of thrombin generation; ETP: endogenous thrombin potential; *p*-values derived from Mann–Whitney–Wilcoxon test for independent samples (comparison of patients versus healthy individuals).

There was no significant difference in the levels of the studied biomarkers between responders and non-responders (Table 2). All studied biomarkers did not show any significant difference between the subgroup of patients under dialysis as compared to non-dialyzed patients. The levels of hypercoagulability biomarkers were not significantly different between the subgroup of patients with high cytogenetic abnormalities and those without.

3.2. Biomarkers of Hypercoagulability and Response to the Antimyeloma Treatment at 3 Months

In the univariate logistic regression analysis performed in the 144 patients (Table 3), poor response or resistance to treatment was associated with longer Procoag-PPL®, higher levels of D-dimer, and higher thrombin Peak in the thrombogram. On the other hand, the rate of poor response or resistance to the treatment was inversely associated with P-Selectin levels. Among patients with ISS stages I, II, and III 17%, 28%, and 23% showed poor responses, respectively. Between group comparisons with respect to poor response did not yield any significant differences; ISS stage II versus stage I: OR 2.11, CI 95% 0.85–5.24, $p = 0.107$, ISS III versus I OR: 1.71, CI 95% 0.79–3.67, $p = 0.172$. Higher rates of response to treatment were seen in patients with PS 0–1 (81%) versus PS 2–4 (68%) but the difference was not statistically significant (PS $\geq$2 versus PS 0–1, OR 1.92, 95% 0.77–4.78, $p = 0.164$). Resistance to treatment was seen in 20% of patients who received PI-based therapy and 29% of patients who received IMiD-based treatment, but the difference was not statistically

significant (p = 0.5). No significant differences in response were observed between patients who were <65 years of age (n = 62), 65–75 years of age (n = 55), or >75 years old (n = 27) (65–75 years group versus <65 years old group OR 1.95, CI 95% 0.79–4.80, p = 0.146 and >75 years old versus <65 years old, OR: 1.82, CI 95% 0.61–5.44, p = 0.284). Patients with one or more adverse cytogenetic abnormalities (n = 28) did not show significantly higher rates of poor response/resistance to treatment compared to patients with none of these cytogenetic abnormalities (OR: 0.72, CI 95% 0.25–2.07, p = 0.537).

Table 3. Univariate logistic regression analysis evaluating associations between the examined biomarkers and poor response/resistance to first line anti-myeloma treatment.

	Compared Categories	**OR (95% CI)**
Cellular-derived Hypercoagulability		
Procoag-PPL® (s)	≥41.7 vs. <41.7	**3.41 (1.45–8.03)**
TFa (ng/mL)	≥0.6 vs. <0.6	0.63 (0.28–1.41)
Heparanase (ng/mL)	≥0.373 vs. <0.373	0.41 (0.13–1.27)
TMa (%)	≥43 vs. <43	0.36 (0.10–1.28)
P-selectin (pg/mL)	≥23477 vs. <23477	**0.23 (0.08–0.65)**
TFPI (ng/mL)	≥35.71 vs. <35.71	1.57 (0.61–4.03)
Blood Coagulation Factors and Natural Inhibitors		
FVIIa (ng/mL)	≥23.31 vs. <23.31	1.95 (0.69–5.54)
FV (%)	≥92 vs. <92	0.50 (0.18–1.36)
ATIII (%)	≥94 vs. <94	0.62 (0.28–1.37)
In vivo Thrombin Generation		
D-Dimer (μg/mL)	≥1.44 vs. <1.44	**3.21 (1.43–7.22)**
FM (μg/mL)	≥5.46 vs. <5.46	1.52 (0.59–3.90)
Thrombogram Parameters		
Lag-time (min)	≥2.75 vs. <2.75	0.59 (0.23–1.52)
ETP (Mxmin)	≥1188.03 vs. <1188.03	0.51 (0.22–1.22)
Peak (nM)	≥181.66 vs. <181.66	**2.73 (1.03–7.23)**
ttPeak (min)	≥7.74 vs. <7.74	0.49 (0.18–1.30)
MRI (nM/min)	≥50.46 vs. <50.46	2.14 (0.81–5.70)

The cut-off levels were set on the basis of the respective ROC curves. Procoag-PPL®: procoagulant phospholipid dependent clotting time; TFa: tissue factor activity; TM: thrombomodulin activity; TFPI: tissue factor pathway inhibitor; FVIIa: activity of factor VII; FV: factor V; ATIII: anti-thrombin; FM: fibrin monomer; ETP: the endogenous thrombin potential; Peak: the peak concentration of thrombin; ttPeak: time to reach the peak concentration of thrombin; MRI: mean rate index of thrombin generation; p-values derived from Mann–Whitney–Wilcoxon test for independent samples.

Multivariate logistic regression analysis for biomarkers (Table 4) demonstrated that Procoag-PPL® ≥ 41.7 versus <41.7 s (OR = 4.06, 95% CI: 1.59–10.38, p = 0.003), D-Dimer ≥1.44 versus <1.44 μg/mL (OR = 2.52, 95% CI: 1.06–6.01, p = 0.037), and thrombin Peak ≥181.66 versus <181.66 nM (OR = 3.29, 95% CI: 1.17–9.26, p = 0.024), were independently associated with poor response or resistance to antimyeloma treatment. The multivariate logistic regression model was described by the equation below:

Table 4. Results of the multivariate logistic regression analysis examining the associations between the biomarkers and poor response/resistance to first line anti-myeloma treatment evaluated at 3 months follow up. Analysis was adjusted for clinical predictors, including dialysis, BMI, age, cardiovascular disease.

