PB1016 | Procoagulant Phospholipid Dependent Clotting Time, Thrombin Generation Test and D-Dimers Are New Biomarkers in the Evaluation of Treatment Failure Risk in Newly Diagnosed Patients with Symptomatic Multiple Myeloma. Results from the Prospective ROADMAP MM Study

G. Gerotziafas[1,2]; D. Fotiou[3]; T. Sergentanis[3]; L. Papageorgiou[4]; P. Van Dreden[5]; K. Stamatelopoulos[3]; E. Kastritis[3]; A. Larsen[6]; A. Falanga[7]; E. Terpos[3]; M. Mohty[1]; M. Dimopoulos[3]

[1]Saint Antoine Hospital, Hôpitaux, Department of Hematology and Cell Therapy, Paris, France, [2]Sorbonne Universities, Faculty of Medicine, Cancer, Haemostasis and Angiogenesis, Paris, France, [3]National and Kapodistrian University of Athens, Department of Clinical Therapeutics, Athens, Greece, [4]Service Hématologie Biologique Hôpital Tenon, Hôpitaux Universitaires de l'Est Parisien, Paris, France, [5]Diagnostica Stago, Clinical Research, Gennevilliers, France, [6]Sorbonne Universities, Faculty of Medicine, Cancer, Haemostasis and Angiogenesis, Paris, Greece, [7]Hospital Papa Giovanni XXIII, Department of Immunohematology and Transfusion Medicine, Bergamo, Italy

Background: Multiple myeloma remains an incurable disease with frequent poor response or resistance to the treatment which impact negatively patients survival and quality of life.

Aims: The prospective, longitudinal observational study ROADMAP-MM CAT conducted in newly diagnosed treatment naïve patients with multiple myeloma assessed a large number of biomarkers of hypercoagulablity to identify those which are clinically relevant for the evaluation of the risk of resistance to antimyeloma treatment.

Methods: Newly diagnosed, treatment naïve symptomatic patients with MM not receiving any antimyeloma or antithrombotic treatment were enrolled and followed for 3 months after treatment initiation. STA®Procoag-PPL, factor VIIa (Staclot® VIIa-rTF), antithrombin (AT), fibrin monomers (FM), free TFPI, D-Dimers, P-selectin, heparanase and thrombin generation with the Calibrated Automated Thrombogram® were measured. The primary study end-point was response to treatment at 3 months.

Results: A total of 144 eligible patients were enrolled and followed. At 3 months 23% (n=33) of the patients showed poor response or resistance to the antimyeloma treatment. At the univariate logistic regression analysis poor response or resistance to the treatment was associated with longer Procoag-PPL, higher levels of D-dimers and higher Peak in the thrombogram. The multivariate analysis led to the derivation of a prognostic model which included the Procoa-PPL, D-Dimers and thrombin generation Peak. Accordingly a new score was created which had 84% sensitivity and 59% specificity to identify patients who showed treatment resistance at 3 months. The AUC corresponding to the ROC analysis for the multivariate model was 0.75.

Conclusions: The prospective ROADMAP-CAT-MM study led to the derivation of an originalrisk assessment model for the identification of patients at risk of poor response orresistance to the antimyeloma treatment which is based on the evaluation of the Procoag-PPL® clotting time, D-Dimers and Peak of thrombin generation.

Procoagulant phospholipid dependent clotting time, thrombin generation test and D-Dimers are new biomarkers in the evaluation of treatment failure risk in newly diagnosed patients with symptomatic multiple myeloma. Results from the prospective ROADMAP MM study.

G. Gerotziafas[1,3,5], D. Fotiou[2], T. Sergentanis [2], L. Papageorgiou[3], P. Van Dreden[4]*, K. Stamatelopoulos[2], E. Kastritis[2], A. Larsen[1], E. Terpos[2], M. Mohty[1,5], M. Dimopoulos[2]

[1]Research Group "Cancer-Angiogenesis-Haemostasis" INSERM U938 Faculty of Medicine, Sorbonne Universities, Paris, France. [2]Department of Clinical Therapeutics, National and Kapodistrian University of Athens, School of Medicine, Athens, Greece. [3] Department of Biological Haematology, Tenon University Hospital, APHP.6, Paris,France. [4] Clinical Research Department, Diagnostica Stago, Gennevilliers, France. [5] Clinical Hemostasis and Thrombosis, Department of Hematology and Cell Therapy, Saint Antoine Hospital, AP-HP.6 Paris, France.

