

# Effect of amniotic fluid on coagulation and platelet function in pregnancy: an evaluation using thromboelastography\*

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## Summary

Amniotic fluid embolism is a rare obstetric complication. The exact pathogenesis of this syndrome remains unknown and significant controversy exists whether coagulopathy should always be present. We used thromboelastography to assess the effect of amniotic fluid on coagulation and platelet function in pregnant women. Different volumes of amniotic fluid (10–60 µl) were added to blood (330 µl) from pregnant women and thromboelastography variables determined. There were three important findings. R time, reflecting time to first clot formation, was significantly decreased with the addition of 10 µl amniotic fluid; platelet function, as determined by Reopro-TEG technique, was increased with the addition of 30 µl of amniotic fluid; and there was no evidence of fibrinolysis in any samples studied. In conclusion, our study substantiates the hypothesis that coagulation profile changes are invariable accompaniments of amniotic fluid embolism.

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Amniotic fluid embolism (AFE) is an often devastating obstetric syndrome, occurring in 1 in 8000 to 1 in 80 000 pregnancies, with an overall mortality rate of 60–80%, and accounting for roughly 10% of all maternal deaths [1]. It usually presents suddenly in the peripartum period with acute onset shortness of breath or cardiovascular collapse, and it is often difficult to make a conclusive diagnosis because there are no distinctive clinical or pathological markers of the disease. Therefore, it has often been labelled anaphylactoid syndrome of pregnancy [2]. Although it is believed that all patients who survive AFE have either clinical or laboratory evidence of a consumptive coagulopathy [2], significant controversy exists whether coagulopathy should be an invariable accompaniment of AFE. Over 50% of animal studies have not demonstrated a coagulopathy [3], and it has been postulated that clear amniotic fluid (AF) may be insufficient to cause significant clinical intravascular coagulation [4].

The purpose of this study is to determine whether amniotic fluid affects coagulation and platelet function in pregnant women as determined by thromboelastography (TEG).

## Methods

After institutional review board approval from the Human Research Committee and written informed consent, we recruited 31 term parturients who were scheduled to undergo an elective Caesarean section and did not have prior bleeding or coagulation abnormalities. Exclusion criteria included a history of pregnancy induced hypertension, liver disease, or recent ingestion of antiplatelet or anticoagulant medications.

Venepuncture for complete blood count and TEG samples was performed at the time of insertion of an 18-gauge intravenous catheter. The first 2 ml of blood was discarded, followed by a 4.5 ml study sample collected into a citrated vacutainer (containing 0.5 ml of buffered citrate solution). All parturients underwent a Caesarean delivery under spinal anaesthesia. The obstetrician collected 10 ml of clear amniotic fluid using a syringe with an 18 G blunt needle inserted into the bulging amniotic sac after uterine incision.

Two physicians (M.H. and D.H.) were trained to perform TEGs, and measured all samples. Two Haemoscope dual-channel Thrombelastograph<sup>®</sup> analysers

(model 5000; Haemoscope Corp., Niles, IL) with four channels (two channels per analyser) and disposable plastic cups and pins were used for this study. Both analysers were calibrated daily. We added 1 ml of blood to the tubes containing 35  $\mu$ l of celite, producing 1% celite activated-blood. We then added 330  $\mu$ l celite-activated blood to each of the four analyser cups prewarmed to 37 °C and containing 20  $\mu$ l of 0.2 M calcium chloride. Calcium chloride was used to reverse the citrate, and celite activated blood was used to hasten TEG activity. In group 1, 0  $\mu$ l, 10  $\mu$ l, 20  $\mu$ l or 30  $\mu$ l of amniotic fluid was added to the blood samples from 10 parturients (four channels). In group 2, 0  $\mu$ l, 20  $\mu$ l, 40  $\mu$ l or 60  $\mu$ l of amniotic fluid was added to the blood sample of another 10 parturients (four channels). All samples were analysed within 30 min of the blood collection.

The effect of amniotic fluid on platelet function was studied using Reopro<sup>®</sup> (Eli Lilly, Indianapolis, IN) in group 3 (11 parturients). Reopro<sup>®</sup> is a platelet IIb/IIIa receptor antagonist (monoclonal antibody fragment c7E3 Fab) that impedes the platelet/fibrinogen interaction, thus blocking the platelet contribution to strengthening of the clot. Four TEG channels were used for this part of the study. We added 330  $\mu$ l citrated and celite-activated blood to each cup containing 20  $\mu$ l of 0.2 M calcium chloride. In addition, 30  $\mu$ l of AF was added to the blood samples of channels 3 and 4. Reopro<sup>®</sup> 5  $\mu$ l was added to channel 2 and 4 to determine platelet function in the

presence and the absence of amniotic fluid. Maximum amplitude (MA) was determined from each channel and platelet function was determined as follows:

- Platelet function without AF = MA (channel 1<sub>whole blood</sub>) – MA (channel 2<sub>whole blood + Reopro<sup>®</sup></sub>);
- Platelet function with AF = MA (channel 3<sub>whole blood+AF</sub>) – MA (channel 4<sub>whole blood + AF + Reopro<sup>®</sup></sub>).