	Compared Categories	**Adjusted OR (95% CI)**
Procoag-PPL® (s)	≥41.7 vs. <41.7	4.06 (1.59–10.38)
D-Dimer (μg/mL)	≥1.44 vs. <1.44	2.52 (1.06–6.01)
Peak (nM)	≥181.66 vs. <181.66	3.29 (1.17–9.26)

log(odds for Poor response or resistance at 3 months) = −3.30 + (1.40 × Procoag-PPL®_binary) + (0.93 × D-Dimer_binary) + (1.19 × Peak_binary)

Based on the equation above, a score was created where the dependent variable is the log(odds for poor response or resistance at 3 months) and the binary predictors: 1 for Procoag-PPL® ≥ 41.7 s, 1 for D-Dimer ≥ 1.44 µg/mL, 1 for thrombin Peak ≥ 181.66 nM or 0 for Procoag-PPL® < 41.7 s, D-Dimer < 1.44 µg/mL, thrombin Peak < 181.66 nM.

The AUC corresponding to the ROC analysis for the multivariate model was 0.75 (Figure 1). The optimal cut-off level in the aforementioned score was equal to −1.18. The rate of poor response or resistance to the treatment at 3 months was 37.7% in patients with score ≥ −1.18 and 7.4% in patients with score < −1.18. The sensitivity and specificity of the score was 83.9% and 59.4%, respectively. According to the Hosmer–Lemeshow test, a value of $p = 0.597$ showed that the model was well calibrated.

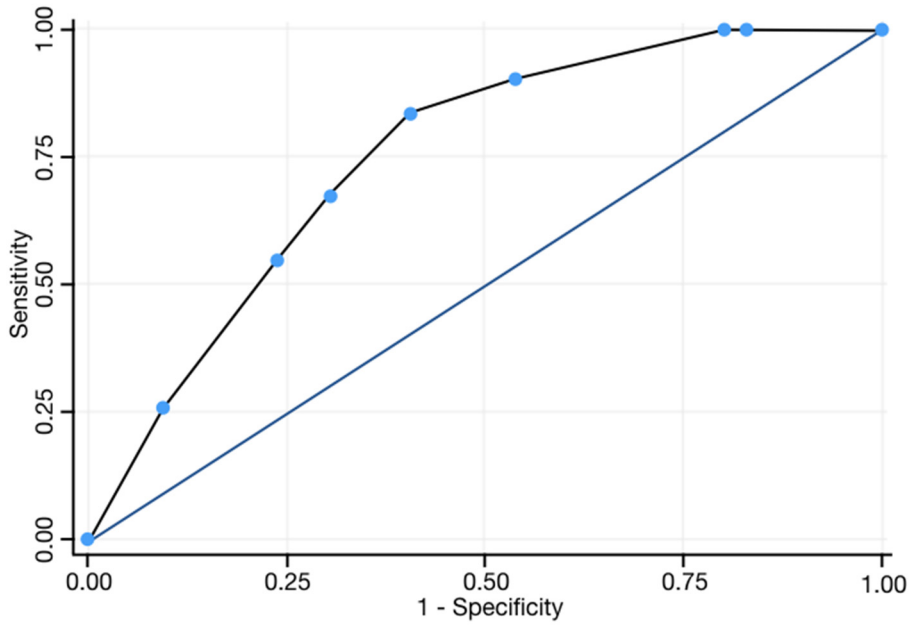

Figure 1. Receiver operating characteristic analysis of the experimental model for poor response or resistance to first line anti-myeloma treatment. The prediction model includes Procoag-PPL® clotting time, D-Dimer, and thrombin Peak and has 84% sensitivity, 60% specificity. Area under the curve = 0.75.

3.3. Biomarkers of Hypercoagulability and Overall Mortality at 3 Months

At 3 months follow-up the overall mortality was 25.7%. Among the 37 patients who died, 23 were responders to the treatment. Only one patient, who was a responder to the treatment, had VTE. Patients who died had significantly higher levels of thrombomodulin (141 ± 86 ng/mL) and TFPI (43 ± 22 ng/mL) and lower levels of antithrombin (88 ± 19%) as compared to the survivors (54 ± 65 ng/mL, 32 ± 14 ng/mL, and 98 ± 17%, respectively; $p < 0.05$). The other biomarkers were not significantly different between survivors and non-survivors. The univariate logistic regression analysis performed in the 144 patients did not show any significant association between the levels of thrombomodulin, TFPI, or antithrombin and mortality.

4. Discussion

The prospective observational ROADMAP-MM-CAT study was conducted in newly diagnosed, treatment naïve patients with symptomatic multiple myeloma and identified Procoag-PPL® clotting time, D-Dimer, and Peak of thrombin (measured with the Thrombinoscope® assay in the presence of PPP-Reagent®) as useful biomarkers for the identification of patients at high risk for poor response or resistance to first line antimyeloma therapy. In the studied cohort of patients, the rate of poor response or resistance to the treatment at 3 months from the start of therapy was similar to that reported in the literature [27].

The interval of 3 months allows a rapid evaluation of the response to the treatment which in future studies could be used for prompt optimization of the antimyeloma therapy.

