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A comparison of global coagulation assays between normal controls and patients with thrombocytopenia

(Global coagulation assays in thrombocytopenia)

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Conflict of interest

The authors have no conflict of interests to declare.

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Abstract

Introduction: Some patients with thrombocytopenia may be at risk of bleeding although quantitative platelet count is not always a sufficient predictive factor. Global coagulation assays such as thromboelastography (TEG[®]), calibrated automated thrombogram (CAT) and overall haemostatic potential (OHP) may provide a better assessment of an individual's haemostatic profile.

Methods: Blood samples were collected from thrombocytopenic patients. TEG[®] was performed on citrated whole blood, while CAT and OHP were performed on platelet-poor plasma. Results were compared to our previously collected normal controls.

Results: Fifty-eight participants (24 immune thrombocytopenia, 34 chemotherapy/malignancy-related) with mean age of 57.5 years were recruited. Compared to normal controls, thrombocytopenic participants had comparable maximum amplitude but reduced clot lysis (0.0% vs 0.6%; $p < 0.001$) on TEG[®] with reduced endogenous thrombin potential on CAT (1252.2 vs 1353.0nM.min; $p = 0.040$). No differences were seen in the OHP parameters. TEG[®] showed significant difference between marked and mild thrombocytopenia groups with minimal differences seen on CAT and OHP. Those with marked thrombocytopenia showed reduced maximum amplitude (47.2 vs 57.8mm; $p = 0.002$) as expected while participants with mild thrombocytopenia (platelet count $100\text{--}150 \times 10^9/\text{L}$) paradoxically demonstrated increased maximum amplitude (66.4 vs 57.8mm; $p < 0.001$).

Conclusion: Global coagulation assays, particularly TEG[®], can detect subtle differences in coagulation in thrombocytopenic patients. While patients with marked thrombocytopenia showed reduced maximum amplitude, patients with mild thrombocytopenia appear to paradoxically show increased maximum amplitude, suggesting compensatory activity within the coagulation pathway which may in part explain why not all thrombocytopenic patients have bleeding complications.

Keywords

Thrombocytopenia, thromboelastography, calibrated automated thrombogram, global coagulation assays, clot lysis

Introduction

Thrombocytopenia, particularly at markedly low levels, may raise concerns of increased bleeding risk. However, it is increasingly observed that platelet count in isolation is an imprecise predictor of bleeding as patients with similarly low platelet count can demonstrate differing tendencies in bleeding [1-3]. The recent Platelet Dose (PLADO) trial found that bleeding occurred over a wide range of platelet counts and that prophylactic platelet transfusion did not prevent bleeding. The authors suggested that other factors might be involved including laboratory predictors such as haematocrit $\leq 25\%$ and coagulation abnormalities [1]. Furthermore, there have been reports of patients, particularly in immune thrombocytopenia (ITP), who appear to be at risk of thrombotic events despite marked thrombocytopenia [4-6]. This suggests that there are other factors within the coagulation system, which are contributing to both haemostasis and thrombosis. Potential reasons may include increased platelet function and procoagulant factors [2, 7, 8], although the underlying pathophysiology remains multifactorial and elusive.

Haemostasis is a complex interplay between various components of the coagulation pathway, platelets as well as endothelial function and blood flow. Hence, a single unidimensional assessment, such as platelet count, prothrombin time (PT) or activated partial thromboplastin time (APTT), is insufficient to predict the risk of bleeding. Furthermore, current available coagulation studies (PT/APTT) are not specifically designed to evaluate bleeding and clotting risk [9-10]. Global coagulation assays may provide a more holistic evaluation of coagulation, particularly in patients with thrombocytopenia, by taking into account other important aspects of the haemostatic system. These assays include thromboelastography (TEG[®]), which evaluates the entire coagulation process in whole blood including clot formation and fibrinolysis [11], calibrated automated thrombogram (CAT), which assesses the generation of thrombin [12] and overall haemostatic potential (OHP), a measure of fibrin generation [13].

