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Assessing maternal clotting function with novel global coagulation assays: a prospective pilot study

Running title: GCAs in pregnancy

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Abstract

Introduction: Women are at higher risk of venous thromboembolism (VTE) during pregnancy and the puerperium. Global coagulation assays (GCAs) including thromboelastography (TEG), thrombin generation using the calibrated automated thrombogram (CAT) and fibrin generation using the overall haemostatic potential assay (OHP) provide a more comprehensive assessment of the coagulation process than conventional coagulation assays. We aimed to evaluate the ability of these GCAs to analyse the coagulability amongst pregnant women of varying VTE risk profile.

Methods: Women undergoing term elective caesarean delivery provided a single pre-delivery blood sample for conventional and novel coagulation testing (TEG, CAT and OHP). Data from 47 healthy non-pregnant women aged 18-45 years were used as controls.

Results: Sixty women with term singleton pregnancies were included. Samples from pregnant women were hypercoagulable on most GCA parameters compared to non-pregnant controls, demonstrating increased maximum amplitude (clot strength) (71.5 vs 60.6mm, *P*<0.001) on whole blood TEG as well as increased endogenous thrombin potential (1895.22 vs 1399.33nM.min, *P*<0.001) and overall coagulation potential (fibrin generation) (57.58 vs 36.21 units, *P*<0.001) on platelet-poor plasma. Pregnant women with booking BMI ≥30kg/m^2 had significantly higher maximum amplitude compared to pregnant women of normal BMI (18.5-25kg/m^2) (73.2 vs 66.1mm, *P*<0.001).

Conclusions: GCAs reliably detect the physiological hypercoagulability of pregnancy. Thromboelastography in particular appears to correlate with obesity in the pregnant population. GCAs may be potential adjuncts to risk factor-based criteria to guide VTE thromboprophylaxis during pregnancy and the puerperium.

Keywords: pregnancy, venous thromboembolism, global coagulation assay, thromboelastography, obesity

Abbreviations

APTT, activated thromboplastin time; BMI, body mass index; CAT, calibrated automated thrombogram; CI, confidence interval; ETP, endogenous thrombin potential; GCA(s), global coagulation assay(s); INR, international normalised ratio; LMWH, low molecular weight heparin; MA, maximum amplitude; OCP, overall coagulation potential; OFP, overall fibrinolytic potential; OHP, overall haemostatic potential assay / overall haemostatic potential; RCOG, Royal College of Obstetricians & Gynaecologists; ROTEM, rotational thromboelastometry; TEG, thromboelastography; VTE, venous thromboembolism.

INTRODUCTION

Venous thromboembolism (VTE) is a leading cause of maternal morbidity and mortality in developed countries, accounting for 9.2-14% of maternal deaths in the United States, United Kingdom and Australia[1-3]. Currently, the key pharmacological strategy in managing VTE risk in pregnancy and the puerperium is the use of low molecular weight heparin (LMWH), with some guidelines recommending up to 85% of women receive postpartum thromboprophylaxis after caesarean delivery[4]. Antenatal and postnatal VTE risk assessment relies on clinical risk factor-based stratification, drawn from case-control studies and consensus opinion. A Cochrane review examining VTE prophylaxis in pregnancy and the puerperium concluded there is insufficient data to guide the routine use of pharmacological VTE prophylaxis in this context[5]. As a result of the limited quality of evidence, recommendations for pharmacological VTE prophylaxis vary widely, carrying with this additional risk of clotting or bleeding[6]. With an increasing prevalence of common risk factors such as obesity and maternal age, further research to refine risk stratification strategies with laboratory testing is warranted.

Standard coagulation assays such as the prothrombin time and activated partial thromboplastin time (APTT) only measure the time to the start of clot formation or 5% of thrombin generation and are unable to detect the physiologically hypercoagulable state of pregnancy and postpartum[7,8]. Newer global coagulation assays (GCAs) such as thromboelastography, thrombin generation and overall haemostatic potential assay, on the other hand, measure the characteristics of total clot formation as well as end-products of the coagulation cascade[8]. Changes in the elastic properties of whole blood during clot formation, propagation and dissolution can be measured with thromboelastography (TEG) and rotational thromboelastometry (ROTEM). Thrombin is a key protein in the coagulation process and thrombin generation measured using the calibrated automated thrombogram (CAT) has previously been shown to predict non-pregnant patients at increased risk of VTE recurrence[9]. Fibrinolysis is less well studied

but previous small studies have observed that hypercoagulable patients have impaired fibrinolysis[10] as measured using the overall haemostatic potential assay (OHP). Current data on GCAs to study the hypercoagulability of pregnancy are often limited by small samples sizes and studies do not evaluate the use of more than one GCA at a time[11]. By providing a more comprehensive assessment of the haemostatic profile, these GCAs may provide additional insight into the pathophysiology of hypercoagulability in the pregnant population and potentially assist VTE risk assessment as well as guide individualised thromboprophylaxis[11-13].

