**In vitro** effects of local anaesthetics on the thromboelastographic profile of parturients

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**Background.** Post-dural puncture headache can be an incapacitating complication of obstetric epidural analgesia/anaesthesia and early or prophylactic epidural blood patch (EBP) is one of the treatment options. Although local anesthetic (LA) agents have been shown to have anticoagulation effects in *vitro*, peri-partum women are known to be hypercoagulable. We postulated that the presence of residual LA might not result in impaired haemostasis of the EBP in parturients.

**Methods.** Blood samples from 10 healthy term parturients were subjected to thromboelastography after the addition of four different LA (lidocaine, bupivacaine, levobupivacaine, and ropivacaine) preparations.

**Results.** There was a significant reduction in reaction (R) and coagulation (K) time (P<0.001, P<0.05) and an increase in α/γ angle (P<0.01) when comparing undiluted blood with the saline control group. Maximum amplitude (MA) and clot lysis (Ly30) did not change significantly despite the 50% dilution. The thromboelastographic parameters of all four LA-treated groups were no different from their saline controls and from each other.

**Conclusion.** At clinical dosages, LA did not cause any hypocoagulable changes on the thromboelastographic profile of healthy parturients.

**Keywords:** anaesthetic techniques, epidural; anaesthetics local, bupivacaine; anaesthetics local, levobupivacaine; anaesthetics local, lidocaine; anaesthetics local, ropivacaine; analgesic techniques, extradural; blood, coagulation; blood, epidural patch; measurement techniques, thromboelastography

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Up to 80% of parturients with inadvertent dural puncture for obstetric epidural block develop post-dural puncture headache (PDPH). Although conservative measures such as bed rest, adequate hydration, and simple analgesics have been the first line of treatment, autologous epidural blood patch (EBP), is one of the most effective treatments for PDPH. Early or prophylactic EBP, however, is not universally adopted. Local anaesthetics (LA) have been shown to significantly impair haemostasis in *vitro*. The anticoagulation effect of residual LA in the epidural space has been suggested to contribute to the higher failure rate of early EBP for non-obstetric patients.

However, peri-partum women have been shown to be hypercoagulable. The effects of LA on the coagulation profile of parturients and their impact on the success of early EBP remain unclear. We aim to determine if four commonly used LA will cause hypocoagulable changes on the thromboelastographic profile of term parturient at clinical doses.

**Methods**

With institutional approval and informed consent, blood samples were obtained from 10 healthy term parturient undergoing elective Caesarean section. Patient exclusion criteria were history of pregnancy-induced hypertension, haemostatic abnormalities (including anti-platelet or anticoagulation therapy), renal or liver dysfunction, smoking or alcohol intake.

Before induction of anaesthesia, an 18-G i.v. cannula was inserted in the operating room. Blood was collected using a two-syringe technique. The initial 2 ml of blood was discarded to prevent tissue thromboplastin contamination. The next 4.5 ml of blood was collected in a citrated tube (0.5 ml of 0.105 M-buffered sodium citrate, Vacutainer®, Becton Dickinson, Franklin Lakes, NJ, USA). Three computerized Thromboelastograph (TEG®) machines (Model 3000, Haemoscope Corporation, Skokie, IL, USA) with dual channels and standard calibrations were used to analyse the coagulation profile of all test specimens.
The following preservative-free LA were used as stock preparation for further dilution with saline 0.9%, bupivacaine 0.5% (Marclair®, AstraZeneca, North Ryde, NSW, Australia), levobupivacaine 0.5% (Chirocaine®, Abbott Laboratories, Queenborough, Kent, UK), ropivacaine 1% (Naropin®, AstraZeneca, North Ryde, NSW, Australia), lidocaine 1% (Xylocaine®, AstraZeneca, North Ryde, NSW, Australia).