We chose 30  $\mu$ l of AF based on the results of MA in groups 1 and 2.

The data in groups 1 and 2 were analysed using ANOVA with Bonferroni's correction for multiple comparisons to determine significant changes in each of the TEG parameters. Paired *t*-test was used to study platelet function of the blood with or without AF; *p* < 0.05 was considered significant.

## Results

We enrolled 31 parturients in the study. The mean (SD) age was 32.5 (5.3) years. The mean (SD) estimated blood loss was 800 (100) ml and none of the parturients received intra-operative blood transfusions. The mean (SD) haematocrit was 35.4 (3.5)%, and the mean (SD) platelet count was 221.2 (57.7)  $\times 10^9 \cdot l^{-1}$ . Tables 1 and 2 illustrate baseline (without amniotic fluid) and amniotic fluid TEG values, with TEG values demonstrating three important findings as compared to baseline. The *R* time was significantly decreased with the addition of

**Table 1** Baseline (blood) and amniotic fluid/blood TEG variables (0, 10, 20, 30  $\mu$ l amniotic fluid). Values are mean (SD) [range].

AF	R time; min	K time; min	$\alpha$ angle; °	M; mm	LY30; %	G; dyn.cm <sup>-2</sup>
0 $\mu$ l	8.5 (2.0) [5.5–11.5]	3.2 (0.8) [2.5–5.5]	68.2 (7.2) [56.0–75.5]	55.9 (3.6) [53.0–63.5]	0.5 (1.1) [0–1.0]	6426 (991) [4901–8698]
10 $\mu$ l	5.3 (1.7)* [3.0–7.0]	2.8 (1.0) [1.5–5.0]	72.1 (5.6) [61.5–76.5]	62.8 (8.4) [55.0–76.0]	3.3 (7.2) [0–3.5]	9056 (3329) [5416–10 384]
20 $\mu$ l	5.4 (2.3)* [2.5–7.5]	2.9 (0.7) [1.5–4.0]	70.6 (5.2) [61.0–74.5]	63.3 (7.1) [55.5–67.5]	3.1 (6.2) [0–3.5]	9034 (2403) [4901–10 384]
30 $\mu$ l	4.3 (1.5)* [2.5–5.5]	2.6 (0.6) [2.0–3.5]	72.6 (3.2) [68.0–77.5]	61.4 (7.5) [54.0–66.5]	2.7 (2.8) [0–4.5]	8369 (2439) [5869–9925]

TEG, thromboelastogram. AF, amniotic fluid. R-time, reaction time. K, time from R to a fixed level of clot firmness.  $\alpha$ , alpha angle. MA, maximal amplitude. LY30, percent lysis at 30 min after MA. G, shear elastic modulus.

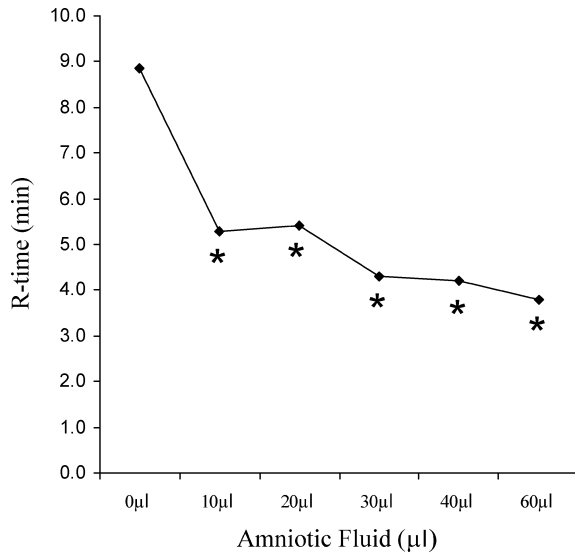
\**p* < 0.05, when compared to baseline (0  $\mu$ l).

**Table 2** Baseline (blood) and amniotic fluid/blood TEG variables (AF 0, 20, 40, 60  $\mu$ l) Values are Mean (SD) [range].