While not clinically validated, current literature suggests that TEG[®] and CAT may be useful for assessing coagulation in thrombocytopenic patients [14-18]. One study looked at the utility of rotational thromboelastometry (ROTEM) to test haemostasis in ITP patients and found that ROTEM was useful in evaluating haemostasis in individual patients [18]. Furthermore, ROTEM has been shown to be able to guide transfusion

choices in acute obstetric haemorrhage [19]. Hence, the addition of global coagulation assays to standard testing will allow for additional assessment of the other components of the haemostatic process, which may help refine the management of thrombocytopenic patients. Data on the use of global coagulation assays to evaluate thrombocytopenia is lacking and to the best of our knowledge, no studies have utilised all the three assays concurrently to evaluate this population. These assays, when used in combination, may provide further insights into the complexity of the haemostatic system.

Hence, we propose evaluating the effect of thrombocytopenia on all three assays (TEG[®], CAT and OHP) in comparison to the normal controls, while also examining the differences between different aetiologies of thrombocytopenia.

Materials and methods

Thrombocytopenic participants (defined as platelet count $< 150 \times 10^9/L$) aged 18 years and older were recruited from the Northern Hospital in Melbourne, Australia. Participants on anticoagulation were excluded. Basic demographic details including age, sex, cardiovascular disease profile and aetiology of thrombocytopenia were recorded.

Baseline laboratory investigations for thrombocytopenia were performed, including full blood count, liver function tests, biochemistry, coagulation studies, D-dimer, von Willebrand studies and Factor VIII level. TEG[®] analysis was performed on citrated whole blood within 4 hours of sample collection and the remaining citrated blood was double spun at 2500G to obtain platelet poor plasma (PPP) and stored at $-80^{\circ}C$ within 2 hours of sample collection for batch testing with the CAT and OHP.

Normal control population

The normal control population data used ($n = 97$, 31 males, 66 females, median age 43.7 years) were from the original study performed by our research group [20]. However, for comparison to this study, 6 normal control participants were removed from analysis as they had platelet count less than $150 \times 10^9/L$ or had platelet clumping.

Coagulation studies, D-dimer, von Willebrand studies and factor VIII

Automated coagulation analysers are used for performing coagulation studies on citrated platelet poor plasma in accordance to laboratory haematology reference manual. The Siemens Dade Behring Innovin assay is used to measure prothrombin time (PT) while the Siemens Dade Actin FSL APTT Kit is used for the measurement of activated partial thromboplastin time (APTT). To measure factor VIII level, a mixture of factor VIII deficient plasma and the patient plasma is tested in the APTT assay and the result is interpreted using a reference curve obtained with dilutions of standard human plasma mixed with deficient plasma. The Clauss fibrinogen method is used for the measurement of fibrinogen. D-dimer is measured on citrated platelet poor plasma using the INNOVANCE[®] D-Dimer assay with the Siemens CS2100i analyser as per manufacturer's guidelines. The INNOVANCE[®] D-Dimer is a particle-enhanced, immunoturbidimetric assay for the quantitative determination of cross-linked fibrin degradation products (D-dimer). The Siemens von Willebrand antigen (vWF:Ag) assay is based on the change in turbidity of a microparticle suspension that is measured by photometry while the immunoturbidimetric Siemens INNOVANCE von Willebrand factor activity assay makes use of the binding of von Willebrand factor to its receptor glycoprotein-1b.

Thromboelastography (TEG[®]) assay

All samples underwent computerized TEG[®] analysis (TEG[®] 5000; Haemonetics) using the citrated kaolin assay as per the manufacturer's guidelines. TEG[®] parameters including R-time (min), K-time (min), maximum amplitude (MA), α -angle ($^{\circ}$) and LY30 (%) were recorded.

Calibrated automated thrombogram (CAT) assay

The calibrated automated thrombogram (CAT, Diagnostica Stago) determines the rate and extent of thrombin generated after a tissue factor stimulus using thawed PPP, as per the manufacturer's guidelines. Parameters of thrombin generation, including lag time (min), endogenous thrombin potential (ETP; nM.min), peak thrombin (nM), time to peak (ttPeak; min) and velocity index (nM/min) were calculated.