The overall aim of this study was to evaluate the hypercoagulability of pregnancy using three GCAs, baseline haematology, biochemical parameters and comprehensive clinical history. Our first objective was to compare TEG, CAT and OHP results in pregnant women to non-pregnant healthy women of childbearing age. Our second objective was to examine the relationship between independent clinical risk factors for VTE and GCA parameters. We hypothesised that pregnant women at increased clinical risk of VTE due to either higher overall VTE risk score or body mass index (BMI) >30 would have more hypercoagulable GCA parameters than pregnant women at lower clinical risk.

MATERIALS AND METHODS

Study design

This was a prospective pilot study of pregnant women with term singleton pregnancies recruited between February 2018 and April 2019 from The Northern Hospital, a level 5 metropolitan maternity centre in Victoria, Australia, that births approximately 3600 infants per annum. Exclusion criteria were: non-English speaking; <18 years; unable to provide written informed consent; pregnancy <37 gestational weeks; multiple pregnancy; and fetal death. All participants were recruited prior to term elective caesarean section except for one participant who was recruited prior to induction of labour. Demographic data was collected including age, BMI at booking and term, country of birth, gravida, parity and smoking status. Booking BMI was collected from

the woman's first recorded antenatal appointment, while BMI at term was collected from the woman's most recent antenatal appointment beyond 37 weeks' gestation. A medical, obstetric and family history was also recorded. Women were specifically asked about a personal history of diabetes, thyroid disease, previous VTE and varicose veins; current medications including insulin, thyroxine, anticoagulants and aspirin; and family history of VTE, cardiovascular disease, blood disorders and diabetes. Indications for caesarean delivery were recorded. Delivery and birth outcomes (primary blood loss, newborn weight and sex, Apgar scores and admissions to special care nursery) were collected after delivery. Each participant's risk of VTE was assessed at the time of blood collection using the Royal College of Obstetricians & Gynaecologists (RCOG) Green-top Guideline 37a based on antenatal clinical risk factors and taking into account their imminent elective caesarean delivery and increase in parity by one (see Table S1 for risk factor scoring)[14]. Additional postpartum risk factors were not included in scoring for this study. According to the RCOG guideline, women with a score of 3 or more can be considered at sufficient risk to be prescribed pharmacological thromboprophylaxis from 28 weeks' gestation, while for postpartum women that threshold is a score of 2.

Data from the healthy non-pregnant comparator group (n=47) were obtained from a previously reported cohort at The Northern Hospital[15]. Women aged 18-45 with no known cardiovascular disease risk factors such as diabetes mellitus, hypertension, hyperlipidaemia and current smoking, no active malignancy and not on anticoagulants were included. Data on BMI was not available for our healthy non-pregnant comparator group.

Laboratory methods

Blood samples were collected by venepuncture or at venous cannulation prior to caesarean delivery or induction of labour. 25-30mL of blood was drawn to fill six 3.2% sodium citrate tubes, one ethylenediaminetetraacetic acid tube and one serum-separating tube. Standard testing including full blood examination, liver function tests,

urea, electrolytes and creatinine, coagulation profile (fibrinogen, international normalised ratio (INR), APTT) and D-dimer were performed. One citrate tube was used for TEG within 4 hours of collection. Four citrate tubes were used to prepare platelet-poor plasma within 2 hours of collection by double centrifugation at 2500 x g for 10 minutes each at room temperature, then stored at -80°C for subsequent batch analysis with CAT and OHP.

Thromboelastography (TEG)

TEG analyses the viscoelastic properties of whole blood during clot formation, propagation and dissolution through measuring the progressive resistance of a clot in an oscillating plastic cuvette relative to a central pin. Samples were tested with the TEG5000 Thromboelastograph Haemostasis Analyzer system (Haemonetics, Braintree, MA, USA)[16] pre-warmed to 37°C. 1mL of whole citrated blood was activated with 40µL of kaolin. A 340µL aliquot of kaolin-activated blood was pipetted into a 37°C cuvette with 20µL of 0.2M calcium chloride for analysis. The following parameters (seen in Figure 1) were assessed: R value (time to clot formation, minutes); K value (time to achieve clot strength of 20mm, minutes); alpha-angle (rate of clot formation, °); maximum amplitude (MA) (maximum clot strength, mm); and LY30 (percentage of clot lysis 30 minutes after MA is achieved, %).

Thrombin generation with calibrated automated thrombography (CAT)

CAT determines the concentration of thrombin generated in a clot over time by measurement of a thrombin-sensitive fluorogenic substrate, which is consumed as thrombin is generated in platelet-poor plasma. A calibrator is simultaneously used to correct for the "inner filter effect" which confounds thrombin generation calculation. Samples were tested with a Fluoroskan Ascent fluorometer (Diagnostica Stago, Parsippany, NJ, USA) at 37°C as per manufacturer guidelines. Thrombinoscope® software outputted the following parameters: lag time (time to thrombin generation, minutes); endogenous thrombin potential (ETP, the amount of thrombin generated,

nM/min); thrombin peak (maximum concentration of thrombin, nM); and velocity index (rate of thrombin generation, nM/min).