INTRODUCTION

Multiple myeloma (MM) figures among malignancies that significantly increase the risk of venous thromboembolism (VTE). The rate of VTE is higher at the time of diagnosis and during the first months following initiation of first line therapy; approximately 10% of newly diagnosed MM (NDMM) will develop a VTE. The risk of VTE in MM patients is linked to patient related clinical factors, type of anti-myeloma therapy and disease-specific mechanisms. The choice of therapy has been shown to affect the risk of VTE to a large extent. Despite adequate thromboprophylaxis as per guidelines, the risk of residual VTE is not eliminated and remains as high as 12%. The significant residual VTE rates reveal that the identification of patients eligible for thromboprophylaxis is probably suboptimal. Hence, an improved routine evaluation of VTE risk emerges as an unmet need.

AIM

The prospective, observational study ROADMAP-MM-CAT (PROspective Risk Assessment and bioMArkers of hypercoagulability for the identification of patients with Multiply Myeloma at risk for Cancer-Associated Thrombosis) aiming to identify in NDMM patients relevant biomarkers of hypercoagulability, variables related with MM and clinical predictors of VTE risk that could be used in combination to risk stratify MM patients and guide thromboprophylaxis.

PATIENTS & METHODS

The study was investigator initiated and designed as a prospective, non interventional trial, 200 patients were recruited from the out-patient day clinic of a tertiary care university hospital. Eligible patients had newly diagnosed multiple myeloma.
The exclusion criteria. Age younger than 18 years, ongoing pregnancy, life expectancy less than 6 months, major psychiatric disorders, recent (<6 months) episode of VTE or acute coronary syndrome, active treatment with UFH, LMWHs, vitamin K antagonists, rivaroxaban, apixaban or dabigatran for any other indication except the prevention of VTE, long-term anticoagulant active anticoagulant treatment at therapeutic dose for any indication, scheduled open elective curative surgery under general anesthesia hospitalization due to stroke, or acute coronary syndrome, or congestive heart failure or acute respiratory failure, liver insufficiency. All patients provided written informed consent. Study approval was obtained from local ethics committee according to national laws. The control group consisted of 30 healthy age & sex-matched individuals.
Procedures. Patients were receiving the recommended anticancer treatment according to the institutional practices. Patients were followed and interviewed at inclusion and at 3, 6 and 12 months after inclusion. Physical interviews were required for patients' inclusion and follow up. At each follow-up visit, patients were routinely assessed with echodoppler of the lower limb veins for the detection of asymptomatic deep vein thrombosis (DVT).
End-points: The primary end-point was the occurrence of any symptomatic and objectively confirmed VTE including deep vein thrombosis (DVT) or pulmonary embolism (PE) or both (DVT and PE) or superficial vein thrombosis (SVT) of the lower limb or central venous catheter (CVC) thrombosis or vein thrombosis of rare localization (i.e. splachnic vein or cerebral vein thrombosis). Any combination of the above mentioned VTE events was also included in the primary end-point. Symptomatic VTE had to be documented, by at least one of the following methods: color echo-Doppler, computerized tomography or magnetic resonance imaging angiography, scintigraphy or computerized tomography scan. Asymptomatic DVT was also included in the end-points of the study. Symptomatic VTE confirmed with the recommended imaging methods, and evolution of the disease were registered during the interview and cross-checked by the analysis of the medical records.
Blood samples. Blood samples were obtained by traumatic puncture of the antecubital vein, using a 20-gauge needle, and placed into siliconized vacutainer tubes containing 0.129 mol/L trisodium citrate (from Becton and Dickinson France) as anticoagulant, in a ratio of 9 parts of blood to 1part of citrate. Platelet poor plasma (PPP) was obtained after double centrifugation of citrated whole blood for 20 minutes at 2000 *g*. Platelet-free plasma was prepared immediately after blood sampling using a 2-step centrifugation procedure: initially at 1500 g for 15 minutes at 20° C to prepare platelet rich plasma and then at 13000g for 2 minutes at 20° C to prepare PFP. Samples were aliquoted and frozen at -80° C and transferred to the Department of Thrombosis and Haemostasis, Service d'Hématologie Biologique, Hôpital Tenon where they were assessed. All measurements were done in thawed plasma samples. Blood anticoagulated with EDTA was used for the determination of complete blood count. This study was approved by the ethics committee of Tenon University Hospital and was performed in accordance with the principles embodied in the Declaration of Helsinki.
Assay for hypercoagulability. Thrombin generation (TGT) in citrated PPP was assessed with the Thrombogram-Thrombinoscope® assay using PPP-reagent® 5 pm TF by Diagnostica Stago. The levels of P-Selectin and heparanase in plasma were measured with ELISA Kit (Cusabio Biotech and R&D Systems respectively). The procoagulant phospholipids clotting time was measured with STA-Procoag-PPL®. Levels of Factor VIIa were measured by Staclot VIIa-rTF®, D-Dimers (DDi) by Liatest D-Di (Diagnostica Stago, France), and Tissue Factor activity (TFa) by specific clotting based home test.