Overall haemostatic potential (OHP) assay

This is a spectrophotometric assessment of fibrin-aggregation in PPP, which provides the details of underlying changes in coagulation and fibrinolysis [13]. For each sample,

there is a corresponding overall coagulation potential (OCP) buffer and an overall haemostatic potential (OHP) buffer consisting of tissue-plasminogen activator. Using the FLUOstar® Optima (BMG Labtech) plate reader operating at 37°C, the minute-by-minute absorbance at 405nm is recorded to construct two fibrin-aggregation curves (OCP and OHP) – the difference between the two areas under the curve reflects the overall fibrinolysis potential (OFP).

Statistical analysis

Statistical analysis was performed on both Microsoft Excel and IBM® SPSS Statistics 23 (IBM Corp, Armonk, New York, USA). Comparisons of the global coagulation assay parameters were made between thrombocytopenic and normal controls, as well as between the different aetiologies (ITP vs chemotherapy-related/cancer patients). Normality of parameters was assessed using the Shapiro-Wilk test, with analysis conducted with independent sample t-tests or Mann-Whitney U tests. Chi squared tests were used to analyse categorical variables. Pearson and Spearman's correlation coefficient were calculated to assess for correlation. Multivariate analysis with binary logistic regression was conducted to assess for the effect of age and gender. Statistical significance was set at $p < 0.05$.

This study was approved by the Human Research Ethics Committee - Austin Health (HREC/16/Austin/460). Written informed consent were obtained from all participants.

Results

Demographics and Baseline characteristics

A total of 58 participants with mean age 57.5 years were recruited (30 males, 28 females), of which there were 34 chemotherapy-related or malignancy related (CM) thrombocytopenic patients (27 haematological and 7 solid organ malignancies), and 24 ITP patients. Demographics and baseline laboratory investigations for thrombocytopenic patients compared to normal controls are displayed in Table 1. The thrombocytopenic group was older with lower haemoglobin count and had more patients with positive D dimer, which may in part be due to a subset of thrombocytopenic patients having malignancy. Interestingly, the thrombocytopenic group showed slightly prolonged prothrombin time with higher factor VIII levels as well

as increased von Willebrand factor antigen levels. The fibrinogen level was comparable between both groups ($p=0.343$).

Overall effects of thrombocytopenia on global coagulation assays

(i) *Thromboelastography (TEG®)*: The mean and median values of the TEG® parameters are described in Table 2. Overall, no difference in maximum amplitude (MA, 59.3 vs 57.8 mm, $p=0.376$) or other parameters were seen apart from α -angle and lysis 30 (LY30). Interestingly, LY30 was significantly decreased in the thrombocytopenic cohort (0.00% vs 0.60%, $p<0.001$) and the significance remained on multivariate analysis ($p<0.001$), accounting for age and gender. LY30 did not appear to correlate with fibrinogen levels ($r = -0.171$, $p=0.212$), abnormal D-dimer ($\chi^2(1) = 0.132$, $p=0.778$), factor VIII levels ($r = 0.167$, $p=0.24$) or von Willebrand factor antigen ($r = -0.091$, $p=0.53$).

(ii) *Calibrated automated thrombogram (CAT)*: Overall, the thrombocytopenic cohort displayed significantly increased lag time (3.7 vs 3.1 min, $p<0.001$) and decreased ETP (1252.2 vs 1353.0 nM.min, $p=0.040$) compared to normal controls (Table 2).

(iii) *Overall haemostatic potential (OHP)*: There was no significant difference shown on fibrin generation between the thrombocytopenic cohort when compared to normal controls (Table 2).