Fibrin generation with overall haemostatic potential assay (OHP)

OHP produces a fibrin-aggregation curve as repeated spectrophotometric measurements are made at 37°C of a platelet-poor plasma sample undergoing fibrin generation and fibrinolysis[17]. Each sample was combined with exogenous thrombin alone (which provides an area under the curve representing fibrin generation as the overall coagulation potential (OCP, units), as well as exogenous thrombin with tissue-type plasminogen activator (tPA) (which provides an area under the curve representing response to tPA as the overall haemostatic potential (OHP, units). The difference between the OCP and OHP reflects the overall fibrinolytic potential (OFP, %). The FLUOstar® Optima (BMG Labtech) plate reader was used. All samples were performed in triplicates and the average derived.

Statistical analysis

Data was analysed using IBM® SPSS Version 25.0 (SPSS Inc., Chicago, IL, USA). Continuous variables were assessed for normality using the Shapiro Wilk test. Univariate comparison of pregnant with non-pregnant samples involved independent samples t-test or Mann-Whitney U test where appropriate. Within the pregnant cohort, analysis of variance (ANOVA) and Kruskal-Wallis tests were used to test for differences across BMI categories where appropriate. Where applicable, multivariable analysis with a generalised linear model was used with a backwards stepwise regression technique. Pearson's and Spearman's tests were used where appropriate to determine correlations between demographic and clinical data, baseline blood test results and GCA results. Statistical significance was defined at $P<0.05$.

Ethical approval

This study was approved by the Austin Health Human Research Ethics Committee, Melbourne, Victoria (HREC/17/Austin329 and AH04977). All women provided written informed consent for participation.

RESULTS

Demographics, clinical characteristics and baseline blood tests

Sixty women with singleton pregnancies were included in the study. Demographic and clinical data are summarised in Table 1. The mean ages of the pregnant and non-pregnant groups were 31.8 and 30.2 years respectively (*P*=0.194). One pregnant participant was on antenatal anticoagulation (enoxaparin) for previous unprovoked VTE, however this was ceased 48 hours prior to sample collection. Four (3.7%) women had a family history of VTE in a first degree relative with no known thrombophilia.

Results of baseline blood tests are summarised in Table S2. Results were significantly different between pregnant and non-pregnant groups consistent with the known physiological changes of pregnancy (*P*-values listed in Table S2). All but one pregnant woman had a D-dimer level above 500mg/L FEU, with a median of 1580mg/L FEU in the pregnant group.

[Insert Table 1]

Global coagulation assay results

Comparison of pregnant cohort with non-pregnant cohort

Results of TEG, CAT and OHP are summarised in Table 2 and Figure 1. All GCA parameters were significantly different between pregnant and non-pregnant groups (*P*-values listed in Table 2). In the pregnant group, all GCA parameters except lag time reflected hypercoagulability. In particular, the pregnant group showed markedly increased maximum amplitude (clot strength) (71.5 vs 60.6mm, *P*<0.001), ETP and peak thrombin (1895.22 vs 1399.33nM.min, *P*<0.001 and 320.91 vs 240.01nM,

P<0.001 respectively) as well as increased fibrin generation (OCP) (57.58 vs 36.21 units, *P*<0.001) and reduced overall fibrinolytic potential (76.25 vs 82.07%, *P*<0.001).

[Insert Table 2] [Insert Figure 1]

Correlations with baseline blood tests

Among pregnant women, there were significant correlations between fibrinogen and fibrin generation parameters: OCP (r_S=0.873, *P*<0.001), OHP (r_S=0.854, *P*<0.001) and OFP (r_S=-0.726, *P*<0.001) as well as TEG parameters: MA (r_S=0.323, *P*=0.013) and alpha-angle (r_S=0.322, *P*=0.013). There were no correlations between fibrinogen and D-dimer, ETP nor LY30. There were also no correlations between fibrin generation parameters and D-dimer.

Analysis of pregnant cohort by BMI and weight category

Results of TEG, CAT and OHP in women of normal booking BMI (18.5-24.9kg/m^2) compared to obese women are summarised in Table 3. At booking, overweight (25.0-29.9kg/m^2), obese (30.0-34.9kg/m^2) and very obese (≥35kg/m^2) women had a higher MA compared to women of normal BMI (*P*<0.001) (Figure 2). Statistical significance of these comparisons was maintained when controlling for age, parity, smoking status and diabetes (B=7.309, 95% confidence interval (CI); 4.26 to 10.36, *P*<0.001). At booking, very obese women had a higher OCP compared to women of normal BMI (62.63 vs 54.51 units, *P*=0.026). At term, very obese women had a higher MA compared to those of normal BMI (73.1 vs 68.0mm, *P*=0.030). No significant differences in CAT or other OHP parameters were seen between weight categories. There were significant correlations between booking BMI and MA (r=0.397, *P*=0.002) and term BMI and MA (r=0.297, *P*=0.022). There were no significant correlations between BMI and CAT or OHP parameters.