From each blood sample, six different preparations were constituted for analysis as shown in Table 1. After incubating the test solution at 37°C for 2 min, 180 µl of citrated blood and 50 µl of 0.025 mmol litre⁻¹ calcium chloride were added to the pre-warmed TEG® metal cuvette. The TEG® pin was raised and lowered five times to ensure uniform mixing of the contents and agitation/activation of test specimens. All samples were run within 15 min of collection and at no point were they refrigerated so as to avoid cold activation of the blood samples.

The TEG® and the following five TEG® variables (R, K, α°, MA, Ly30) were recorded. Reaction time (R) is the distance measured from the beginning of the trace (time from sample placement in the cuvette) to the point of 2-mm divergence of the tracing amplitude. It corresponds to an early stage of clot formation and is influenced by activities of coagulation factors.

Coagulation time (K) is the time from the end of R to a point at 20-mm divergence in amplitude and represents the time the formed clot takes to achieve a fixed degree of viscoelasticity. The parameter K is influenced by the activities of coagulation factors, platelet count, platelet function, and fibrinogen level.

Alpha angle (α°) is the angle formed by the slope of the TEG® tracing from the R to the K value. This is an indicator of the rate of solid clot formation and is dependent on platelet count and fibrinogen.

Maximum amplitude (MA) is the width of the curve at its maximal divergence and represents the strength of the clot. It is influenced by platelet count and activity and correlates with tests of platelet aggregation responses to adenosine diphosphate and collagen.

Clot lysis (Ly30), defined as clot lysis at 30 min after the MA.

The Student’s paired t-test was used to compare the parameters from citrated blood and saline control groups. Analysis of variance (ANOVA) with post-hoc Tukey test was applied to data from LA-treated groups and their saline controls. Significance was taken at P<0.05.

### Results

In total, 60 TEG® analyses were performed for this study; the results are presented in Table 2. As anticipated, the TEG® parameters of undiluted blood of parturients suggest a hypercoagulable state when compared with the local normal range obtained from the general population. Comparing citrated blood with that of the saline control group, there was a further increase in coagulability as evidenced by a significant reduction in R and K time and an increase in α°. MA and Ly30 did not change significantly despite the 50% dilution.

Comparing LA-treated groups with the saline control group, there was no significant difference in R, K, MA, α°, or Ly30. All five TEG® variables were not significantly different between the different LA-treated groups either.

### Discussion

Autologous EBP was first used by Gormley for treatment of PDPH. The proposed mechanism is to prevent a decrease in...
subarachnoid pressure from cerebrospinal fluid (CSF) leak,\(^8\) and distension on pain-sensitive intracranial structures\(^7\) and distortion of venous sinuses\(^10\) by direct tamponade of the dural tear with a blood clot. This resulted in the restoration of CSF pressure and volume and the eventual repair of the dura.

Two separate case series reported a 100% efficacy for prophylactic EBP.\(^{11,12}\) In the series by Quaynor and colleagues, prophylactic EBP was instituted within 15 min of accidental dural puncture through the epidural needle inserted at a different interspace with 15–18 ml of autologous blood in seven non-obstetric patients. Five patients had received epidural anaesthesia before the patch and two received subarachnoid LA before the EBP. In three of these patients, further top-ups of LA were given through the epidural catheter. No spinal headache occurred in any of the patients. Ackerman and colleagues injected 15 ml of autologous blood via an epidural catheter inserted one interspace above the puncture site in six parturients within 15–20 min after delivery; none developed PDPH. However, Loeser reported a failure rate of 71% for early EBP within 24 h of puncture in patients receiving only i.v. maintenance fluids for treatment of PDPH, using only 10 ml of autologous blood.\(^{13}\) Palahniuk also reported no significant difference in the incidence of PDPH (54 vs 59%) after prophylactic EBP in obstetric patients when compared with conservative first-line management. There was no conversion to spinal analgesia, and no patient complained of a headache before prophylactic EBP. However, the decision for prophylactic EBP was made solely by the attending anaesthetist, using only 5–10 ml of autologous blood via the indwelling epidural catheter. Only 11 of the 86 parturients were selected for EBP while the rest received conservative management.\(^{14}\)