Table 2

Impact of severity of thrombocytopenia on global coagulation assays

Table 3 shows the breakdown between the different severities of thrombocytopenia and how this may impact on global coagulation assays. A sub-analysis of severely thrombocytopenic patients (platelet count $< 50 \times 10^9/L$) showed hypocoagulability, when compared to patients with mild thrombocytopenia as evaluated by TEG, with reduced α -angle (41.2° vs 57.9° , $p=0.001$) and MA (47.2 vs 57.8 mm, $p=0.002$) as well as corresponding reduced LY30 (0.00% vs 0.60%, $p<0.001$) observed. Of note, two patients with platelet count $14 \times 10^9/L$ and $42 \times 10^9/L$ had an MA above the mean of normal control, 65.8 mm and 60.3 mm respectively. Both patients were receiving chemotherapy. Interestingly and paradoxically, participants with platelet count $100\text{--}150 \times 10^9/L$ demonstrated more hypercoagulable TEG parameters compared to normal controls, with increased MA (66.4 vs 57.8 mm, $p<0.001$) and decreased LY30 (0.00% vs 0.60%, $p=0.003$). The difference in MA between different severities of

thrombocytopenia is represented in Figure 1. MA did not correlate with factor VIII levels ($r^2=0.028$), fibrinogen ($r^2=0.164$) or von Willebrand antigen levels ($r^2=0.009$). In addition, the severity of thrombocytopenia also did not appear to significantly influence factor VIII levels (mild thrombocytopenia – average 124.9% vs moderate thrombocytopenia – average 187.4% vs marked thrombocytopenia – average 155.9%; $r^2=0.239$) or von Willebrand antigen levels (mild thrombocytopenia – average 196.0% vs moderate thrombocytopenia – average 222.3% vs marked thrombocytopenia – average 176.8%; $r^2=0.002$).

Figure 1

On the other hand, severity of thrombocytopenia did not appear to significantly influence the majority of the thrombin and fibrin generation parameters although patients with mild thrombocytopenia showed a trend towards increased fibrin generation ($p=0.081$) and fibrinolysis ($p=0.023$) with otherwise preserved OFP. Of note, there were no significant differences seen in the derived fibrinogen levels across the three categories of severities (3.30 (mild) vs 3.13 (moderate) vs 3.45 (severe)).

Table 3

Effect of ITP and Chemotherapy/Malignancy Cohorts on global coagulation assays

There were no significant differences seen between the two aetiologies across global coagulation says. The TEG parameters were largely comparable between patients with ITP ($n=24$; average platelet count $82.2 \pm 38.8 \times 10^9/L$) and CM patients ($n=24$; average platelet count $77.6 \pm 41.1 \times 10^9/L$) apart from clot lysis time – R-time (6.9 vs 7.2 min, $p=0.136$), α -angle (55.2 vs 51.5, $p=0.661$), MA (60.8 vs 58.3, $p=0.427$) and LY30 (0.1% vs CM 0.0%, $p=0.018$). The aetiology of thrombocytopenia also did not affect the CAT parameters significantly – lag time (3.6 vs 3.7 min, $p=0.608$), ETP (1301.5 vs 1217.0 nM.min, $p=0.322$), thrombin peak (236.5 vs 209.7 nM, $p=0.238$) and velocity index (74.1 vs 66.0 nM/min, $p=0.242$). Similarly, there was no difference seen in OHP parameters between the groups and there was no correlation between OHP parameters and D-dimer levels.

Discussion

Management of thrombocytopenia remains a clinical challenge, particularly in modulating bleeding risk, with various platelet count thresholds being recommended for different disease indications [21-23]. This pilot study demonstrates the utility of global coagulation assays (TEG®, CAT, OHP) in detecting subtle differences in coagulation

parameters in the thrombocytopenic population. This may help provide clinicians with additional tools in managing the complex risk assessment of these patients. To our knowledge, this is also the first study to use all three assays in combination to compare between different aetiologies of thrombocytopenia.

The patients with severe thrombocytopenia (platelet count $< 50 \times 10^9/L$) demonstrated both reduced maximum amplitude and endogenous thrombin potential, suggesting an overall hypocoagulable state. Interestingly, two patients with severe thrombocytopenia had MA above the mean for the normal controls suggesting variability in response to thrombocytopenia, both of whom were receiving chemotherapy. This is consistent with our clinical experience of increased bleeding risk in patients with platelet count $< 50 \times 10^9/L$ and may also explain thrombotic complications which occur despite severe thrombocytopenia [6]. We note with interest that lysis time, or clot breakdown at 30mins using TEG[®], was significantly reduced compared to the normal population, which may in part explain the preservation of fibrin generation in patients with marked thrombocytopenia (30.0 vs 28.7, $p=0.50$).

