Original Article

Reference ranges for thromboelastography (TEG®) and traditional coagulation tests in term parturients undergoing caesarean section under spinal anaesthesia*

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Summary

There has been little published work defining ‘normal’ thromboelastography (TEG®) values in healthy parturients, and few large studies defining reference ranges for traditional coagulation tests in this patient group. Our aim was to establish peri-operative reference ranges for TEG and for standard laboratory coagulation tests in our pregnant population. Fifty healthy term parturients presenting for elective caesarean section under spinal anaesthesia had blood samples taken pre-operatively, on arrival in the recovery room and, in a subset of 33 women, 4 h after routine thromboprophylaxis with enoxaparin 40 mg. All three samples had TEG analysis, the first and second having standard laboratory coagulation tests in addition. Reference ranges for our pregnant population were established, demonstrating a hypercoagulable state in term parturients and a significant effect of enoxaparin. The standard coagulation reference ranges were within 98% of the local non-pregnant ranges. These reference ranges provide a useful comparator for peri-operative TEG and routine coagulation analysis in term parturients.

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The recent decline in direct maternal deaths in the UK has been attributed largely to reductions in deaths due to thromboembolism, and, to a lesser extent, haemorrhage [1]. With increased awareness and more widespread use of thromboprophylaxis, and the ever-present risk of maternal haemorrhage, greater importance is being placed on the assessment of coagulation status and blood product replacement during the peripartum period.

Thromboelastography (TEG®) is a relatively new near-patient monitor of coagulation in UK labour wards, although its use in cardiac and liver surgery is long-standing [2, 3]. The TEG evaluates the mechanical strength of clot during its formation, via the torsion in a pin suspended in an oscillating heated cup that contains the blood sample [4], leading to a graphical output (Fig. 1). Compared with traditional laboratory coagulation tests, the TEG takes into account the dynamic interaction of plasma (clotting factors) and cellular (platelets) elements that occurs during in vivo clotting, thus indicating overall ‘clot quality.’ Test protocols can
also use reagents that selectively inhibit heparin, platelets or fibrinolysis to help determine the cause of a coagulopathy. Use of a ‘point-of-care’ analyser such as TEG avoids some of the logistical problems associated with laboratory-based testing. The equipment is easy to use and produces a reliable result in 30 min [5].

Pregnancy is a hypercoagulable state that protects the mother against peripartum haemorrhage. Reference ranges for the obstetric population for the related point-of-care ROTEM® thromboelastometry device have recently been published [6, 7]. Principal differences between the ROTEM and TEG are the use of an optical detector system, initiation of movement of the cup/pin unit by the pin rather than the cup and the variables measured. There have been limited data published on ‘normal’ values for TEG in late pregnancy, particularly during the peri-operative period when antithrombotic prophylaxis is routine and concerns over bleeding are not uncommon. Our principal aim was to establish ‘normal’ reference ranges for TEG parameters in healthy women presenting for elective caesarean section in our obstetric unit. During our research of this topic, it became evident to our surprise that there are also few published data on reference ranges for standard coagulation tests in late pregnancy − it being our anecdotal experience that non-pregnant reference ranges are used universally − so our secondary aim was to establish reference ranges for these tests too.

Methods
Following Research Ethics Committee and Trust Research and Development approval, and with written informed consent, we recruited 61 women of ASA physical status 1–2, aged 16–45 years, with an uncomplicated singleton pregnancy of > 38 weeks’ gestation presenting for elective caesarean section under spinal anaesthesia. Exclusion criteria included a history of thromboembolic/clotting disorders, cardiovascular, renal, liver or malignant disease, a positive smoking history, current treatment with anticoagulants or non-steroidal anti-inflammatory drugs, pre-eclampsia or HELLP syndrome, a history of alcohol ingestion > 20 U.week⁻¹, a recent history of active bleeding, blood transfusion or surgery within the last 28 days and an expected peripartum blood loss > 500 ml.

All patients were fasted for at least 6 h. On arrival in the operating theatre, a 16-G iv cannula was placed in the hand or arm under local anaesthesia and approximately 500 ml Hartmann’s solution was given as a fluid preload. Patients received a spinal or combined spinal-epidural anaesthetic with intrathecal injection of 2.0–2.5 ml heavy bupivacaine 0.5% and diamorphine 300 μg. Boluses of phenylephrine (100 μg) were given during the operation to maintain blood pressure. On delivery of the baby, 5 IU oxytocin was given, followed by a 40-IU infusion over 5 h. At the end of surgery, all patients received 100 mg rectal diclofenac.