[Insert Table 3] [Insert Figure 2]

Analysis of pregnant cohort by clinical VTE risk

Of the seven women who theoretically would have qualified for antenatal thromboprophylaxis from 28 weeks' gestation under the RCOG Green-top Guideline 37a[14], five were obese at booking and only one had been prescribed antenatal thromboprophylaxis. Forty-two women (70%) theoretically would have qualified for postpartum thromboprophylaxis taking into account their imminent elective caesarean delivery and increase in parity by one. There were no differences in GCA parameters between women who qualified for antenatal or postnatal thromboprophylaxis under RCOG guidelines and those who did not. The most common postpartum risk factors were elective caesarean delivery, obesity, parity ≥3 and age > 35 (n=59 (98.3%), n=25 (41.7%), n=22 (36.7%) and n=18 (30%) respectively). Of all GCA parameters, only thrombin peak correlated with RCOG antenatal and postpartum raw VTE risk scores (r_s=0.267, *P*=0.039 and r_s=0.324, *P*=0.012 respectively).

There were no differences in any GCA parameters between women with and without a history of previous VTE (n=3 and n=57 respectively). There were no correlations between age and any GCA parameters other than thrombin peak (r_s=0.257, *P*=0.048). OCP and OHP were negatively correlated with parity (r_s=-0.296, *P*=0.023 and r_s=-0.336, *P*=0.009 respectively) while OFP was positively correlated with parity (r_s=0.312, *P*=0.016). Women with any form of diabetes mellitus did not have significant differences in any GCA parameters compared to non-diabetic women, other than a significantly decreased lag time (means: 3.00 vs 3.28 min, *P*=0.028).

Clinical outcomes

All women had live births and other birth outcome data have been summarised in Table 1. Two women (3.3%) had an intra-operative blood loss estimate of 1000mL, however the only difference in GCA parameter was a lower thrombin peak compared to those who did not (medians: 248.40 vs 319.03 nM, *P*=0.028). Three women were prescribed postnatal thromboprophylaxis for six weeks, based on The Northern Hospital postpartum VTE risk assessment guidelines[18]. No women experienced a

postpartum VTE prior to discharge and none had any medical record of presenting to The Northern Hospital with VTE during the six-week puerperium.

DISCUSSION

This prospective observational study is the only study of its kind to simultaneously investigate three different GCA in pregnant women, providing a comprehensive profile of coagulation. Our results provide a comprehensive analysis of the hypercoagulability of pregnancy and detected a correlation between GCA parameters and BMI among the pregnant cohort.

Compared to non-pregnant women, all three GCAs showed hypercoagulability in term pregnant women, a difference not apparent on routine INR and APTT testing. TEG demonstrated overall hypercoagulability in term pregnancy consistent with previous literature, including earlier and more rapid clot formation, stronger clot strength, and slower clot breakdown[19-23]. Our CAT findings are similar to those which studied women at any point in third trimester, demonstrating an increased thrombin peak and ETP compared to early pregnancy or non-pregnant women[24-27], suggesting an upregulation of thrombin generation in pregnancy. We note that lag time was slightly more prolonged in the pregnant population although the absolute difference to non-pregnant women was minimal (3.3 vs 2.9 min). The OHP assay demonstrated increased fibrin generation (OCP) in pregnancy and reduced fibrinolysis (OFP), consistent with previous studies[10,28]. Of interest, in our study D-dimer levels did not correlate with any fibrin generation parameters using OHP, despite being a fibrin degradation product, highlighting a current knowledge gap in the complexity of the fibrinolytic pathway, which warrants further investigation.

BMI and weight category were key risk factors of interest in the study, given the global obesity epidemic and its well-recognised ability to drive increased thrombotic disorders through all three arms of Virchow's triad[29]. The finding of increased clot strength in overweight and obese pregnant women compared to those of normal BMI

is a novel finding and contributes to our understanding of the mechanisms of VTE in obese pregnant women. Other studies examining obesity in pregnancy had variable results. Morgan et al[30] found no difference in any TEG parameters amongst obese and non-obese women >36 weeks' gestation, nor did Lee at al[31] find any association between BMI and ROTEM parameters in pregnant women at term. However, Sharma et al[32] found a trend of increasing clot strength with BMI in pregnancy, though this did not meet statistical significance. Whilst not significant, a trend of increasing ETP and OCP in obese pregnant women compared to those of normal BMI may also suggest a role of obesity in VTE risk. Some studies have suggested that obesity-driven chronic inflammation involves key modulators of prothrombotic pathways and impaired fibrinolysis has been reported to be a major effector mechanism of thrombosis[33]. Whilst we were able to demonstrate hypofibrinolysis in the pregnant cohort compared to the non-pregnant group, with decreased LY30 using TEG and decreased OFP using OHP, there were no differences in these parameters between obese women at term and those of normal BMI. Our analysis of BMI was limited to the pregnant cohort, as data for BMI in the non-pregnant comparator group was not available.