Previous clinical studies have demonstrated hypocoagulable parameters (particularly reduced maximum amplitude) [15, 24] in patients with severe thrombocytopenia despite no significant differences in PT and APTT, which is similar to our TEG[®] experience. However, the data for thrombin generation is less clear with Álvarez-Román et al. [18] observing an elevated ETP (1696.4 vs 1223.8, $p<0.05$) in 42 chronic ITP patients with platelet count $< 50 \times 10^9/L$, in contrast to our ITP patients with marked thrombocytopenia. One possible explanation for this discrepancy is the small number of ITP patients with severe thrombocytopenia ($n=5$) in our pilot study. Álvarez-Román et al. also reported increased amount of platelet- and red cell-microparticles with microparticles-associated thrombogenic ability inversely correlated with platelet count as well as increased resistance to protein C through performing CAT with and without thrombomodulin. This study also reported augmented levels of plasminogen activator inhibitor-1 (PAI-1) and E-selectin, which may reflect endothelial damage in ITP [18]. In contrast to the TEG performed in our study, the authors performed rotational thromboelastometry (ROTEM) on their patients, which coincidentally also showed increased maximum clot firmness and reduced lysis, suggesting complex and paradoxical interactions in the coagulation system, which require further evaluation.

In addition, to the best of our knowledge, this is the first study to evaluate thrombocytopenia using OHP assays. The OHP assays use an excess of thrombin to evaluate fibrin generation as well as the fibrinolytic breakdown capacity in the setting of tissue plasminogen activator (tPA). Interestingly, thrombocytopenia does not appear to impact on the final fibrin generation pathway, which may explain the continued manifestation of thrombotic complications, particularly venous thrombosis, despite severe thrombocytopenia, potentially if there are situations where excess thrombin is generated [6]. Álvarez-Román et al. reported that clot formation in severe ITP may be more resistant to fibrinolysis due to augmented levels of (PAI-1) although there were no differences observed in other measured fibrinolysis-related proteins (tPA, urokinase plasminogen activator (uPA), thrombin activatable fibrinolysis inhibitor (TAFI)) [18].

Intriguingly, the picture in patients with mild to moderate thrombocytopenia ($50\text{--}149 \times 10^9/\text{L}$) is also significantly different. In this subgroup, their MA is actually markedly higher compared to normal controls (62.7 vs 57.8 mm, $p < 0.001$), with patients with mild thrombocytopenia having the highest MA (mean 66.4 mm). These results were similar to a study by Roeloffzen et al. [15] which observed a trend, albeit a non-significant one, towards more hypercoagulable TEG[®] parameters in patients on with platelets $\geq 100 \times 10^9/\text{L}$ on consolidation chemotherapy. Hypercoagulable parameters are also seen with fibrin generation with increased OHP levels. These results may represent overcompensation occurring in mild to moderate thrombocytopenia and may also reflect the underlying pathophysiology causing the individual's thrombocytopenia. However, it does highlight the need to individually assess thromboprophylaxis in this subgroup of patients if clinically required, rather than to be driven by just platelet count.

Another unique finding in this study is LY30, which has seldom been investigated in the literature. LY30, which reflects clot breakdown at 30 mins using TEG[®] was markedly decreased compared to normal controls (0.00 vs 0.60, $p < 0.001$) and did not appear to significantly correlate with fibrinogen or D-dimer levels. Interestingly, all but two patients with marked thrombocytopenia did not have recordable LY30. This finding is contrary to the hypocoagulable state, which typifies thrombocytopenia, though the exact pathophysiology for this phenomenon is unclear. This reduction in clot lysis could be indicative of a compensatory mechanism in response to thrombocytopenia and may explain why not all thrombocytopenic patients experience bleeding diatheses. Moreover, this could also explain the preserved fibrin generation parameters seen.