Given the reciprocal relationship between cancer and blood coagulation activation, biomarkers of hypercoagulability are a promising tool for the evaluation of treatment efficacy and cancer outcomes [28,29]. The procoagulant potential of the malignant cells is based on cancer type and TF expression [30]. According to this rationale, our group followed a "cancer specific strategy" and focused exclusively on symptomatic patients with newly diagnosed multiple myeloma. The prospective design of the ROADMAP-MM-CAT study and the homogeneity of the studied cohort of patients regarding the phase of myeloma disease allowed the identification of three hypercoagulability biomarkers as more clinically relevant in this setting. Procoag-PPL® clotting time, D-Dimer, and Peak of thrombin, when assessed before any treatment administration, are significant predictors of the response to the first line of treatment. The presence of renal insufficiency, cardiovascular disease, hypertension, or obesity did not influence the levels of the studied biomarkers.

Procoag-PPL® clotting time correlates with the concentration of procoagulant phospholipids and microparticles derived from platelets or other vascular cells (i.e., endothelial cells, monocytes, etc.) [31]. All patients enrolled in the study had Procoag-PPL® clotting time shorter than the lower normal levels of this test indicating that vascular and blood cell activation is a constitutional background phenomenon in multiple myeloma patients. Interestingly, patients with Procoag-PPL® clotting time $\geq$41.7 s showed about 4-fold higher risk of resistance or poor response to treatment compared to those with lower values. Along the same lines, low levels of P-selectin, a marker of platelet activation, was a predictor of poor response or treatment resistance. Longer Procoag-PPL® clotting time and lower P-selectin levels may reflect a state of chronic platelet activation which has been previously described as "exhausted platelet syndrome" corroborating the concept that sustained platelet activation is part of the disease process in MM [32,33]. A plausible hypothesis would be that this phenomenon reflects the presence of "tumor educated platelets"; the most aggressive clones of plasma cells induce sustained platelet activation leading them to an "exhausted" status which is reflected by a more prolonged Procoag-PPL® clotting time and decreased P-Selectin levels in plasma [34]. In addition, patients showed significantly decreased levels of soluble thrombomodulin as compared to healthy individuals. The levels of P-selectin did not correlate with the platelet count. The decrease in P-selectin and thrombomodulin is rather puzzling. An increase in these biomarkers was expected since multiple myeloma is characterized by hypercoagulability. A previous study in 44 newly diagnosed patients with multiple myeloma reported increased levels of P-selectin [34].

To understand this unexpected finding we should focus on the role of P-selectin in cell adhesion and the origin of thrombomodulin. During the inflammatory process, P-selectin, a membrane glycoprotein existing in the Weibel-Palade bodies of endothelial cells and the α-granules of platelets, plays a key role in platelet activation and neutrophil adhesion to the endothelium [35]. Soluble P-selectin in plasma reflects primarily platelet activation. During the inflammatory process, P-selectin binds to activated leucocytes with leukocyte P-selectin glycoprotein ligand 1 (PSGL-1) and to endothelial cells. The P-selectin/PSGL-1 axis has a supporting role in the platelet rolling on the activated endothelium [36]. In addition, soluble P-selectin may bind to activated monocytes and regulate homing of multiple myeloma cells in the bone marrow microenvironment [37]. Thus, we can hypothesize that in the chronic inflammatory status induced by multiple myeloma, platelets are exhausted and P-selectin is depleted from the plasma component towards the support of cellular interactions. This may result in decreased levels of P-selectin in plasma. The design of the present study does not allow to explore this hypothesis.

Thrombomodulin is an endothelial cell surface protein essential for the activation of protein C by thrombin [38]. It has been reported that following acute endothelial cell activation, plasma thrombomodulin levels are initially increased, perhaps through cleavage from the cell surface. Then, after chronic endothelial cell activation plasma thrombomodulin levels are decreased as a consequence of downregulation of its production [39]. The

combined decrease in P-Selectin and thrombomodulin levels presumably reflects enhanced chronic endothelial cell activation. We hypothesize that the intensity of the inflammation and the interval between the initiation of the inflammatory process by the myeloma cells and the diagnosis of the disease (when these biomarkers were measured) might be a parameter that influences the levels of P-selectin and thrombomodulin measured in patients' plasma. Nevertheless, due to the design of the study it is not possible to prove this hypothesis since this interval was not recorded, nor was the intensity of the inflammatory state evaluated.

In newly diagnosed patients with multiple myeloma both TFPI and TFa were significantly increased. Both proteins are released by activated endothelial cells. This finding further corroborates the concept that endothelial cell activation is an inherent vascular alteration associated with multiple myeloma.

Almost all patients had D-Dimer levels higher than the upper normal limit adapted for age, BMI, renal function, and the presence of cardiovascular risk factors or disease. This is in accordance with previous studies conducted on cancer patients [28,29]. D-Dimer is the degradation product of cross-linked fibrin, indicating enhanced fibrin formation, activation of the fibrinolytic system, increased levels of fibrinogen, and likely reflects the biological activity of cancer cells. A significant association between elevated plasma levels of D-Dimer and advanced tumor stage and poor prognosis has been reported in patients with solid cancers and is not necessarily mediated by the increased risk of VTE [40]. The data presented herein establish a cut-off value for the prognostic use of D-Dimer in the evaluation of response to the antimyeloma treatment in newly diagnosed treatment naïve patients. A value of D-Dimer $\geq$1.44 μg/mL at multiple myeloma diagnosis, is an independent predictor of primary resistance to the antimyeloma treatment. Similarly, the threshold value of thrombin Peak is situated at 182 nM.