Residual LA at the time of EBP was postulated to induce hypocoagulopathy and further dilute the blood injected, impairing clot formation. Interestingly, our study showed that a 50% dilution of blood with saline in vitro produced a hypercoagulable state. This result is consistent with other studies.\(^{15,16}\) The greater impact of moderate haemodilution on the concentration of anti-coagulation factors (e.g. anti-thrombin III) than pro-coagulation factors was postulated to be the cause.\(^{17}\) The saline control group was used for comparison in order to account for the dilutional effect of LA and to specifically investigate the effect of LA on haemostasis.

In our institution, lidocaine 1.5% is used as an initial test dose for labour epidural analgesia; bupivacaine 0.1% or levobupivacaine 0.1% and ropivacaine 0.1–0.2% are used for continuous epidural infusion and a bolus of ropivacaine 0.25%, bupivacaine 0.25%, or levobupivacaine 0.25% is given for rescue. The LA volume and concentration in the epidural space at the time of EBP are difficult to determine as they are affected by CSF leak and the relative systemic absorption of LA and normal saline. We therefore chose to investigate the effects of lidocaine 0.5% and bupivacaine 0.25%, levobupivacaine 0.25%, and ropivacaine 0.25%. We used a 50% dilution admixture as it could occur at the time of early prophylactic EBP, that is 10–20 ml of epidural LA and 10–20 ml of blood for the epidural patch.

Our study showed no significant change in TEG\(^\circledast\) variables between LA-treated groups and their saline control, suggesting this is not the case in healthy term parturients. In fact, the LA-treated groups have TEG\(^\circledast\) variables consistent with a hypercoagulable state relative to that of the local general population.

It would be ideal to use fresh whole blood for TEG\(^\circledast\) analysis. However, as time was required to transport, aliquot, and further dilute the various admixtures before analysis, we opted to use re-calculated citrated blood without celite. In our study, all TEG\(^\circledast\) analyses were started within 15 min of venepuncture. Although Roche and colleagues had raised the issue of reliability of using citrated blood instead of fresh whole blood in studies of in vitro haemodilution, it was in the context of using stored citrated blood.\(^{18}\) Their samples were analysed 2 h after blood collection, which allowed time for contact activation to trigger the initial intrinsic pathway. We did not take into account the presence of CSF in the epidural space in this study. It was suggested that larger CSF leak at early stages post-dural puncture might have impaired coagulation.\(^{2}\) This was refuted when CSF was shown to have procoagulant effects greater than accountable by haemodilution alone.\(^{19,20}\) Hence, the presence of CSF would have accelerated clot formation. The effect of epinephrine, a procoagulant, was not investigated, as we do not routinely incorporate epinephrine into our labour epidural regime. We have also assumed uniform mixing of LA and blood in the epidural space after EBP.

When considering the factors that influence the success of an EBP, the size of the dura tear, the pressure gradient across it,\(^{21}\) the volume of blood injected and extent of its spread,\(^{22}\) the changes in craniospinal elasticity,\(^{23}\) and the presence of intrathecal amide LA\(^{24}\) need to be included. In vitro testing using TEG\(^\circledast\) does not take into account interactions between tissue factors and components of blood, limiting the extrapolation of our results to clinical practice. A randomized controlled clinical study should help to clarify the efficacy of early prophylactic EBP in the obstetric patient, given the advantages of early bonding of mother and child, timely ambulation, and discharge from hospital.

In conclusion, amide LA, at clinical dosages, did not cause any hypocoagulable changes on the thromboelastographic profile of healthy parturients, in vitro. This suggests that the presence of residual LA in the epidural space may not result in a higher failure rate when early prophylactic EBP is performed for obstetric patients with PDPH.

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