However, we note that Katori et al had previously performed TEG on platelet-poor plasma (PPP) and platelet-rich plasma (PRP) with an increasing platelet count (range 50 – 1200 × 10⁹/L) with or without abciximab and found that the clot lysis increased with higher platelet counts but not in the abciximab samples [25]. Morphological changes of clots were also examined in this study and it was noted that clot retraction was observed in the PRP but not in PPP or PRP pre-treated with abciximab, which led to the conclusion that the clot lysis may be due to platelet-mediated clot retraction [25]. This may explain the lower lysis in our cohort, due to artefactual impact of clot retraction. However, this does not explain reduced lysis seen in older normal controls, not on any antiplatelet agents [20] nor the lack of impact of aspirin on clot lysis in patients with myeloproliferative neoplasm [26]. Hence, given the contradictory findings, further clinical and laboratory studies are warranted, including assessment of other fibrinolytic markers to explore the significance of this observation, as an underlying compensatory mechanism should not be excluded.

We recognise that this is a pilot study with a small sample size (n=58) and as such multivariate analysis of subgroup and comparison between the chemotherapy and ITP subgroup was not possible. Hence, the contribution of underlying aetiology on the global coagulation assays could not be fully evaluated. Moreover, the number of patients with severe thrombocytopenia was also small hence, differences in global coagulation assays, particularly thrombin generation, may not have been demonstrable. Nonetheless, this study does highlight significant differences in global coagulation assays between thrombocytopenic patients and normal controls. Specifically, differences seen with lysis and fibrin generation parameters have not been previously described. Hence, we propose a larger study, including functional platelet assays, to further validate these data.

Conclusion

This pilot study has demonstrated that global coagulation assays can identify unique differences in coagulation between normal and thrombocytopenic populations, and maybe useful for risk assessment in thrombocytopenic patients. The presence of hypocoagulable parameters in patients with marked thrombocytopenia confirms our clinical paradigm of classifying these patients as high bleeding risk. However, the increased thrombin generation and TEG parameters seen in the mild to moderate

thrombocytopenia, together with preserved fibrin generation and reduced clot lysis, suggests that there are other mechanisms and interactions that regulate overall thrombotic risk, regardless of platelet count. Further clinical studies to evaluate the role of global coagulation assays are warranted.

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Table 1. Demographics and baseline laboratory investigations for both control and thrombocytopenic groups. Normal ranges for tests as indicated.

Table 2. Global coagulation parameters of the overall thrombocytopenic cohort in comparison to normal controls

Table 3. Global coagulation parameters comparing different severities of thrombocytopenic cohort to normal controls

Figure 1. Patients with mild thrombocytopenia shows paradoxically higher maximum amplitude compared to normal control ($p < 0.001$) while those with severe thrombocytopenia shows reduced maximum amplitude ($p = 0.002$)

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Tables

Table 1. Demographics and baseline laboratory investigations for both control and thrombocytopenic groups. Normal ranges for tests as indicated.