In the studied cohort of patients, a "paradoxical" prolongation of the initiation and propagation phase of thrombin generation and decrease in the ETP and Peak of thrombin were observed. A similar profile of thrombin generation in newly diagnosed patients with multiple myeloma has been previously reported by Legendre et al. in a study conducted in a small group of patients [38]. Along the same lines, the data published by Chalayer et al., who measured thrombin generation in NDMM patients using the PPP-reagent Low (1 pM TF and 5 pM procoagulant phospholipids) showed levels of ETP and Peak thrombin in the same range as in our study [41]. In contrast, other studies in small groups of patients with multiple myeloma and using different concentrations of TF showed increased thrombin generation [42–44]. A methodological approach is required for the interpretation of these findings. The Thrombogram-Thrombinoscope® assay is performed with exogenously added optimal concentrations of TF and procoagulant phospholipids (5 pM and 4 μM, respectively, in our study). Thus, the sensitivity of the test to variations in plasma concentrations of TF or procoagulant phospholipids is limited by the amount of TF present in the reagent. In contrast, with the reagents used in this study, the test is sensitive to the variations in TFPI and/or TM levels in plasma (data from in vitro experiments not shown). Correlation analysis showed that the attenuation of thrombin generation correlates with the increased plasma concentration of TFPI. Accordingly, in patients with NDMM, the attenuation of thrombin generation should be interpreted as a reflection of endothelial cell activation rather than an indicator of plasma hypercoagulablity, i.e., an imbalance between clotting factors and natural coagulation inhibitors. Other plasma parameters, such as the levels of albumin and monoclonal immunoglobulin, might influence thrombin generation kinetics, Peak, and ETP [45] The impact of paraproteinemia on thrombin generation in patients with multiple myeloma needs to be explored further.

Interestingly, multiple myeloma patients with higher thrombin generation capacity were those who had about 3-fold higher risk of poor response or resistance to treatment as compared to those with lower levels of thrombin generation. The design of the ROADMAP-MM-CAT study does not allow the exploration of this interesting observation. A hypothesis can, however, be generated. Tumor cells possess procoagulant activities that induce local

activation of blood coagulation and deposition of fibrin, which has an important role in the formation of tumor stroma and hematogenous spread of tumor cells [46]. Thrombin generation assay simulates, ex vivo, the response of the blood coagulation mechanism when triggered by physiologically relevant concentrations of TF and procoagulant phospholipids. The potential of myeloma cells to activate platelets and to organize the fibrin network in the bone marrow microenvironment, probably reduces the likelihood of effective target reach by the antimyeloma agents [47,48].

The research on the biological significance of hemostatic biomarkers in cancer progression is ever growing. The hypothesis that biomarkers of hypercoagulability could reflect more aggressive cancer or tumor bulk and might be linked to poorer treatment responses is becoming increasingly relevant [49]. The data provided by the ROADMAP-MM-CAT study shows that endothelial cell activation is among the early systematic alterations of multiple myeloma disease. Modeling studies published by our group and others show that cancer-cell derived microparticles expressing TF induce a procoagulant shift of endothelial cells. Recent evidence shows that myeloma plasma cells indirectly induce blood-borne hypercoagulability through the release of microparticles rich in TF [50]. Endothelial cells, upon exposure, display TFa and enhance thrombin generation. These properties are transferred to daughter generations [51,52]. These data lead to the hypothesis that endothelial cell activation and hypercoagulability of the microenvironment of plasma cells results in platelet adhesion and aggregation as well as fibrin clot formation which reduces the access of the antimyeloma treatment towards the target cells. This mechanism is under investigation by our group.

Sub-optimal treatment administration in patients with VTE could be a contributing factor for the suboptimal responses to treatment in some patients. Indeed, in 60% of the patients with VTE, thrombosis occurred within the first 3 months from treatment initiation. Nevertheless, the occurrence of VTE was not associated with either treatment resistance or mortality.

The administration of thromboprophylaxis with aspirin or LMWH slightly, but not significantly, decreased the risk of resistance to treatment. This finding needs to be explored in a prospective study with a higher number of patients.

The derived biological score was not predictive for the overall survival at 3 months after treatment initiation. Nevertheless, patients who died, as compared to the survivors, had an important imbalance of the natural coagulation inhibitors as documented by the significantly higher levels of TFPI and thrombomodulin and the lower levels of AT. This imbalance was not predictive for the overall 3-month mortality risk. TFPI and TM increase is a marker of endothelial cell dysfunction and AT decrease indicates impaired control of hypercoagulability. The impact of the combined alteration of endothelial cell function and hypercoagulability at the risk of early death in newly diagnosed patients with multiple myeloma needs to be thoroughly investigated.

The prospective design of the study led to the derivation of an original score based on pertinent hemostatic biomarkers. The application of the rule of thumb (1–10 event per variable), prevented erroneous inclusion of predictors into the model. The proposed score is well balanced and identifies patients with poor response or resistance to anti-myeloma treatment with 84% sensitivity, 60% specificity, and 0.75 value of the AUC of ROC curve.

