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Letter to the Editors-in-Chief

Global coagulation assays in patients with multiple myeloma and monoclonal gammopathy of unknown significance

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Dear Editors,

Both multiple myeloma and monoclonal gammopathy of undetermined significance (MGUS) are associated with increased venous thromboembolism (VTE) and arterial thrombosis [1]. The thrombogenicity of myeloma is multi-fold with current VTE risk stratification focusing predominantly on clinical factors. Key to augmenting our clinical risk assessment model to identify patients who may benefit from thromboprophylaxis is a predictive biomarker. Current coagulation testing are inadequate as they measure only the time to clot formation, making them poor indicators of a hypercoagulable state.

Global coagulation assays (GCA) such as thromboelastography (TEG) and thrombin generation, have been shown promise in being more superior predictors of VTE risk in cancer patients. A study of 1033 cancer patients found that elevated peak thrombin was associated with increased risk of VTE (11% vs 4%; $p = 0.002$) [2]. One study demonstrated that myeloma patients who developed thrombosis had significantly higher endogenous thrombin potential (ETP) and peak thrombin ($p = 0.001$) [3]. To further evaluate the role of GCA in VTE risk assessment in plasma cell dyscrasia, we examined the role of a combination of thromboelastography, thrombin generation using calibrated automated thrombogram (CAT) and fibrin generation using overall haemostatic assay (OHP), in patients with active myeloma as well as those with the precursor conditions of MGUS and smouldering myeloma compared to normal controls.

In our study, patients with plasma cell dyscrasia were prospectively recruited from the Northern Hospital in Melbourne, Australia and categorised into MGUS, smouldering multiple myeloma (sMM) and multiple myeloma based on the International Myeloma Working Group (IMWG) guidelines. Participants on anticoagulation and active infection/inflammation were excluded. Basic demographics were recorded and blood samples were sent for routine laboratory tests and experimental assays which included:

- Thromboelastography (TEG®): All samples were tested using citrated kaolin assay on TEG 5000® (Haemonetics, USA) within 4 h of collection. Routine parameters included R-time (time to clot onset), maximum amplitude (strength of fibrin clot, MA), α-angle (rate of clot formation), and clot lysis (rate of clot breakdown, LY30).
- Thrombin generation: The rate and extent of thrombin generated in double-centrifuged platelet-poor plasma after a tissue factor stimulus were measured using CAT (Diagnostica STAGO). Parameters calculated included lag time, ETP, peak thrombin and velocity index.
- OHP: This is a spectrophotometric assessment of fibrin-aggregation in double-centrifuged platelet-poor plasma using the FLUOstar® Optima (BMG Labtech) plate reader. For each sample, there is a corresponding overall coagulation potential (OCP) buffer of Tris/NaCl/$CaCl_2$/thrombin and an overall haemostatic potential (OHP) buffer of Tris/NaCl/$CaCl_2$/thrombin/tissue-plasminogen activator and the difference between the two areas under the curve reflects the overall fibrinolysis potential (OFP).

Results from these studies were compared to an age-matched normal population (age > 45 years) identified through previously collected normal cohort [4]. These controls did not have significant cardiovascular and thrombotic history and were not on anti-platelet therapy, anticoagulant or oral contraceptive pill. Statistical analyses were performed with Stata version 14.0 (StataCorp, College Station, Texas, USA). Univariate comparisons between the normal control population and the study population were performed, using either the chi-squared test or Fisher's Exact test for categorical variables, the student's test for normally distributed continuous variables or the Mann-Whitney (rank-sum) test for skewed (non-normally distributed) continuous variables. For all CAT, TEG and OHP variables, age, gender and ethnicity were included in the multivariate analysis. Statistical significance was set at p-value < .05. This study was approved by the Austin Health Human Research Ethics Committee (H2013/04977) and the Northern Health Human Research Ethics Committee (P5/13).

