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Letter to the Editors-in-Chief

Significant age, race and gender differences in global coagulation assays parameters in the normal population

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Sir,

Thrombosis and cardiovascular disease are major causes of morbidity and mortality. While there are well-established clinical risk factors for thrombosis and cardiovascular disease, including increasing age and male gender amongst others, the risk of thrombotic events still varies significantly. However, despite advancement of research in thrombosis and haemostasis, there currently remain no laboratory tests to evaluate this risk. Global coagulation assays such as thromboelastography (TEG®) and thrombin generation via the calibrated automated thrombogram (CAT) may be a better surrogate measure of an individual's thrombosis risk.

The influence of age and gender on thrombosis is well known with both these factors being independent risks for cardiovascular disease [1]. Similarly, individuals of East Asian origin (Chinese and Japanese) have been reported to have a significantly lower risk of venous thromboembolism [2], though recent literature have challenged this perception particularly in the post-operative setting [3]. Likewise, rates of cardiovascular disease are significantly higher in the South Asian population [4], but not in the East Asian population when compared to the Caucasian population [5]. Unfortunately, these differences in thrombotic risk is not reflected in routine coagulation testing [6].

Hence, we performed a study evaluating CAT and TEG in a normal population and evaluated specifically the impact of age, gender and race on these assays.

1. Materials & methods

Normal controls, aged 18 to 80 years, currently not on anticoagulation and without history of thrombotic disease, were prospectively recruited. Volunteers on hormone replacement therapy, anticoagulant, antiplatelet agents, or with abnormal blood test results on routine screening were excluded. Patients with active cardiovascular risk factors, such as hypertension, diabetes and hypercholesterolaemia, on medical therapy were also excluded.

Blood was obtained using standard venipuncture techniques using a 21G needle and butterfly. Normal laboratory tests to exclude underlying thrombosis risk factors were performed; these included a full blood count, biochemistry, standard coagulation testing, thrombophilia screen (factor V Leiden, prothrombin gene mutation, protein C, protein S, antithrombin III), von Willebrand studies, fasting lipids and glucose.

1.1. TEG® assay

All samples were tested using citrated kaolin assay using manufacturer suggested guidelines and underwent computerized TEG® analysis (TEG® 5000; Haemonetics) within 4 h of sample collection. Routine TEG parameters including R (mins), K (mins), maximum amplitude (MA), α-angle (°) and LY30 (%) were recorded.

1.2. CAT assay

80 μL of platelet-poor plasma was manually dispensed into a 96 well-microtitre plate, together with 20 μL of PPP standard reagent (5pM Tissue factor reagent). The starting reagent FluCA Kit (Diagnostica Stago) containing 20 μL of fluorogenic substrate (Z-Gly-Gly-Arg-AMC HCl)/calcium chloride buffered solution at 37 °C was automatically dispensed by the fluorometer (Thermo Fluroskan Ascent). A dedicated software program, Thrombinoscope BV (Diagnostica Stago), was used to calculate thrombin activity against the calibrator and display thrombin activity versus time. Parameters of thrombin generation, including endogenous thrombin potential (ETP), thrombin peak, velocity index, lag time, were derived using CAT.

1.3. Statistical analysis

Statistical analyses were performed using Microsoft Excel and Stata version 14.0 (StataCorp, College Stations, Texas, USA). Comparisons between genders, ethnicity and age, dichotomised at 50 years of age, were conducted using *t*-tests or Analysis of Variance (ANOVA) for those variables which were normally distributed. Mann-Whitney (rank-sum) or Kruskal-Wallis tests were performed for those variables found to be non-normally distributed. Categorical variables were assessed using the Chi-squared test or Fishers Exact test on occasions where frequencies in groups were fewer than 5.0. The differences in gender, ethnicity and age were also assessed via multivariate analysis, to determine the independent associations with the TEG and CAT variables. Pearson or Spearman correlation coefficients were estimated for the comparison between selected TEG and CAT variables. Statistical significance was set at $p < 0.05$.

This study was approved by the Human Research Ethics Committee - Austin Health (H2013/04977) and Northern Health (P5/13).

2. Results

Ninety-seven normal volunteers, 31 males and 66 females, with a median age of 44 years (range: 20–79), were recruited, of which 64 volunteers (21 males, 43 females) had assessment for both CAT and TEG parameters. All volunteers had a normal coagulation profile and platelet count, and no other significant blood test abnormality.

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2.1. Gender differences (Table 1)

Overall, the female population had a more prothrombotic profile for both CAT and TEG parameters, including a higher ETP and MA. On multivariate analysis, including correction for age and ethnic differences, these statistically significant differences between genders were maintained.

2.2. Effect of Age (Table 1)

The older age group (age >50 years) had more hypercoagulable parameters across all TEG parameters, but with minimal differences on CAT parameters. On multivariate analysis, age was not found to have a statistical significant association with R time or maximum amplitude, after adjusting for gender, but other statistical differences by age group were maintained. Lysis time was significantly lower in the older population ($p = 0.001$) on multivariate analysis with 27/31 (87%) of those >50 compared to 24/37 (64%) having LY30 <2% ($p = 0.03$). Low LY30 did not correlate with other risk factors.

2.3. Effect of Race (Table 2)

East Asian persons, defined as ethnic Chinese in this cohort, were found to have less prothrombotic CAT parameters including a lower ETP than both Caucasian and "Other Asian". No difference between ethnicity and any TEG parameters were seen after adjusting for age and gender. Other differences seen were longer aPTT as well as lower protein C and S levels. While the numbers of "Other Asian" persons were small, they also had significantly higher ETP compared to Caucasians ($p = 0.004$).