The present study has some limitations. The control group was selected, and this might introduce a bias in the comparison of the studied biomarkers with the normal reference range. However, the comparisons of the biomarkers between the responder and non-responder group, the calculation of the relative risk for resistance to the treatment, and the final score are not influenced by any potential bias introduced by the selection of the control group. The inclusion of patients from a single center did not allow the evaluation of the potential influence of other therapeutic practices or supportive treatments on the predictive capacity of the studied biomarkers. Indeed, 32% of patients received IMiD-based therapy. This might not be representative for countries where triplet therapy

(VRd-bortezomib, lenalidomide, dexamethasone) is standard of care or for countries in which these combinations are not used or not approved yet.

The size of the cohort and the number of events with primary poor response or resistance to treatment provides sufficient statistical power for the derivation of a score. However, the sample size did not allow any internal validation of the model and this is the aim of the ongoing ROADMAP-MM-CAT. The rate of poor response or resistance to treatment was not significantly different between patient subgroups. The ISS stage and the performance status tended to be associated with treatment response but were not included in the model as they did not achieve statistical significance. The low number of patients with high risk cytogenetic abnormalities did not allow to evaluate their association with the hypercoagulability biomarkers which could improve the precision of the score. This issue must be investigated in a larger cohort of patients. Nevertheless, it is important to underline that ISS stage and high-risk cytogenetics are mandatory for the survival rather than for the response to the treatment.

The actual number of events does not allow investigation of the performance of the score according to the pre-treatment probability of response based on the established criteria which include clinical characteristics and cytogenetics of multiple myeloma patients. Moreover, this study does not allow us to evaluate whether the findings apply to a particular type of antimyeloma treatment. These are the aims of the ongoing ROADMAP-MM-CAT study. The findings of the present study are restricted to the assessment of the Procoag-PPL® clotting time, D-Dimer, and thrombin generation in plasma using only the assays and the reagents employed. The performance of other methods that measure thrombin generation (i.e., in-house assays, other combinations of reagents, or other techniques available in the market) should be assessed in suitably designed prospective studies.

The feasibility and the clinical application of the score presented herein is a challenge in the context of the new strategy for the optimization of antimyeloma treatment. The assessment of the score at 3 months from treatment initiation could be, following a prospective clinical validation of the score, an additional element for prompt optimization of the antimyeloma treatment. Procoag-PPL® clotting time and D-Dimer are commercially available, user-friendly, fully automated, quick, and reproducible assays that can be installed in any blood coagulation analyzer. The measurement of Peak of thrombin generation performed in PPP with PPP-Reagent® using the Calibrated Automated Thrombogram-Thrombinoscope® assay is a semiautomated, standardized technique, available in the market worldwide. Important steps towards the standardization of the external quality control procedure were accomplished. The new version of the Calibrated Automated Thrombogram-Thrombinoscope® analyzer recently presented by the manufacturer, will render this method accessible to hematological laboratories which are not highly specialized in blood coagulation exploration. A financial analysis must be performed so that the benefits of the new score will not be restricted by the cost of the laboratory assessment.

In conclusion, the prospective ROADMAP-MM-CAT study leads to an original concept for prompt identification of NDMM patients with primary poor response or resistance to the anti-myeloma treatment. The identified biomarkers, Procoag-PPL® clotting time, D-Dimer, and Peak of thrombin generation, need to be correlated in future studies with established clinical prognostic parameters in multiple myeloma patients and then validated clinically within the context of prospective clinical trials to address the question of their role in clinical practice and antimyeloma treatment optimization.

Author Contributions: Conceptualization, G.T.G.; methodology P.V.D.; formal analysis, T.N.S., P.V.D.; investigation D.F.; resources D.F.; data curation, D.F., T.N.S., L.P.; writing—original draft preparation, G.T.G., P.V.D.; review and editing, J.F., A.F., M.S., L.G., I.E.; supervision, E.T., M.A.D.; project administration, D.F.; funding acquisition, P.V.D. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Identifier: clinicalTrials.gov (accessed on 29 December 2021) NCT03405571.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: Data are available by the principal investigator upon formal request.

Acknowledgments: The authors would like to thank Matthieu Grusse for his skillful technical assistance.

Conflicts of Interest: The authors declare no competing financial interest for this study.