RESULTS

Table 1. ***Baseline demographic, clinical characteristics of multiple myeloma patients***

Patients' clinical characteristics	
Age (years)	66.0±12.0 (36-86)
Male/female	76/68 (53%/47%)
BSA (m^2)	1.85±0.20 (1.46-2.50)
BMI (kg/m^2)	25.9±5.0 (17.2-44.8)
ISS stage - n(%)	
I	46 (32%)
II	33 (23%)
III	65 (45%)
Anti-myeloma treatment - n(%)	
PI-based	92 (64%)
IMiD-based	46 (32%)
Other	6 (4%)
Other	
Dialysis at diagnosis - n(%)	14 (10%)
Bone disease present - n(%)	102 (71%)
High risk cytogenetics- n(%)	27 (19%)
Comorbidities and VTE risk factors non related with the cancer - n(%)	
Active pulmonary disease	13 (9%)
CV risk factors	110 (76.4%)
EPO use	50 (35%)
GFR<30ml/min	22(15%)
Thromboprophylaxis after enrollment in the study - n(%)	
None	47 (33)
Aspirin	74 (51.0)
LMWH (tinzaparin)	23 (16)

Study population
The demographics and clinical characteristics of the patients at the time of inclusion are summarized in Table 1. The control group consisted of 30 healthy individuals; 15 women and 15 men. The mean age of the control group was not significantly different as compared to the patient group.
Analytical data on patients with VTE are shown in Table 2.

At inclusion PDC as compared to the control group showed :

- Increased levels of TFa without a corresponding increase of FVIIa indicating reselase of TF which is not associated by activation of TF clotting pathway.
- Increased levels of D-Dimers and Fibrin Monomers indicating sustained *in vivo* thrombin generation.
- Attenuated initiation and propagation phase of thrombin generation (lag-time and MRI respectively) associated with a decreased peak and endogenous thrombin potential (ETP) indicating down-regulation of thrombin generation process.
- Shorter Procoag-PPL clotting time indicating the presence of high concentrations of procoagulant microparticles in plasma.
- Decreased levels of P-Selecting probably associated with an "exhausted platelet" status.
- Increased levels of heparanse indicating increased endoglycosidase activity and potential depolymerisation activity against heparan sulfate molecules.
- Increased Tissue pathway Inhibitor (TFPI) and Thrombomodulin (TM) which were positively correlated with attenuated thrombin generation.

The aforementioned results are shown in Table 3.

Multivariate logistic regression analysis demonstrated that ETP <1087.43 nMxmin versus ≥1087.43 nMxmin (OR=4.04, 95% CI 1.18-13.84, p=0.026) and Procoag-PPL® ≥46.9 versus <46.9 sec (OR=3.01, 95% CI 0.93-9.78, p=0.066), were independently associated with VTE occurrence.(Table 4)
Regarding clinical factors, pulmonary disease and lower M-peak emerge as independent risk factors for VTE.

Table 3. ***Profile of hypercoagulability in patients at diagnosis of MM prior to treatment initiation. 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).

	Healthy subjects (n=30)	MM (n=144)	p
Cellular derived hypercoagulability			
Procoag-PPL (sec.)	62.8±8.6	45.6±.22.6	<0.0001
TFa (pM)	0.26±0.13	3.97±13.10	<0.0001
Heparanase (ng/ml)	0.13±0.03	0.34±0.52	0.476
TM (%)	90±18	39.25±68.1	<0.005
P-selectin (ng/ml)	62660.3±10390.6	38122±31785	<0.0001
TFPI (ng/ml)	18 ± 4 ng/ml	31±18.5	0.02
Blood coagulation factors and natural inhibitors			
FVIIa (U/ml)	50.9±10.6	74.1±147.6	0.022
FV (%)	90±12	78±11	0.23
ATIII (%)	92±12.0	95.4±17.7	<0.005
In vivo fibrin formation/lysis			
D-Dimers (µg/ml)	0.31±0.08	1.80±3.41	<0.0001
FM (µg/ml)	2.5 ± 0.5	14.29±31.8	<0.0001
Thrombogram parameters			
Lag-time (min)	2.53±0.43	4.20±2.16	<0.0001
ttPeak (min)	5.28±0.73	7.33±2.76	<0.0001
Peak (nM)	287.8±35.7	214.4±80.1	<0.0001
MRI (nM/min)	109.9±24.5	80.2±45.7	<0.0001
ETP (nMxmin)	1496.8±191.4	1181.8±398.4	<0.0001