AF	R time; min	K time; min	$\alpha$ angle; °	M; mm	LY30; %	G; dyn.cm <sup>-2</sup>
0 $\mu$ l	9.2 (1.9) [6.0–12.5]	3.4 (1.0) [2.0–3.5]	67.7 (4.5) [60.0–70.5]	53.9 (3.7) [51.0–60.5]	1.2 (1.5) [0–2.5]	5909 (892) [4615–6494]
20 $\mu$ l	4.8 (1.5)* [3.0–6.5]	2.5 (0.7) [1.5–3.5]	73.0 (4.0) [68.5–78.5]	63.0 (6.3) [56.5–67.5]	3.1 (3.8) [0–3.5]	8864 (2456) [6765–10 384]
40 $\mu$ l	4.2 (1.3)* [2.5–6.0]	2.4 (1.1) [1.5–3.0]	73.8 (5.1) [71.5–76.5]	58.8 (5.9) [55.0–65.5]	3.4 (5.0) [0–4.0]	7372 (1750) [5989–10 151]
60 $\mu$ l	3.8 (1.2)* [2.5–5.5]	2.5 (1.1) [1.5–3.5]	73.7 (6.0) [6.08–78.5]	52.0 (6.8) [51.0–61.0]	2.9 (5.2) [0–5.5]	5587 (1346) [4259–7820]

TEG, thromboelastogram. AF, amniotic fluid. R-time, reaction time. K, time from R to a fixed level of clot firmness.  $\alpha$ , alpha angle. MA, maximal amplitude. LY30, percent lysis at 30 min after MA. G, shear elastic modulus.

\**p* < 0.05, when compared to baseline (0  $\mu$ l).



**Figure 1** Increasing amounts of amniotic fluid resulted in a decrease in the R-time. There was no additional change in the R-time with more than 30 µl of amniotic fluid. \*Significantly decreased when compared to baseline.

incremental amounts of amniotic fluid in both groups ( $p < 0.05$ ). Since there was no difference in the R-value at 0 µl and 20 µl between groups 1 and 2 (Student's *t*-test), the data from these two groups were combined to reflect the incremental effect of amniotic fluid (0–60 µl) on the R time (Fig. 1). There was a trend towards increasing MA with the addition of the smallest amounts of amniotic fluid (10 and 20 µl, Tables 1 and 2) although this did not reach statistical significance. However, the platelet function as determined in group 3 by the addition of Reopro<sup>®</sup> was significantly increased in the presence of 30 µl amniotic fluid: mean (SD) MA = 32.7 (10.1) mm vs. 22.7 (5.5) mm,  $p < 0.001$ . Finally, there was no fibrinolysis in any of the samples studied.

## Discussion

The aetiology of the coagulopathy associated with amniotic fluid embolism remains somewhat obscure, with investigations attempting to clarify the mechanism yielding inconclusive and sometimes contradictory results. Although amniotic fluid contains activated coagulation factors II, VII and X, the concentrations of these factors are well below those found in maternal serum at term [5]. Furthermore, amniotic fluid has been shown to have a direct factor X activating property and thromboplastin like effect [3, 6, 7]. Tissue factor present in amniotic fluid might be responsible for these effects, with potential sources being sloughed skin and epithelial cells derived from fetal respiratory, gastrointestinal and genito-

urinary tract mucosa [5]. Tissue factor activates the extrinsic pathway by binding with factor VII, and then triggers clotting by activating factor X. This activation of the clotting cascade in the pulmonary vasculature will cause local thrombin generation, which could then cause vasoconstriction, microvascular thrombosis and secretion of vascular endothelium. Although these suggested mechanisms explain some of the presenting features of AFE such as hypoxia and pulmonary hypertension, they do not elucidate the cause of the bleeding diathesis associated with AFE. There is controversy whether the aetiology of bleeding is due to a consumption coagulopathy or to massive fibrinolysis. Using TEG, we demonstrated that the addition of amniotic fluid to blood accelerated clot formation but there was no evidence of fibrinolysis (LY30%), suggesting that the primary cause of bleeding is a consumption coagulopathy. Thromboelastography is a sensitive test for the global assessment of coagulation. It measures the viscoelastic properties of blood and the strength of the clot is displayed graphically over time. TEG values include R time (time to first clot formation), K time (rate of clot strengthening) and  $\alpha$  angle (rate of clot strengthening), the maximum amplitude (MA) indicates the maximum strength of developed clot, LY30 (percent lysis at 30 min after MA is reached) and G (actual measure of clot firmness, measured in  $\text{dyn.cm}^{-2}$ ). In addition, it is a test of platelet function, plasma factor activity, and activators and inhibitors of coagulation [8–10].

Liu *et al.*, who used TEG to study the effect of mid- and final-trimester amniotic fluid (5, 10 and 20 µl) on blood, also determined that amniotic fluid significantly accelerated coagulation but did not cause fibrinolysis [11]. However, they studied the effect of amniotic fluid on the blood of female, non-pregnant volunteers. This is not an ideal patient population as AFE is specific to pregnancy and may even have an immunological component specific to parturients [12]. Furthermore, pregnancy itself is associated with increased coagulation factors as well as decreased inhibitor factors. The alpha angle ( $\alpha$ ), representing fibrinogen activity, was increased in their study. We did not find this change in our study, most likely due to the already increased  $\alpha$  in pregnant subjects as a result of increased fibrinogen during pregnancy [13].