References

1. Ziogas, D.C.; Dimopoulos, M.A.; Kastritis, E. Prognostic factors for multiple myeloma in the era of novel therapies. *Expert Rev. Hematol.* **2018**, *11*, 863–879. [CrossRef]
2. Majithia, N.; Vincent Rajkumar, S.; Lacy, M.Q.; Buadi, F.K.; Dispenzieri, A.; Gertz, M.A.; Hayman, S.R.; Dingli, D.; Kapoor, P.; Hwa, L.; et al. Outcomes of primary refractory multiple myeloma and the impact of novel therapies. *Am. J. Hematol.* **2015**, *90*, 981–985. [CrossRef] [PubMed]
3. Dutta, A.K.; Hewett, D.R.; Fink, J.L.; Grady, J.P.; Zannettino, A.C.W. Cutting edge genomics reveal new insights into tumour development, disease progression and therapeutic impacts in multiple myeloma. *Br. J. Haematol.* **2017**, *178*, 196–208. [CrossRef] [PubMed]
4. Diamandis, E.P. The failure of protein cancer biomarkers to reach the clinic: Why, and what can be done to address the problem? *BMC Med.* **2012**, *10*, 87. [CrossRef] [PubMed]
5. Falanga, A.; Russo, L.; Milesi, V.; Vignoli, A. Mechanisms and risk factors of thrombosis in cancer. *Crit. Rev. Oncol. Hematol.* **2017**, *118*, 79–83. [CrossRef] [PubMed]
6. Rousseau, A.; Larsen, A.K.; Van Dreden, P.; Sabbah, M.; Elalamy, I.; Gerotziafas, G.T. Differential contribution of tissue factor and Factor XII to thrombin generation triggered by breast and pancreatic cancer cells. *Int. J. Oncol.* **2017**, *51*, 1747–1756. [CrossRef] [PubMed]
7. Ruf, W.; Disse, J.; Carneiro-Lobo, T.C.; Yokota, N.; Schaffner, F. Tissue factor and cell signalling in cancer progression and thrombosis. *J. Thromb. Haemost.* **2011**, *9* (Suppl. S1), 306–315. [CrossRef] [PubMed]
8. Owens, A.P., 3rd; Mackman, N. Microparticles in hemostasis and thrombosis. *Circ. Res.* **2011**, *108*, 1284–1297. [CrossRef] [PubMed]
9. Klemm, F.; Joyce, J.A. Microenvironmental regulation of therapeutic response in cancer. *Trends Cell Biol.* **2015**, *25*, 198–213. [CrossRef] [PubMed]
10. Guglietta, S.; Rescigno, M. Hypercoagulation and complement: Connected players in tumor development and metastases. *Semin. Immunol.* **2016**, *28*, 578–586. [CrossRef] [PubMed]
11. Fotiou, D.; Sergentanis, T.N.; Papageorgiou, L.; Stamatelopoulos, K.; Gavriatopoulou, M.; Kastritis, E.; Psaltopoulou, T.; Salta, S.; Van Dreden, P.; Sangare, R.; et al. Longer procoagulant phospholipid-dependent clotting time, lower endogenous thrombin potential and higher tissue factor pathway inhibitor concentrations are associated with increased VTE occurrence in patients with newly diagnosed multiple myeloma: Results of the prospective ROADMAP-MM-CAT study. *Blood Cancer J.* **2018**, *8*, 102. [PubMed]
12. De Stefano, V.; Za, T.; Rossi, E. Venous thromboembolism in multiple myeloma. *Semin. Thromb. Hemost.* **2014**, *40*, 338–347. [PubMed]
13. Crowley, M.P.; Eustace, J.A.; O'Shea, S.I.; Gilligan, O.M. Venous thromboembolism in patients with myeloma: Incidence and risk factors in a "real-world" population. *Clin. Appl. Thromb. Hemost.* **2014**, *20*, 600–606. [CrossRef] [PubMed]
14. Palumbo, A.; Rajkumar, S.V.; Dimopoulos, M.A.; Richardson, P.G.; San Miguel, J.; Barlogie, B.; Harousseau, J.; Zonder, J.A.; Cavo, M.; Zangari, M.; et al. Prevention of thalidomide- and lenalidomide-associated thrombosis in myeloma. *Leukemia* **2008**, *22*, 414–423. [CrossRef] [PubMed]
15. Terpos, E.; Kleber, M.; Engelhardt, M.; Zweegman, S.; Gay, F.; Kastritis, E.; van de Donk, N.W.; Bruno, B.; Sezer, O.; Broijl, A.; et al. European Myeloma Network guidelines for the management of multiple myeloma-related complications. *Haematologica* **2015**, *100*, 1254–1266. [CrossRef]
16. Rajkumar, S.V.; Dimopoulos, M.A.; Palumbo, A.; Blade, J.; Merlini, G.; Mateos, M.V.; Kumar, S.; Hillengass, J.; Kastritis, E.; Richardson, P.; et al. International Myeloma Working Group updated criteria for the diagnosis of multiple myeloma. *Lancet Oncol.* **2014**, *15*, e538–e548. [CrossRef]
17. Van Dreden, P.; Rousseau, A.; Fontaine, S.; Woodhams, B.J.; Exner, T. Clinical evaluation of a new functional test for detection of plasma procoagulant phospholipids. *Blood Coagul. Fibrinolysis* **2009**, *20*, 494–502. [CrossRef]
18. Van Dreden, P.; Rousseau, A.; Savoure, A.; Lenormand, B.; Fontaine, S.; Vasse, M. Plasma thrombomodulin activity, tissue factor activity and high levels of circulating procoagulant phospholipid as prognostic factors for acute myocardial infarction. *Blood Coagul. Fibrinolysis* **2009**, *20*, 635–641. [CrossRef]
19. Schneider, P.; Dreden, P.V.; Rousseau, A.; Kassim, Y.; Legrand, E.; Vannier, J.P.; Vasse, M. Increased levels of tissue factor activity and procoagulant phospholipids during treatment of children with acute lymphoblastic leukaemia. *Br. J. Haematol.* **2010**, *148*, 582–592. [CrossRef]