Venous blood samples were taken pre-operatively, on arrival in the recovery room, and, in a subset of 33 women (depending on timing and staffing levels as the laboratory service was limited outside of normal working hours), 4 h after administration of 40 mg subcutaneous enoxaparin (itself given 3–4 h after surgery). The pre-operative blood sample (15 ml) was taken via the iv

Figure 1 Typical thromboelastography trace, showing reaction time (R), clot formation time (K; arbitrarily set as the time from the end of the R time until the trace reaches an amplitude of 20 mm), alpha angle (α), maximum amplitude (MA) and percentage lysis at 30 min post-MA (Ly30).
cannula that was placed on arrival in the operating theatre, before commencing any IV fluids. This sample underwent laboratory measurement of full blood count and standard coagulation tests (prothrombin time (PT), activated partial thromboplastin time (APTT) and fibrinogen levels), and TEG analysis. The second and third blood samples (10 and 5 ml, respectively) were taken postoperatively either via a second, smaller cannula sited in the contralateral arm on arrival in the recovery room, or via venepuncture with a needle and syringe, depending on the patient’s preference. The second sample underwent standard laboratory coagulation tests and TEG analysis, whilst the third sample underwent TEG analysis only. The first 2 ml blood from each sample was discarded; 1 ml was used for TEG analysis, and the remainder was used for laboratory tests when indicated. The TEG analysis was started immediately after sampling.

For each TEG sample, 1 ml whole blood was pipetted into a vial containing kaolin (Haemoscope, Niles, IL, USA). All vials were stored at 2–10 °C but allowed to warm to room temperature before use. After adequate mixing using five gentle inversions, 360 μl kaolin-activated blood was pipetted into a plain cup and analysed using the TEG® 5000 Hemostasis Analyzer System (Haemonetics Corporation, Niles, IL, USA), calibrated and prewarmed to 37 °C before use. The third blood sample (post-enoxaparin) underwent additional TEG analysis with the addition of heparinase in order to eliminate the effect of heparin. These two TEG samples (one plain, one with heparinase) were analysed simultaneously.

Data collection included TEG parameters (reaction time (R), clot formation time (K), maximum amplitude (MA), alpha angle (α), percentage lysis at 30 min post-MA (Ly30) and coagulation index (CI; a calculated indicator of overall coagulation) (Fig. 1). Other data collected included age, body mass index, gestation, parity, comorbidities and concomitant drugs. Intra-operative fluid type and volume, estimated blood loss, time of enoxaparin administration and sampling times were also recorded.

A sample size of 50 women was based on previous studies [5, 8–11], assuming a normal distribution of MA and with 95% CI for the upper and lower limits that approximated to SD. Values at each time point were compared using one-way ANOVA with Tukey post-hoc tests. Since the sample size from which the non-pregnant reference ranges were calculated were unknown, single-sample t-tests were used to compare values at each time point with the mid-point of the reference range. Values for p < 0.05 were taken to denote statistical significance.

Results
A total of 61 patients were recruited into the study. Eleven patients were not studied: one due to a technical error with the pre-operative TEG; one had her operation too late in the day to process the samples; and the remaining nine women withdrew their consent after the pre-operative blood sample had been taken. Baseline characteristics and obstetric/intra-operative details for the remaining 50 women are shown in Table 1. The booking body mass index ranged from 18 to 46 kg.m⁻², with a mean of 24.9 kg.m⁻². Indications for caesarean section were previous caesarean section (34), breech presentation (8), previous third or fourth degree perineal tear (4) or other (4). Mean (SD) pre-operative haemoglobin concentration, white cell count and platelet count were 11.8 (1.0) g.dl⁻¹, 8.5 (1.7) · 10⁹ l⁻¹ and 218 (51) · 10⁹ l⁻¹, respectively. No patients received blood transfusions peri-operatively.

Reference ranges for pre-operative, postoperative and post-enoxaparin TEG are given in Table 2. Reference ranges are expressed as mean (2SD) in keeping with previous studies and consistent with our

| Table 1 Patients’ characteristics and obstetric/intra-operative details. Values are mean (SD) or number (proportion). |
|---|---|
| Age; years | 34.7 (4.1) |
| Height; cm | 165.2 (7.3) |
| Weight; kg | 68.3 (17.4) |
| BMI; kg.m⁻² | 24.9 (5.2) |
| Gestation; weeks | 38.7 (1.0) |
| Parity; | |
| 0 | 13 (26%) |
| 1 | 23 (46%) |
| 2 | 11 (22%) |
| > 2 | 3 (6%) |
| Crystalloid; ml | 1778 (488) |
| Colloid; ml | 700 (274) |
| Estimated blood loss; ml | 560 (216) |

BMI, body mass index.
Table 2  Pre-operative, postoperative and post-enoxaparin (uncorrected [plain cup] and corrected [heparinase cup]) thromboelastography parameters in parturients undergoing caesarean section, with the manufacturer’s non-pregnant reference ranges for comparison. Data are mean (2SD) (2SD only for non-pregnant).