No significant differences in GCA parameters between the three women at highest clinical risk of VTE (those with prior VTE) were seen compared to the rest of the pregnant cohort, nor were there any differences between those who theoretically qualified for antenatal or postnatal thromboprophylaxis and those who did not based on the RCOG Green-top Guideline 37a[14]. However, thrombin peak appeared to correlate to raw VTE risk scores. We acknowledge that no definitive conclusion can be drawn as the statistical power of our comparisons of GCA parameters between these groups was limited by unequal numbers in each group. It is important to note, however, that clinical risk assessments incorporate a wide range of factors. GCAs only measure the 'hypercoagulability' arm of Virchow's triad and it is likely that the other two components, endothelial dysfunction and stasis, also contribute significantly to the overall hypercoagulable state in pregnancy[30,34]. Even with a larger sample size, a previous study found no difference in CAT parameters between women at high clinical

risk of VTE compared to normal pregnancies[35]. These findings highlight the complexity of coagulation pathways and suggest that multimodal risk algorithms incorporating both clinical risk factors and biomarkers of coagulation such as GCAs should be investigated in future studies.

Results of standard coagulation profile tests (INR and APTT) confirmed that they are unhelpful in discerning hypercoagulability in pregnancy. D-dimer and fibrinogen levels were increased above non-pregnant reference range to a similar extent previously described in pregnancy[36]. That 98.3% of women had a raised D-dimer level and none experienced a postpartum VTE confirms that D-dimer level on its own is not appropriate for the general risk assessment of pregnancy-associated VTE.

A key strength of this study lies in its simultaneous evaluation of three different GCAs alongside baseline haematology and biochemical parameters. We were able to see significant hypercoagulable changes when comparing pregnant and non-pregnant women, despite a relatively small sample size. The comprehensive collection of clinical data allowed thorough assessment of antenatal and postpartum VTE risk using RCOG guidelines. The timeframe of the study also allowed us to follow up the medical records of women six weeks postpartum to ensure none re-presented to the maternity centre with VTE.

One of our study limitations was that BMI was not available for our non-pregnant cohort, and we were therefore unable to adjust for this factor when comparing the non-pregnant group to the obstetric cohort. As our recruitment was largely from elective caesarean theatre lists, we acknowledge that our pregnant cohort may not represent the general obstetric population as caesarean births are known to be associated with higher maternal age, parity and BMI[37,38]. Our pregnant cohort also had a relative high rate of gestational diabetes mellitus (GDM) (31.7%) when compared with our institution's general obstetric population (14.7%)[37]. This was likely due to the timing of study recruitment at the beginning of the morning operating lists, when women with GDM are preferentially scheduled for their caesarean births.

While this study represents one of the largest cohorts of pregnant women at term for this field of study, larger prospective studies are still needed to establish reference values for GCAs in pregnancy. Reference ranges for healthy pregnancy and postpartum have been established for TEG and ROTEM[13,39], however this remains an area of need for CAT and OHP as studies are limited by small sample sizes[10,26]. Further clinical trials would then be required to validate their accuracy before incorporating them into routine use. Only then would a large-scale prospective study be able to validate a risk assessment tool incorporating GCAs.

Several other challenges which must be addressed before GCAs could be considered for implementation into routine VTE risk scores. CAT and OHP, still in their infancy, can be labour and time intensive with inter-operator variability and lack of inter-laboratory standardisation[8]. Ensuring standardised protocols for all GCAs in regard to collection, processing, storage and substrates used would be of benefit to minimise variability in future studies.

CONCLUSION

This pilot study, to our knowledge, is the first of its kind to simultaneously evaluate TEG, CAT and OHP in pregnant women. Unlike currently validated coagulation assays, each GCA was able to consistently detect the physiological hypercoagulability of pregnancy previously described in the literature. Our results suggest that GCAs hold promise for understanding the mechanisms underlying the relationship between BMI and VTE risk during pregnancy, particularly with TEG. Further large clinical studies in pregnant women are warranted to evaluate the role of GCAs in the prediction of pregnancy-associated VTE.

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Conflicts of interest

The authors have no conflicts of interest to declare.