20. Rousseau, A.; Favier, R.; Van Dreden, P. Elevated circulating soluble thrombomodulin activity, tissue factor activity and circulating procoagulant phospholipids: New and useful markers for pre-eclampsia? *Eur. J. Obstet. Gynecol. Reprod. Biol.* **2009**, *146*, 46–49. [CrossRef]
21. Gerotziafas, G.T.; Depasse, F.; Busson, J.; Leflem, L.; Elalamy, I.; Samama, M.M. Towards a standardization of thrombin generation assessment: The influence of tissue factor, platelets and phospholipids concentration on the normal values of Thrombogram-Thrombinoscope assay. *Thromb. J.* **2005**, *3*, 16. [CrossRef] [PubMed]
22. Spronk, H.M.; Dielis, A.W.; De Smedt, E.; van Oerle, R.; Fens, D.; Prins, M.H.; Hamulyák, K.; Ten Cate, H. Assessment of thrombin generation II: Validation of the Calibrated Automated Thrombogram in platelet-poor plasma in a clinical laboratory. *Thromb. Haemost.* **2008**, *100*, 362–364. [PubMed]
23. Dargaud, Y.; Wolberg, A.S.; Luddington, R.; Regnault, V.; Spronk, H.; Baglin, T.; Lecompte, T.; Ten Cate, H.; Negrier, C. Evaluation of a standardized protocol for thrombin generation measurement using the calibrated automated thrombogram: An international multicentre study. *Thromb. Res.* **2012**, *130*, 929–934. [CrossRef] [PubMed]
24. Kumar, S.; Paiva, B.; Anderson, K.C.; Durie, B.; Landgren, O.; Moreau, P.; Munshi, N.; Lonial, S.; Bladé, J.; Mateos, M.V.; et al. International Myeloma Working Group consensus criteria for response and minimal residual disease assessment in multiple myeloma. *Lancet Oncol.* **2016**, *17*, e328–e346. [CrossRef]
25. Peduzzi, P.; Concato, J.; Kemper, E.; Holford, T.R.; Feinstein, A.R. A simulation study of the number of events per variable in logistic regression analysis. *J. Clin. Epidemiol.* **1996**, *49*, 1373–1379. [CrossRef]
26. Wasson, J.H.; Sox, H.C.; Neff, R.K.; Goldman, L. Clinical prediction rules. Applications and methodological standards. *N. Engl. J. Med.* **1985**, *313*, 793–799. [CrossRef] [PubMed]
27. Gavriatopoulou, M.; Musto, P.; Caers, J.; Merlini, G.; Kastritis, E.; van de Donk, N.; Gay, F.; Hegenbart, U.; Hajek, R.; Zweegman, S.; et al. European myeloma network recommendations on diagnosis and management of patients with rare plasma cell dyscrasias. *Leukemia* **2018**, *32*, 1883–1898. [CrossRef]
28. Falanga, A.; Marchetti, M. Hemostatic biomarkers in cancer progression. *Thromb. Res.* **2018**, *164* (Suppl. S1), S54–S61. [CrossRef] [PubMed]
29. Streetly, M.; Hunt, B.J.; Parmar, K.; Jones, R.; Zeldis, J.; Schey, S. Markers of endothelial and haemostatic function in the treatment of relapsed myeloma with the immunomodulatory agent Actimid (CC-4047) and their relationship with venous thrombosis. *Eur. J. Haematol.* **2005**, *74*, 293–296. [CrossRef]
30. Gerotziafas, G.T.; Galea, V.; Mbemba, E.; Khaterchi, A.; Sassi, M.; Baccouche, H.; Prengel, C.; van Dreden, P.; Hatmi, M.; Bernaudin, J.F.; et al. Tissue factor over-expression by human pancreatic cancer cells BXPC3 is related to higher prothrombotic potential as compared to breast cancer cells MCF7. *Thromb. Res.* **2012**, *129*, 779–786. [CrossRef] [PubMed]
31. Patil, R.; Ghosh, K.; Shetty, S. A simple clot based assay for detection of procoagulant cell-derived microparticles. *Clin. Chem. Lab. Med.* **2016**, *54*, 799–803. [CrossRef] [PubMed]
32. Riedl, J.; Kaider, A.; Marosi, C.; Prager, G.; Eichelberger, B.; Koder, S.; Panzer, S.; Pabinger, I.; Ay, C. PO-63-Exhausted platelets in cancer patients with high risk of venous thromboembolism and poor prognosis. *Thromb. Res.* **2016**, *140* (Suppl. S1), S199–S200. [CrossRef]
33. Mannucci, P.M.; Cattaneo, M.; Canciani, M.T.; Maniezzo, M.; Vaglini, M.; Cascinelli, N. Early presence of activated ('exhausted') platelets in malignant tumors (breast adenocarcinoma and malignant melanoma). *Eur. J. Cancer Clin. Oncol.* **1989**, *25*, 1413–1417. [CrossRef]
34. Lemancewicz, D.; Bolkun, L.; Mantur, M.; Semeniuk, J.; Kloczko, J.; Dzieciol, J. Bone marrow megakaryocytes, soluble P-selectin and thrombopoietic cytokines in multiple myeloma patients. *Platelets* **2014**, *25*, 181–187. [CrossRef] [PubMed]
35. Stenberg, P.E.; McEver, R.P.; Shuman, M.A.; Jacques, Y.V.; Bainton, D.F. A platelet alpha-granule membrane protein (GMP-140) is expressed on the plasma membrane after activation. *J. Cell. Biol.* **1985**, *101*, 880–886. [CrossRef] [PubMed]
36. Coenen, D.M.; Mastenbroek, T.G.; Cosemans, J. Platelet interaction with activated endothelium: Mechanistic insights from microfluidics. *Blood* **2017**, *130*, 2819–2828. [CrossRef] [PubMed]
37. Chimen, M.; Evryviadou, A.; Box, C.L.; Harrison, M.J.; Hazeldine, J.; Dib, L.H.; Kuravi, S.J.; Payne, H.; Price, J.M.; Kavanagh, D.; et al. Appropriation of GPIbalpha from platelet-derived extracellular vesicles supports monocyte recruitment in systemic inflammation. *Haematologica* **2020**, *105*, 1248–1261. [CrossRef] [PubMed]
38. Owen, W.G.; Esmon, C.T. Functional properties of an endothelial cell cofactor for thrombin-catalyzed activation of protein C. *J. Biol. Chem.* **1981**, *256*, 5532–5535. [CrossRef]
39. Cacoub, P.; Karmochkine, M.; Dorent, R.; Nataf, P.; Piette, J.C.; Godeau, P.; Gandjbakhch, I.; Boffa, M.C. Plasma levels of thrombomodulin in pulmonary hypertension. *Am. J. Med.* **1996**, *101*, 160–164. [CrossRef]
40. Ay, C.; Dunkler, D.; Pirker, R.; Thaler, J.; Quehenberger, P.; Wagner, O.; Zielinski, C.; Pabinger, I. High D-dimer levels are associated with poor prognosis in cancer patients. *Haematologica* **2012**, *97*, 1158–1164. [CrossRef] [PubMed]
41. Chalayer, E.; Tardy-Poncet, B.; Karlin, L.; Chapelle, C.; Montmartin, A.; Piot, M.; Guyotat, D.; Collet, P.; Lecompte, T.; Tardy, B. Thrombin generation in newly diagnosed multiple myeloma during the first three cycles of treatment: An observational cohort study. *Res Pr. Thromb. Haemost.* **2019**, *3*, 89–98. [CrossRef] [PubMed]
42. Nielsen, T.; Kristensen, S.R.; Gregersen, H.; Teodorescu, E.M.; Pedersen, S. Prothrombotic abnormalities in patients with multiple myeloma and monoclonal gammopathy of undetermined significance. *Thromb. Res.* **2021**, *202*, 108–118. [CrossRef]