Forty-nine patients (median age 72 years) including 22 (45%) multiple myeloma, 14 (29%) sMM and 13 (26%) MGUS were recruited. IgG heavy chain ($n = 37$, 76%) and kappa light chain were the predominant subtype ($n = 31$, 63%). One patient had a previous history of VTE. Basic demographics, investigations and GCA parameters for study

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Table 1
Comparison of basic demographics and investigations as well as global coagulation assay parameters between study participants (MGUS, smouldering myeloma and myeloma) to normal controls > 45 years old.

	Normal Control	All study patients	p-Value	Breakdown of study patients						
				MGUS	p-Value*	Multiple myeloma^	p-Value**	Smouldering myeloma	p-Value***	p-Value****
Number of patients	55	49		13		22		14		
Basic demographics										
Age (years), median (IQR)	58 (52–62)	72 (63–76)	< 0.001	67 (63–74)		71 (63–77)		75 (71–80)		
Male, n (%)	14 (25%)	24 (50%)	< 0.001	5 (42%)		13 (59%)		6 (50%)		
Pre-existing cardiovascular risks	0	38 (78%)		10 (77%)		17 (77%)		11 (79%)		
Concurrent aspirin	0	15 (31%)		5 (38%)		5 (23%)		5 (36%)		
Paraprotein (median, g/L)	–	8.0 (3.0–19.0)		2.0 (2.0–5.0)		10.7 (4.0–37.0)		17.0 (7.0–22.0)		
Basic investigations										
Prothrombin time (PT) (s), Mean (SD)	10.7 (0.8)	11.5 (1.0)	< 0.001	11.6 (1.2)	0.002	11.5 (1.0)	0.001	11.4 (0.9)	0.005	0.68
Activated partial thromboplastin time (APTT) (s), Mean (SD)	27.3 (3.5)	26.4 (3.9)	0.19	26.8 (3.4)	0.55	26.3 (4.5)	0.25	26.1 (3.6)	0.21	0.74
D-dimer (ng/ml FEU), median (IQR)	205 (140–350)	540 (290–880)	< 0.001	340 (260–420)	0.008	770 (390–2330)	< 0.001	505 (270–730)	< 0.001	0.048
Factor VIII (%),Mean (SD)	122.3 (35.5)	163.4 (54.2)	< 0.001	149.7 (39.1)	< 0.001	187.3 (57.8)	< 0.001	136.6 (45.9)	0.016	0.046
vWF antigen, Mean (SD)	128.7 (45.2)	154.6 (59.5)	0.014	141.2 (38.7)	0.023	188.5 (63.0)	< 0.001	113.9 (36.9)	0.91	0.020
Thromboelastography (TEG)										
R-time (mins), mean (SD)	6.4 (1.8)	4.9 (1.7)	< 0.001	4.5 (1.8)	0.002	5.2 (1.7)	0.018	4.8 (1.7)	0.005	0.26
K-time (mins), median (IQR)	2.2 (1.8–2.3)	1.6 (1.3–1.8)	< 0.001	1.5 (1.2–1.8)	0.006	1.4 (1.3–1.8)	0.002	1.7 (1.3–2.1)	0.041	0.83
α-angle (°), mean (SD)	60.4 (8.8)	60.2 (10.1)	0.89	64.5 (7.5)	0.14	58.9 (11.5)	0.56	58.0 (9.4)	0.38	0.13
Maximum amplitude (mm), mean (SD)	60.6 (5.7)	67.4 (8.0)	< 0.001	67.6 (6.6)	< 0.001	66.5 (10.2)	0.005	68.5 (5.5)	< 0.001	0.73
Clot lysis, LY30 (%),median (IQR)	0.5 (0.1–1.1)	0.0 (0.0–0.6)	0.003	0.0 (0.0–0.4)	0.030	0.0 (0.0–0.8)	0.024	0.1 (0.0–0.6)	0.053	0.84
LY30 (%) ≥ 0.5	20 (51%)	14 (29%)	0.036	3 (23%)	0.076	6 (29%)	0.090	5 (36%)	0.32	0.72
Calibrated automated thrombogram (CAT)										
Lag time (min), median (IQR)	3.3 (3.0–4.0)	3.2 (2.9–3.7)	0.099	3.4 (3.0–3.7)	0.93	3.5 (2.9–3.7)	0.54	3.0 (2.8–3.2)	0.004	0.64
Endogenous thrombin potential (ETP, nM.min), mean (SD)	1338.0 (230.0)	1420.6 (242.9)	0.078	1436.5 (160.6)	0.15	1378.2 (277.6)	0.32	1472.3 (251.9)	0.060	0.50
Peak (nM), mean (SD)	219.1 (63.1)	253.8 (58.7)	0.005	237.7 (45.8)	0.32	254.7 (56.5)	0.024	267.4 (72.0)	0.015	0.36
Velocity index (nM/min), mean (SD)	66.5 (30.9)	88.1 (37.5)	0.002	73.8 (34.7)	0.45	90.4 (35.3)	0.004	97.9 (42.9)	0.003	0.18
Overall haemostatic potential (OHP)										
Overall coagulation potential (%), mean (SD)	65.0 (10.3)	62.3 (13.8)	0.28	65.7 (14.8)	0.86	65.2 (14.5)	0.97	55.2 (9.3)	0.002	0.92
Overall haemostatic potential (%), median (IQR)	30.2 (27.1–37.1)	29.6 (25.1–38.0)	0.64	28.2 (27.5–42.7)	0.99	29.8 (24.8–38.1)	0.77	29.5 (24.5–37.3)	0.50	0.77
Overall fibrinolytic potential (%), mean (SD)	50.2 (8.2)	48.3 (9.1)	0.29	46.5 (12.0)	0.20	49.6 (8.5)	0.77	48.3 (7.0)	0.42	0.39