3. Discussion

This study documents the normal range for CAT and TEG parameters in the normal multicultural Australian population. Importantly, it also highlights significant variation in global coagulation assays parameters with basic epidemiological factors such as age, gender and race being major contributors. Our results confirms the previously published association [7,8] between the female gender and more hypercoagulable TEG parameters. Females showed more prothrombotic TEG and CAT parameters, with the exception of lag time, which may explain the lack of difference seen with routine PT and aPTT, which also estimates the time to the start of clot formation. Importantly, our study excluded women on the oral contraceptive pill (OCP) or hormone replacement, as these have been associated with increase in absolute clot strength and also VTE risk [8]. However, these findings are contradictory to clinical epidemiological data, which consistently show that women not on hormonal replacement are at lower thrombotic and cardiovascular risk compared to their male counterparts. Hence, further studies to evaluate the rationale behind this contradiction are warranted.

Similarly, older individuals aged above 50 years had more hypercoagulable TEG® parameters than their younger counterparts affecting all TEG parameters including a greater MA consistent with prior TEG® [8] and ROTEM studies [9]. However, interestingly, these differences were not apparent with CAT parameters with the exception of lag-time, which was conversely longer in the older population (Table 1). However, the preservation of platelet-poor CAT parameters, despite substantial differences seen with TEG parameters, may suggest that platelets and other factors independent of the coagulation cascade, play a more significant role in age-related thrombosis.

Interestingly, LY30, a marker of clot breakdown, was significantly reduced in the older population ($p = 0.001$ on multivariate analysis) and was not associated with D-dimer or fibrinogen levels and independent of gender and race (Tables 1, 2). We note that a number of studies have evaluated fibrinolytic markers such as tissue type plasminogen activator (TPA), plasmin activator inhibitor (PAI-1) and D-dimer, but the results remain conflicting and the utility of the fibrinolytic markers remains limited [10]. One reason could be that many of these biomarkers are end-products of fibrinolysis, and do not take into account in-vivo thrombin/fibrin generation nor the effects of platelets and leukocytes in both clot formation and destruction. Our study finding suggests a potential important role of clot stability in thrombotic risk and highlights the need for further investigation.

Finally, this study demonstrates a pronounced difference in platelet-poor thrombin generation, which was significantly lower in the East Asian population when compared to Europeans and other Asians (Table 2). No statistically significant differences were seen with whole blood TEG assays nor other routine coagulation assays. The only routine coagulation results that were discriminating were protein C and S levels, which were higher in the European population, and contrary to the higher thrombin generation seen in this population. Given that the differences were isolated to CAT assays, it suggests that the non-platelet coagulation factors play an important factor in race differences, and may explain the lower venous thromboembolism rate in East Asians.

We recognise that our study population is small ($n = 97$), and not all of our controls ($n = 67$) were tested using both global coagulation assays. Moreover, there was a female predominance ($n = 43$ or 68%) and both genders were not age-matched. However, we note that the

Table 1
TEG parameters with age and gender comparisons.

	Overall	MRR	Tests below MRR (%)	Tests above MRR (%)	Female	Male	p-Value	Age <50	Age >50	p-Value
Calibrated automated thrombogram (CAT)										
Number of patients	96				65	31		55	41	
ETP	1347.5 ± 255.6				**1390.2 ± 272.7**	**1258.0 ± 189.8**	**0.017**	1336.2 ± 283.2	1362.7 ± 215.6	0.617
Velocity index	65 (45.8–99.9)				**74.1 (47.9–109)**	**52 (31.5–77)**	**0.003**	65.3 (43–101.1)	64.7 (47–93.5)	0.903
Thrombin peak	222.6 ± 66.6				**236.7 ± 69.0**	**193.2 ± 50.7**	**0.002**	219.1 ± 70.8	227.3 ± 61.1	0.554
Lag time, Med (IQR)	3.1 (2.7–3.7)				3.1 (2.7–3.7)	3.1 (2.9–3.7)	0.683	3 (2.6–3.3)	**3.4 (3–4.1)**	**<0.001**
Thromboelastography (TEG®)										
Number of patients	67				47	20		36	31	
R time (min)	7 (5.8–8.5)	2–8	0 (0.0%)	21 (31.3%)	**6.3 (5.2–8.2)**	**8 (6.8–8.9)**	**0.012**	**7.9 (6.2–9.2)**	**6.3 (5.2–7.5)**	**0.006**
K time (min)	2.3 (1.9–2.9)	1–3	0 (0.0%)	14 (20.9%)	**2.1 (1.8–2.4)**	**2.9 (2.6–3.2)**	**<0.001**	**2.6 (2.2–3.2)**	**2.1 (1.6–2.4)**	**<0.001**
a-angle (deg)	56.7 ± 10.8	55–78	25 (37.3%)	1 (1.5%)	**59.1 ± 11.0**	**51.1 ± 8.1**	**<0.001**	**51.5 ± 10.8**	**62.8 ± 7.0**	**<0.001**
Maximum amplitude (mm)	57.9 ± 6.2	51–69	9 (13.4%)	2 (3.0%)	**59.4 ± 5.8**	**54.3 ± 5.6**	**0.001**	**56.0 ± 6.1**	**60.1 ± 5.5**	**0.007**
LY 30 (%)	0.6 (0–2.2)	0–8%	0 (0.0%)	4 (6.0%)	0.7 (0.1–2.2)	0.1 (0–1.8)	0.067	1 (0–5.8)	0.4 (0.1–1.1)	0.214
TTG	710.4 ± 64.5	584–796	3 (4.4%)	4 (6.0%)	**724.9 ± 60.1**	**676.5 ± 53.2**	**0.004**	**685.5 ± 62.9**	**739.3 ± 54.2**	**<0.001**

Results highlighted in bold are statistically significant results.
*MRR = manufacturer's reference range (Haemonetics).