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Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Table 1: Demographic and clinical data of pregnant cohort and birth outcomes

Variable	Pregnant women (n = 60)
Age, years (mean ± SD)	31.8 ± 4.1

Gestation, weeks (median (IQR))	39 (1)
Parity (median)	1
- Nulliparous (%)	7 (11.7)
- Multiparous (%)	53 (88.3)
Medical history	---
- BMI ≥ 30kg/m^2 at booking n, (%)	25 (41.7)
- GDM managed with insulin n, (%)	12 (20.0)
- GDM managed with diet control n, (%)	7 (11.7)
- Any current diabetes n, (%)	20 (33.3)
- Previous VTE n, (%)	3 (5.0)
Mode of delivery	---
- Caesarean delivery (%)	59 (98.3)
- Normal vaginal delivery (%)	1 (1.7)
Smoking status	---
- Never smoked (%)	43 (71.7)
- Current smoker (%)	6 (10.0)
- Ex-smoker (%)	11 (18.3)
Body mass index at booking (kg/m^2; median (IQR))	28.5 (7.0)
Body mass index at booking (kg/m^2)	---
- 18.5-24.9 kg/m^2 (%)	13 (21.7)
- 25-29.9 kg/m^2 (%)	22 (36.7)
- 30-34.9 kg/m^2 (%)	17 (28.3)
- ≥ 35 kg/m^2 (%)	8 (13.3)
Body mass index at term (kg/m^2; median (IQR))	32.0 (6.0)
Body mass index at term (kg/m^2)	---
- 18.5-24.9 (%)	3 (5.0)
- 25-29.9 (%)	15 (25.0)
- 30-34.9 (%)	24 (40.0)
- ≥ 35 (%)	18 (30.0)
Country of birth	---
- Australia (%)	27 (45.0)
- India (%)	15 (25.0)

- Other[a] (%)	18 (30.0)
Indication for caesarean delivery (n=59)	---
- Previous caesarean delivery (%)	42 (70.0)
- Breech or transverse lie (%)	7 (11.7)
- Placenta previa (%)	3 (5.0)
- Patient choice or previous complicated vaginal delivery (%)	6 (10.0)
- Other (%)	1 (1.7)
Primary blood loss, mL (median (IQR))	325 (150)
Newborn weight, grams (mean ± SD)	3426.17 ± 508.94
Newborn outcomes	---
- Male (%)	29 (48.3)
- Female (%)	31 (51.7)
Number immediately admitted to special care nursery (%)	8 (13.3)
Number with APGAR <7 at 5 mins (%)	0 (0.0)

Abbreviations: BMI, body mass index; GDM, gestational diabetes mellitus; IQR, interquartile range; SD, standard deviation; VTE, venous thromboembolism

[a] Other countries of birth: New Zealand, Iran, Nepal, Ghana, Greece, South Africa, Vietnam and Philippines

Table 2: GCA results in pregnant vs non-pregnant women

Assay	Variable		Pregnant		Non-pregnant*	*P*-value
TEG	**R value**	n=59	5.8 ± 1.6	n=37	6.9 ± 2.4	0.013
	K value		1.4 (0.5)		2.2 (0.9)	<0.001
	Alpha angle		65.8 ± 6.5		54.1 ± 11.3	<0.001
	Maximum amplitude		71.5 ± 5.1		60.6 ± 6.6	<0.001
	Lysis 30	n=58	0.0 (0.1)		0.6 (2.1)	<0.001
CAT	**Lag time**	n=60	3.28 ± 0.61	n=47	2.89 ± 0.60	0.001
	Endogenous thrombin potential		1895.22 ± 380.32		1399.33 ± 286.13	<0.001

	Thrombin peak		320.91 ± 64.31	240.01 ± 71.63	<0.001
	Velocity index		110.70 ± 38.24	83.97 ± 40.28	0.001
OHP	**Overall coagulation potential**	n=59	57.58 ± 10.53	36.21 ± 7.71	<0.001
	Overall haemostatic potential		13.44 (6.41)	6.65 (3.28)	<0.001
	Overall fibrinolytic potential		76.25 (6.64)	82.07 (5.48)	<0.001

Normally distributed variables are reported as mean ± SD. Non-normally distributed variables are reported as median (IQR).

Statistical significance of normally distributed variables determined with independent samples t-test; statistical significance of non-normally distributed variables determined with Mann-Whitney U test.

Abbreviations: CAT, calibrated automated thrombogram; OHP, overall haemostatic potential assay; TEG, thromboelastography.