<table>
<thead>
<tr>
<th>Pregnant reference ranges</th>
<th>Pre-operative (n = 50)</th>
<th>Postoperative (n = 50)</th>
<th>Post-enoxaparin (n = 33)</th>
<th>Non-pregnant reference range</th>
</tr>
</thead>
<tbody>
<tr>
<td>R time; min</td>
<td>7.0 (1.0–13.0)</td>
<td>6.6 (2.4–10.8)</td>
<td>8.2 (3.2–13.2)</td>
<td>4–8</td>
</tr>
<tr>
<td>K time; min</td>
<td>2.0 (0.2–3.8)</td>
<td>1.8 (0.4–3.2)</td>
<td>2.2 (0.4–4.0)</td>
<td>0–4</td>
</tr>
<tr>
<td>MA; mm</td>
<td>75.4 (64.6–86.2)</td>
<td>76.4 (66.8–86.0)</td>
<td>72.8 (62.8–82.8)</td>
<td>54–72</td>
</tr>
<tr>
<td>Alpha angle;°</td>
<td>64.8 (47.6–82.0)</td>
<td>67.3 (53.5–81.1)</td>
<td>63.2 (45.0–81.4)</td>
<td>47–74</td>
</tr>
<tr>
<td>Ly30; %*</td>
<td>1.6 (0–8.8)</td>
<td>0.7 (0–4.9)</td>
<td>0.7 (0–4.5)</td>
<td>0–8</td>
</tr>
<tr>
<td>CI</td>
<td>1.2 (–5.4–7.8)</td>
<td>1.8 (–3.4–7.0)</td>
<td>–0.2 (–6.4–6.0)</td>
<td>–3–3</td>
</tr>
</tbody>
</table>

See Fig. 1 for explanation of variables.

*Where the lower limit of the reference range for Ly30 < 0, this was recorded as 0.

Reference ranges in pregnancy

Table 3  Pre-operative and postoperative standard laboratory coagulation tests in parturients undergoing caesarean section. Data are mean (2SD) (2SD only for non-pregnant).

<table>
<thead>
<tr>
<th>Pregnant reference ranges</th>
<th>Pre-operative (n = 47)</th>
<th>Postoperative (n = 49)</th>
<th>Non-pregnant reference range</th>
</tr>
</thead>
<tbody>
<tr>
<td>PT; s</td>
<td>9.5 (9.1–9.9)</td>
<td>9.8 (9.2–10.4)</td>
<td>9.6–11.6</td>
</tr>
<tr>
<td>APTT; s</td>
<td>27.1 (22.5–31.7)</td>
<td>28.4 (19.3–36.5)</td>
<td>24.0–32.0</td>
</tr>
<tr>
<td>Fibrinogen; g.l⁻¹</td>
<td>5.0 (3.7–6.4)</td>
<td>4.4 (3.0–5.7)</td>
<td>1.5–4.0</td>
</tr>
</tbody>
</table>

PT, prothrombin time; APTT, activated partial thromboplastin time.

Reference ranges for the standard laboratory coagulation tests are given in Table 3. Four coagulation tests (three pre-operative, one postoperative) could not be processed by the laboratory because the sample tubes were underfilled. Prothrombin time was significantly shorter and fibrinogen concentration greater in pregnant subjects, compared with the non-pregnant reference ranges.

Discussion

Use of the TEG has the potential to assist the obstetric anaesthetist in haemostatic decision-making when a routine coagulation screen and full blood count may not provide enough information. These situations include deciding whether a woman is haemostatically fit for regional analgesia/anaesthesia when she has a haemostatic defect or is taking anti-thrombotic medication.
and guiding blood product replacement in major haemorrhage [13]. However, in order for TEG to be a useful test during the peripartum period, appropriate reference ranges are required.

‘Normal’ reference ranges for TEG parameters provided by the manufacturer of the device are based on data provided from patients in a number of hospitals in the USA [14]. However, pregnancy is known to result in a prothrombotic state [5]. Our study has established normal ranges for TEG in healthy women in late pregnancy undergoing elective caesarean section in our maternity unit, both pre-operatively and postoperatively. Whilst it may not be practical to refer to three different reference ranges in routine practice, we were concerned that a single ‘pregnant’ reference range would be an oversimplification and thus hinder interpretation of TEG results in different settings, for example, pre-operatively compared with postoperatively/after enoxaparin. We have also defined a ‘caesarean section’ reference range for standard laboratory coagulation tests (PT, APPT and fibrinogen).