	Normal range	Control	Thrombocytopenic	p-value
Number		91	58	
Age, Mean $\pm$ SD		43.1 $\pm$ 16.8	57.5 $\pm$ 18.7	<0.001
Female, n (%)		63 (69.1)	28 (48.3)	0.008
Haemoglobin (g/L), Median (IQR)	115 – 165	145.4 (136.0 – 159.0)	126.5 (91.8 – 139.0)	<0.001
Platelet count ($\times 10^9$ /L), Mean $\pm$ SD	150 – 450	244.7 $\pm$ 43.8	79.8 $\pm$ 39.7	<0.001
White cell count ($\times 10^9$ /L), Median (IQR)	4.0 – 11.0	5.7 (5 – 6.6)	5.2 (3.5 – 7.6)	0.105
Neutrophils ($\times 10^9$ /L), Median (IQR)	2.0 – 8.0	3.1 (2.4 – 4.0)	3.2 (1.5 – 4.6)	0.442
Prothrombin time (PT) (s), Median (IQR)	9.0 – 13.0	10.8 (10.0 – 11.0)	11.4 (10.9 – 11.9)	<0.001
Activated partial thromboplastin time (APTT) (s), Median (IQR)	25.0 – 35.0	27.5 (25.7 – 30.0)	27.2 (25.2 – 27.2)	0.709
Fibrinogen (g/L), Median (IQR)	2.0 – 4.0	2.9 (2.5 – 3.4)	3.3 (2.3 – 4.0)	0.343
D-dimer (ng/ml FEU), normal (N)	<500	80 (87.9%)	21 (36.2%)	<0.001
D-dimer (ng/ml FEU), abnormal (N)		11 (12.1%)	37 (63.8%)	
Factor VIII (%), Median (IQR)	50 – 200	101.1 (85.5 – 130.0)	144.0 (117.0 – 185.0)	<0.001
von Willebrand factor (vWF) antigen between 50 – 200, n (%)	50 – 200	89 (97.8%)	34 (58.6%)	<0.001
vWF Ag over > 200, n (%)		2 (2.2%)	24 (41.4%)	
Sodium (mmol/L), Median (IQR)	135 – 145	141.0 (139.0 – 142.0)	140.0 (139.0 – 141.0)	0.012
Potassium (mmol/L), Mean $\pm$ SD	3.5 – 5.2	4.3 $\pm$ 0.26	4.2 $\pm$ 0.41	0.080
Creatinine (μ mol/L), Median (IQR)	45 – 90	67.0 (59.0 – 80.0)	76.5 (56.8 – 89.5)	0.163
Estimated glomerular filtration rate (eGFR) (mL/min/1.73m ²)	≥ 90	68 (74.7%)	32 (55.2%)	0.009
	60 – 89	20 (22.0%)	14 (24.1%)	
	30 – 59	3 (3.3%)	9 (15.5%)	
	15 – 29	0	2 (3.4%)	
	< 15	0	1 (1.7%)	
Albumin (g/L), Median (IQR)	35 – 50	42.0 (40.0 – 44.0)	36.0 (33.0 – 39.0)	<0.001
Alkaline phosphatase (ALP) (U/L), Median (IQR)	40 – 130	74.0 (57.8 – 84.0)	81.0 (59.0 – 111.3)	0.029
Bilirubin (unit), Median (IQR)	< 25	9.0 (7.0 – 13.0)	11.0 (8.0 – 16.3)	0.045
Gamma-glutamyl transferase (GGT) (U/L), Median (IQR)	< 51	18.0 (13.0 – 26.0)	32.0 (14.0 – 87.3)	0.001
Alanine aminotransferase (ALT) (U/L), Median (IQR)	< 41	23.0 (18.0 – 32.3)	27.5 (15.8 – 38.3)	0.258

IQR = interquartile range; SD = standard deviation

Table 2. Global coagulation parameters of the overall thrombocytopenic cohort in comparison to normal controls

	Normal Control	Thrombocytopenic	p-value
Thromboelastography (n)	63	57	
R-time (mins), median (IQR)	7.1 (5.8 – 8.6)	7.1 (5.8 – 8.4)	0.989
K-time (mins), median (IQR)	2.3 (1.9 – 2.9)	2.1 (1.7 – 3.2)	0.552
α -angle ($^{\circ}$), median (IQR)	57.8 (50.8 – 62.7)	53.5 (42.3 – 62.2)	0.036
Maximum amplitude (mm), mean (SD)	57.8 $\pm$ 5.9	60.3 $\pm$ 11.4	0.364
Clot lysis, LY30 (%), median (IQR)	0.60 (0.10 – 2.25)	0.00 (0.00 – 0.10)	<0.001
Calibrated automated thrombogram (n)	90	48	
Lag time (min), median (IQR)	3.1 (2.7 – 3.7)	3.7 (3.3 – 4.3)	<0.001
Endogenous thrombin potential (ETP, nM.min), mean (SD)	1354.8 $\pm$ 261.2	1252.2 $\pm$ 288.4	0.036
Peak (nM), mean (SD)	223.0 $\pm$ 67.0	220.9 $\pm$ 76.9	0.864
Velocity index (nM/min), median (IQR)	65.0 (45.2 – 99.9)	72.4 (45.0 – 98.9)	0.394
Overall haemostatic potential (n)	89	53	
Overall coagulation potential (%), mean (SD)	59.5 $\pm$ 11.9	60.4 $\pm$ 13.4	0.610
Overall haemostatic potential (%), mean (SD)	28.7 $\pm$ 6.5	31.1 $\pm$ 9.8	0.086
Overall fibrinolytic potential (%), mean (SD)	50.5 $\pm$ 8.2	48.0 $\pm$ 11.6	0.154