* = non-pregnant population defined as non-pregnant women aged 18-45 with no cardiovascular disease risk factors, no active malignancy and not on anticoagulants

Table 3: GCA results in pregnant women of normal vs obese BMI at booking

Assay	Variable	Normal BMI at booking (18.5-24.9kg/m²)		Obese at booking (≥30kg/m²)	*P*-value
TEG	**R value**	6.0 ± 2.1	n=24	5.8 ± 1.3	0.810
	K value	1.8 (0.6)		1.3 (0.5)	**0.008**
	Alpha angle	64.1 ± 5.4		67.0 ± 7.1	0.207
	Maximum amplitude	66.1 ± 4.6		73.2 ± 4.1	**<0.001**
	Lysis 30	0.0 (0.1)	n=23	0.0 (0.2)	0.948
CAT	**Lag time**	3.33 (0.63)	n=25	3.33 (0.56)	0.523
	Endogenous thrombin potential	1778.27 ± 276.65		1939.43 ± 364.83	0.172

	Thrombin peak		314.27 (45.22)		323.06 (53.38)	0.504
	Velocity index		101.64 (38.08)		110.70 (33.35)	0.738
OHP	**Overall coagulation potential**	n=12	54.51 ± 7.61	n=25	61.47 ± 10.98	0.056
	Overall haemostatic potential		11.93 (5.52)		15.36 (8.45)	0.109
	Overall fibrinolytic potential		76.25 (5.46)		75.89 (9.12)	0.471

Normally distributed variables are reported as mean ± SD. Non-normally distributed variables are reported as median (IQR).

Statistical significance of normally distributed variables determined with independent samples t-test; statistical significance of non-normally distributed variables determined with Mann-Whitney U test.

Abbreviations: CAT, calibrated automated thrombogram; OHP, overall haemostatic potential assay; TEG, thromboelastography.

Figure legends

Figure 1: Graphical output of TEG (A), CAT (B) and OHP (C) results in pregnant and non-pregnant groups.

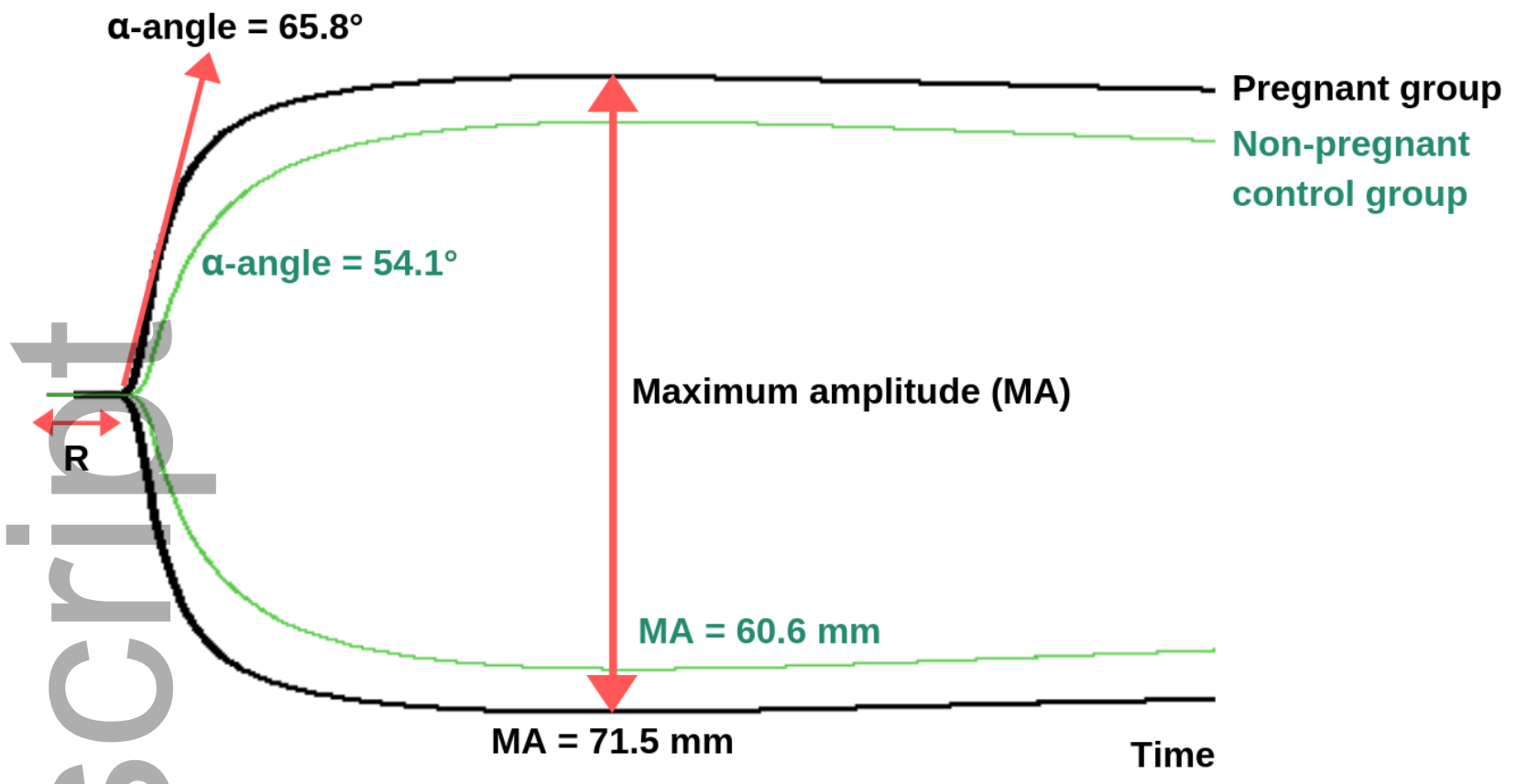
Figure 2: Box plot of maximum amplitude (using TEG) according to booking BMI category among pregnant women. Box plots represent the range of data from 25th to 75th percentile; the bar in the middle of each box plot represents the median value. The whiskers extending from each box plot represent the range of values obtained excluding outliers. * = P<0.001 compared with normal BMI (18.5-24.9kg/m^2). ° = outlier (1.5 x the IQR).