Previous studies have used TEG to demonstrate the hypercoagulable state of pregnancy [10, 11, 15–17], and our study confirms these findings, with increased MA, alpha angle and CI compared with non-pregnant values. The MA reflects the strength of the clot and is a function of the interaction between platelets and fibrin. Although caesarean section is thought to increase this hypercoagulable state further, we found that pre-operative TEG parameters were similar to those immediately postoperatively and 4 h after enoxaparin (corrected sample). Sharma and Philip also found similar TEG parameters before and immediately following caesarean section under spinal anaesthesia [11]. Conversely, Boyce et al. recently demonstrated a significantly shorter R time, shorter K time and wider alpha angle following caesarean section compared with pre-operative values [18]. However, the greatest increase in coagulability in their study was found at 3 h. Since our postoperative samples were taken either immediately postoperatively, or 4 h following enoxaparin administration (itself given 3–4 h following surgery), we may have missed the peak hypercoagulable period. Other investigators have found shorter R times in pregnant women compared with non-pregnant [11, 15–17, 19]; the mean values for R time in our study were still within the non-pregnant reference range though slightly increased from the latter’s mid-point. This might be due to inadequate mixing of blood with the kaolin activator or peri-operative fluid therapy affecting blood viscosity. Some of our reference ranges were wide compared with the non-pregnant ones, and the range for R time particularly so; whether this suggests greater variability in pregnancy or reflects our small sample size would require further work.

A heparin effect has been defined as a TEG R time > 25% longer than the heparinase-corrected control [18, 19]. Of the 33 patients who had post-enoxaparin TEG samples, only 17 (52%) showed this effect, although 24 (73%) were hypocoagulable compared to their pre-operative samples, with increased R times, decreased MA and decreased CI values. A recent study found that the majority of patients given 7500 IU subcutaneous unfractionated heparin immediately before elective caesarean section remained hypercoagulable compared to pre-operative samples [19]. These findings suggest that 40 mg enoxaparin has a greater anticoagulant effect than 7500 IU unfractionated heparin, an observation that has been made previously [20].

Since the hypercoagulable state of pregnancy is so well described [5, 10, 11, 15–17, 21], we were surprised to find few papers on reference ranges for haematology tests in pregnancy [7, 22, 23], although data do exist for other standard laboratory tests in pregnancy [21, 24–26]. It is reassuring that the current study has demonstrated broadly similar reference ranges for PT and APTT in pregnancy compared with the laboratory ranges, but our finding of higher fibrinogen levels in pregnancy should be considered when interpreting standard coagulation tests in the peripartum period.

There are a number of limitations to our study. Our reference ranges only apply to healthy parturients at term. Other factors such as systemic disease, drug therapy, labour, gestation, age and body mass index may affect ‘normal’ parameters for TEG and standard coagulation tests. We did not exclude obesity but could not find any other study of TEG that did, though Armstrong et al. (using ROTEM) excluded parturients with weights < 50 kg or > 100 kg [6]. Potential confounding factors include oxytocin [27], the duration of surgery and blood loss [28]. In several cases, there were difficulties with aspiration of blood, particularly from the small cannula sited for the second and third blood
samples. This may have resulted in artificial activation of coagulation, and may have delayed the time taken to place the sample in the TEG device, but care was taken to start the analysis within 2 min in all cases. The ‘premature splitting’ in 19 samples led to exclusion of their alpha angles and CIs; although the correct alpha angles could have been measured manually, we felt that this could have been inaccurate. We have noted that other investigators have encountered the same problem [12]. We also did not control the volume of iv fluid administered intra-operatively, although Butwick et al. have observed no significant effects on TEG parameters following preloading with 1500 ml Hartmann’s solution [9]. Finally, we did not compare our reference ranges in pregnancy to those of matched non-pregnant female controls, instead using the manufacturer’s reference ranges for TEG and our haematology laboratory’s reference ranges for full blood count and standard coagulation tests. This decision was based on the additional time and resources that such a control group would require. We were therefore unable to compare directly the values from pregnant and non-pregnant groups, since we did not have access to the actual non-pregnant measurements, just the reference ranges, hence our use of single-sample t-tests. However, we were less concerned with the statistical comparison per se and more with the actual ranges from our patient population.

In conclusion, our reference ranges for TEG provide us with a useful comparator for healthy women undergoing caesarean section in our maternity unit, both pre- and postoperatively. For standard laboratory coagulation tests, non-pregnant reference ranges may be used for APTT, although we found a shorter PT and higher fibrinogen levels.

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