Table 4. ***Univariate logistic regression analysis evaluating associations between the examined biomarkers and VTE. The cut-off levels were set on the basis of the respective ROC curves. PPL-ct (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.***

F: *p=value derived from Fisher's exact test*

	Compared categories	OR (95% CI)	P
Cellular derived hypercoagulability			
Procoag-PPL (sec)	≥46.9 vs. <46.9	3.49 (1.13-0.82)	0.030
TFa (pM)	≥0.03 vs. <0.03	0.49 (0.09-2.50)	0.389
Heparanase (ng/ml)	≥0.678 vs. <0.678	Not estimable due to zero events in the upper category	0.215[F]
TMa (%)	≥41.95 vs. <41.95	4.93 (0.97-24.99)	0.054
P-selectin (pg/ml)	≥46700 vs. <46700	2.69 (0.71-10.26)	0.147
TFPI (ng/ml)	≥39.08 vs. <39.08	7.75 (1.51-39.70)	0.014
Blood coagulation factors and natural inhibitors			
FVIIa (ng/ml)	≥56.81 vs. <56.81	0.34 (0.07-1.59)	0.172
FV (%)	≥103 vs. <103	0.15 (0.02-1.18)	0.071
ATIII (%)	≥87 vs. <87	2.33 (0.50-10.84)	0.282
In vivo thrombin generation			
D-Dimers (µg/ml)	≥2.1 vs. <2.1	2.52 (0.82-7.69)	0.105
FM (µg/ml)	≥8.4 vs. <8.4	2.07 (0.61-6.95)	0.241
Thrombogram parameters			
Lag-time (min)	≥6.5 vs. <6.5	Not estimable due to zero events in the upper category	0.612 [F]
ETP (Mxmin)	≥1087.43 vs. <1087.43	0.25 (0.07-0.83)	0.024
Peak (nM)	≥253.16 vs. <253.16	1.50 (0.49-4.61)	0.479
ttPeak (min)	≥10 vs. <10	Not estimable due to zero events in the upper category	0.364 [F]
MRI (nM/min)	≥120.82 vs. <120.82	1.40 (0.36-5.49)	0.625

Table 2. ***Venous thromboembolism events among study population. M: male, F: female, DVT: deep vein thrombosis of the lower limb; CVC: central venous catheter insertion; IJV: internal jugular vein; LL: lower limb; UL: upper limb, PR: partial response; VGPR: very good partial response; PD: progressive disease; SD: stable disease; ASCT: autologous stem cell transplant; RAD: revlimid, adriamycin and dexamethasone; VMP: velcade, melphalan and prednisone; CTD: cyclophosphamide, thalidomide and dexamethasone; VCD: velcade, cyclophosphamide and dexamethasone; RD: revlimid and dexamethasone.***

age	Localization	Time of event from diagnosis (days)	Disease status at follow up	Thrombo-prophylaxis	Anti-myeloma treatment
50	IJV thrombosis post CVC insertion	150	PR	no	ASCT
46	IJV thrombosis post CVC insertion	90	VGPR	no	ASCT
40	Superficial UL vein thrombosis	90	PR	Aspirin	RAD
76	Superficial LL vein thrombosis	60	PR	no	VMP
78	distal DVT	45	PR	LMWH	CTD
81	distal DVT	45	PR	Aspirin	VMP
68	distal DVT	15	PR	no	VCD
55	PE	180	PD	Aspirin	RD
88	Mesenteric vein thrombosis	90	SD	LMWH	CTD
62	distal DVT	360	VGPR	Aspirin	RD
73	distal DVT	270	PR	LMWH (prior to event)	RD
71	distal DVT	330	PR	aspirin	RD
43	IJV thrombosis – CVC insertion	135	PR	no	ASCT
81	distal DVT	30	SD	aspirin	RD
59	PE	10	non evaluable	none	none

CONCLUSION

The prospective ROADMAP-CAT-MM study demonstrates the presence of pronounced cellular hypercoagulability in newly diagnosed chemotherapy naïve patients with symptomatic multiple myeloma,characterized by decreased Procoag-PPL® clotting time, enhanced endothelial cell activation, and exhausted thrombin generation. Among a large number of biomarkers of hypercoagulability, the Procoag-PPL clotting time and the ETP of thrombin generation were found to be independently associated with the risk of VTE and formulated a new score that accurately stratifies patients to high- and intermediate/low-level of VTE risk. The evaluation of these biomarkers is feasible in most hospitals and should be taken into consideration when designing phase III clinical trials that evaluate the efficacy and safety of pharmacological thromboprophylaxis in outpatients with multiple myeloma.