Supporting information

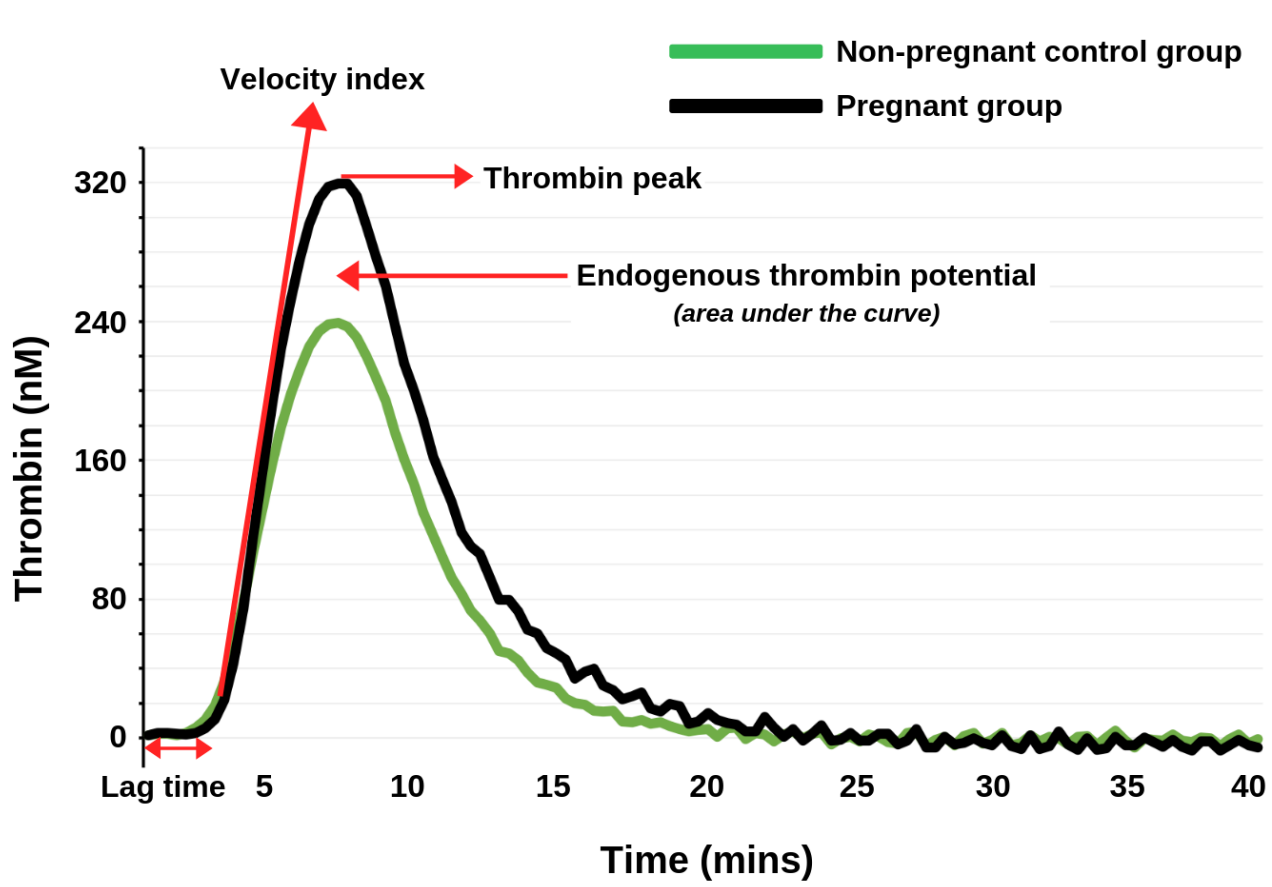
Table S1: Risk factors for VTE in pregnancy and the puerperium, and risk assessment for VTE, adapted from 2015 Royal College of Obstetricians & Gynaecologists Green-top Guidelines[14].

Table S2: Baseline blood and coagulation profile results in pregnant vs non-pregnant women

A



B



C