IQR: interquartile range; SD: standard deviation; ^13/22 patients were receiving chemotherapy at time of sample collection − bortezomib-based (n = 7), lenalidomide-based (n = 2), maintenance thalidomide (n = 1), melphalan-based (n = 1), carfilzomib (n = 1), PCAB (prednisolone-cyclophosphamide-doxorubicin-carmustine) (n = 1). 7/22 had chemotherapy commenced post sample collection including bortezomib-based (n = 4) and lenalidomide-based (n = 3) therapies.

* p-Value comparing MGUS to normal controls.

** p-Value comparing multiple myeloma to normal controls.

*** p-Value comparing smouldering myeloma to normal controls.

**** p-Value comparing MGUS to multiple myeloma

patients compared to controls are displayed in Table 1. Overall, the study patients demonstrated significantly higher factor VIII, von Willebrand's antigen and D-Dimer levels ($p < .05$) as well as hypercoagulable TEG (increased maximum amplitude ($p < .001$)) and CAT parameters (increased thrombin peak ($p = 0.005$) and velocity index ($p = 0.003$)). Fibrin generation parameters were comparable and did not appear to correlate with D-dimer or LY30.

Impact of disease status and treatment: Although myeloma patients had higher factor VIII levels ($p = 0.046$) and von Willebrand (vWF) antigen ($p = 0.02$) compared to MGUS, there were no significant differences across the GCA assays. Paraprotein levels did not significantly influence most of the GCA parameters apart from velocity index ($r = 0.33$). The heavy and light chain subtypes and treatment of myeloma also did not impact the GCA parameters although we note the majority of patients were on bortezomib-containing regimen.