IQR = interquartile range; SD = standard deviation

Table 3. Global coagulation parameters comparing different severities of thrombocytopenic cohort to normal controls

	Normal Control	Thrombocytopenia					
		Platelet < 50 x 10 ⁹ /L	p-value*	Platelet 50 - 100 x 10 ⁹ /L	p-value**	Platelet > 100 x 10 ⁹ /L	p-value***
Thromboelastography (n)	63	13		27		17	
R-time (mins), median (IQR)	7.1 (5.8 – 8.6)	7.8 (6.6 – 9.9)	0.237	7.1 (6.6 – 8.3)	0.709	6.2 (5.2 – 7.6)	0.268
K-time (mins), median (IQR)	2.3 (1.9 – 2.9)	3.4 (2.4 – 4.9)	0.002	2.1 (1.8 – 3.1)	0.712	1.7 (1.5 – 2.1)	<0.001
α-angle (°), median (IQR)	57.8 (50.8 – 62.7)	41.2 (30.6 – 53.0)	0.001	52.3 (42.7 – 60.8)	0.025	63.7 (57.6 – 67.6)	0.051
Maximum amplitude (mm), mean (SD)	57.8 ± 5.9	47.2 ± 9.8	0.002	60.6 ± 9.9	0.176	66.4 ± 6.5	<0.001
Clot lysis, LY30 (%), median (IQR)	0.60 (0.10 – 2.25)	0.0 (0.00 – 0.00)	<0.001	0.0 (0.00 – 0.40)	<0.001	0.00 (0.00 – 0.45)	0.003
Calibrated automated thrombogram (n)	90	11		22		15	
Lag time (min), median (IQR)	3.1 (2.7 – 3.7)	4.0 (3.0 – 5.4)	<0.001	3.7 (3.3 – 4.1)	0.012	4.0 (3.2 – 4.7)	0.005
Endogenous thrombin potential (ETP, nM.min), mean (SD)	1354.8 ± 261.2	1184.0 ± 358.7	0.053	1280.0 ± 277.5	0.237	1261.1 ± 258.4	0.201
Peak (nM), mean (SD)	223.0 ± 67.0	209.2 ± 97.7	0.542	237.7 ± 76.0	0.372	204.7 ± 59.2	0.323
Velocity index (nM/min), median (IQR)	65.0 (45.2 – 99.9)	50.4 (43.3 – 87.0)	0.731	88.8 (53.7 – 113.9)	0.069	66.1 (44.5 – 93.3)	0.394
Overall haemostatic potential (n)	89	11		26		16	
Overall coagulation potential (%), mean (SD)	59.5 ± 11.9	59.9 ± 16.0	0.816	57.5 ± 14.0	0.735	65.1 ± 10.4	0.066
Overall haemostatic potential (%), mean (SD)	28.7 ± 6.5	30.0 ± 8.4	0.502	30.2 ± 10.4	0.377	33.3 ± 11.1	0.024
Overall fibrinolytic potential (%), mean (SD)	50.5 ± 8.2	48.8 ± 10.4	0.246	46.8 ± 13.1	0.183	49.2 ± 11.4	0.611

* p-value compares normal controls to patients with platelet <50 x 10⁹/L

** p-value compares normal controls to patients with platelet 50 – 100 x 10⁹/L

*** p-value compares normal controls to patients with platelet $>100 - <150 \times 10^9/L$

IQR = interquartile range; SD = standard deviation

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Figures

Figure 1. Patients with mild thrombocytopenia shows paradoxically higher maximum amplitude compared to normal control ($p<0.001$) while those with severe thrombocytopenia shows reduced maximum amplitude ($p=0.002$).

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