43. Baccouche, H.; Hadhri, M.; Aissi, W.; Chakroun, A.; Bahri, D.; Mahjoub, S.; Ben Romdhane, N. The hypercoagulable state in multiple myeloma: The contribution of thrombin generation test. *Int. J. Lab. Hematol.* **2019**, *41*, 684–690. [CrossRef]
44. Tiong, I.S.; Rodgers, S.E.; Lee, C.H.; McRae, S.J. Baseline and treatment-related changes in thrombin generation in patients with multiple myeloma. *Leuk. Lymphoma.* **2017**, *58*, 941–949. [CrossRef]
45. Paar, M.; Rossmann, C.; Nusshold, C.; Wagner, T.; Schlagenhauf, A.; Leschnik, B.; Oettl, K.; Koestenberger, M.; Cvirn, G.; Hallström, S. Anticoagulant action of low, physiologic, and high albumin levels in whole blood. *PLoS ONE* **2017**, *12*, e0182997. [CrossRef]
46. Palumbo, J.S.; Kombrinck, K.W.; Drew, A.F.; Grimes, T.S.; Kiser, J.H.; Degen, J.L.; Bugge, T.H. Fibrinogen is an important determinant of the metastatic potential of circulating tumor cells. *Blood* **2000**, *96*, 3302–3309. [CrossRef] [PubMed]
47. Kumar, R.; Godavarthy, P.S.; Krause, D.S. The bone marrow microenvironment in health and disease at a glance. *J. Cell. Sci.* **2018**, *131*, jcs201707. [CrossRef] [PubMed]
48. Kawano, Y.; Moschetta, M.; Manier, S.; Glavey, S.; Görgün, G.T.; Roccaro, A.M.; Anderson, K.C.; Ghobrial, I.M. Targeting the bone marrow microenvironment in multiple myeloma. *Immunol. Rev.* **2015**, *263*, 160–172. [CrossRef] [PubMed]
49. Falanga, A.; Marchetti, M.; Russo, L. Hemostatic Biomarkers and Cancer Prognosis: Where Do We Stand? *Semin. Thromb. Hemost.* **2021**, *47*, 962–971. [CrossRef] [PubMed]
50. Papageorgiou, L.; Hussen, K.A.; Thouroude, S.; Mbemba, E.; Cost, H.; Garderet, L.; Elalamy, I.; Larsen, A.; Van Dreden, P.; Dimopoulos, M.A.; et al. Modelization of Blood-Borne Hypercoagulability in Myeloma: A Tissue-Factor-Bearing Microparticle-Driven Process. *TH Open.* **2019**, *3*, e340–e347. [CrossRef] [PubMed]
51. AmraneDjedidi, R.; Rousseau, A.; Larsen, A.K.; Elalamy, I.; Van Dreden, P.; Gerotziafas, G.T. Extracellular vesicles derived from pancreatic cancer cells BXPC3 or breast cancer cells MCF7 induce a permanent procoagulant shift to endothelial cells. *Thromb. Res.* **2020**, *187*, 170–179. [CrossRef] [PubMed]
52. Faulkner, L.G.; Alqarni, S.; Maraveyas, A.; Madden, L.A. Isolated tumour microparticles induce endothelial microparticle release in vitro. *Blood Coagul. Fibrinolysis* **2020**, *31*, 35–42. [CrossRef] [PubMed]