Overall outcomes: Over a median follow-up period of 14 months, one patient was diagnosed with pulmonary embolism four months after commencement of PCAB therapy. Four patients suffered from cardiovascular events including three MGUS patients (2 myocardial infarction, 1 stroke) and one sMM patient (myocardial infarction). There were no significant differences between the MA ($p = 0.99$), thrombin peak ($p = 0.20$) and velocity index ($p = 0.30$) between those who had arterial events compared to patients who did not. However, the OHP was significant elevated (45.8 vs 32.0 units, $p = 0.012$) with reduced OFP (36.6 vs 49.4%, $p = 0.006$) in those who had arterial events. There were no recorded clinically significant bleeding. Eleven patients had progressive disease during the follow-up period. There were 3 disease-related deaths.

To the best of our knowledge, this pilot study provides the first evaluation of a combination of three global coagulation assays in patients with MGUS, smouldering myeloma and multiple myeloma. We have found that, overall, myeloma patients demonstrated hypercoagulable TEG and CAT parameters, consistent with our clinical experience showing increased thrombotic risk in this population. Interestingly, however, prothrombotic parameters were also identified in MGUS, suggesting that this relatively benign condition may be associated with increased thrombotic risk consistent with previous clinical studies [1].

We observed a significantly increased maximum amplitude ($p < .001$) in active myeloma and MGUS patients compared to normal controls, consistent with a study by Gracheva et al. [5]. In terms of thrombin generation, the peak thrombin and velocity index were increased in our study patients compared to normal controls, similar to previous studies [6,7]. We note that the predictive value of ETP vs thrombin peak in VTE risk, particularly in malignancy, remains unclear. One study demonstrated that myeloma patients with increased ETP were at higher risk of VTE [3] while the Vienna Cancer and Thrombosis Study of 1033 cancer patients found thrombin peak to be a better predictor [2]. The lack of difference in fibrin generation is in contrast to previously postulated mechanism that increased blood viscosity and immunoglobulin in myeloma may interfere with fibrin polymerisation and impair fibrinolysis [8]. However, despite the small numbers, the four patients who had arterial thrombosis demonstrated hypofibrinolysis (increased OHP with blunted response to tPA and reduced OFP), which is an important finding that may herald a potential biomarker to be considered for future risk stratification models.

One of the key findings was the lack of differences between MGUS and active myeloma, with both groups demonstrating prothrombotic TEG and CAT characteristics compared to normal controls. Crowley et al. [9] demonstrated that there was no difference in thromboelastography parameters between normal controls, MGUS and myeloma patients although they reported significantly higher peak thrombin generation in patients with myeloma and MGUS compared to normal controls [6]. The lack of correlation of paraprotein levels with the GCA parameters in our study is consistent with clinical studies demonstrating no significant correlation between the paraprotein level and DVT occurrence in myeloma patients [1] although other studies have shown that monoclonal protein > 16 g/L was associated with increased VTE risk [10].

In addition to disease biology, the treatment of myeloma such as immunomodulatory agents in combination with high-dose dexamethasone also increases the risk of VTE [8]. In the small proportion ($n = 13$) of myeloma patients receiving therapy, there was no difference when compared to those not receiving therapy. We acknowledge that our study is not powered to study the effect of myeloma treatment on the patients' coagulation state, however we note that previous studies also did not find significant differences in thrombin generation between these two groups [3,7].

We recognise that this is a pilot study with small numbers and heterogenous sampling of patients at different time points since their diagnosis of myeloma, smouldering myeloma or MGUS. Additionally, the low numbers of thromboembolic outcomes limited our ability to correlate the GCA parameters to VTE risk stratification. A strength of our study, however, lies in its novel study design which uses three different GCA assays to evaluate different aspects of the coagulation cascade. Our study also contributes to an area that is less well-documented and provides direction for future research. Our findings in combination with other studies collectively suggest the need to further investigate the role of GCA parameters in the assessment of hypercoagulability and clinical VTE risk. This may help further refine our clinical risk assessment model allowing clinicians to target individual patients who may benefit from primary thromboprophylaxis.

Declaration of competing interest

No conflict of interest to declare.

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