Amniotic fluid contains enhanced levels of plasminogen activator, urokinase-like plasmin activator and thrombin–antithrombin complexes [14]. Increased fibrinolysis has been shown to occur in amniotic fluid embolism [15] and it has been suggested that a grossly elevated plasminogen activator inhibitor-1 antigen in the amniotic fluid may become active in the maternal blood and contribute to disseminated intravascular coagulation (DIC) in AFE [16]. However, our study did not

demonstrate an increase in fibrinolysis with the addition of amniotic fluid. We hypothesise that the increased markers of enhanced fibrinolytic activity observed in other studies may be due to the overall enhanced coagulation associated with AFE.

A trend towards an increased MA observed in our study, and by Liu *et al.* [11], may reflect increased platelet activation with the addition of amniotic fluid. This prompted us to assess platelet function specifically using Reopro<sup>®</sup>. Amniotic fluid enhanced platelet function significantly, as demonstrated by the increased MA platelet. This is an important finding, in addition to the procoagulant effect of AF in enhancing the coagulation cascade. This enhanced platelet function may be a result of term amniotic fluid causing irreversible platelet aggregation by enhancing platelet thromboxane B<sub>2</sub> production [17]. The thromboxane enhancing property of amniotic fluid appears to be distinct from its thrombin generating property [17]. Salem *et al.*, using centrifuged amniotic fluid that was concentrated 3–5 fold, found significant platelet aggregation as a result of a platelet aggregation factor, which was found to be type 1 collagen [18]. Although the authors of this study demonstrated procoagulant effect with both unconcentrated and concentrated amniotic fluid, no significant platelet aggregation was observed with unconcentrated amniotic fluid. However, in our study, using the TEG-ReoPro technique, we were able to demonstrate enhanced platelet function as a result of increased platelet activation produced by unconcentrated amniotic fluid.

It is interesting to note that despite our results demonstrating AF as being procoagulant and enhancing platelet function while not causing fibrinolysis, bleeding is the primary manifestation of amniotic fluid embolism. This is a result of the initial thromboplastic like effect where tissue factor triggers acute disseminated intravascular coagulation (DIC) via the extrinsic pathway. Tissue factor activates the conversion of factor VII to VIIa and forms VIIa/TF complex, which in turn initiates activation of factors IX and X, leading to thrombin formation and DIC [19]. The continuous exposure to excess tissue factor that typically occurs under disease conditions associated with DIC, exhausts the available tissue factor pathway inhibitor, leading to rampant thrombin generation, persistent feedback activation of factor XI by the generated thrombin, and hence virtually uncheckable ongoing fibrin generation [20]. This is often followed by fibrinolysis – unrelated to a direct effect of amniotic fluid, hypofibrinogenaemia, and a haemorrhagic state accompanied by a consumption coagulopathy.

Limitations of our study include the fact that TEG may not be sufficiently specific for platelet function; however, we used Reopro<sup>®</sup> to compensate for this. Also, some

might argue that different volumes of amniotic fluid may lead to different results, including fibrinolysis. Our study range of amniotic fluid, which corresponds to 160–850 ml amniotic fluid in a blood volume of 6 l, reveals no fibrinolysis. However, it is possible that prothrombotic effect can occur at lower concentrations, which requires further evaluation.

In conclusion, we used TEG to assess the effect of amniotic fluid on coagulation. TEG, unlike other tests of isolated portions of the coagulation cascade, assesses the interaction of clotting factors and cellular elements. Our results demonstrate that the presence of amniotic fluid in the blood of pregnant women causes a hypercoagulable state related to the procoagulant activity of amniotic fluid and to the enhanced platelet function. Therefore, activation of the coagulation cascade can be used as a marker in the diagnosis of amniotic fluid embolism.

## References

- 1 Morgan M. Amniotic fluid embolism. *Anaesthesia* 1979; **34**: 20–32.
- 2 Clarke SL, Hankins GD, Dudley DA, Didly GA, Porter TF. Amniotic fluid embolism: analysis of the national registry. *American Journal of Obstetrics and Gynecology* 1995; **172**: 1158–67.
- 3 Clark SL. New concepts of amniotic fluid embolism: a review. *Obstetrical and Gynecological Survey* 1990; **45**: 360–8.
- 4 Phillips LL, Davidson EC. Procoagulant properties of amniotic fluid. *American Journal of Obstetrics and Gynecology* 1972; **113**: 911–9.
- 5 Lockwood CJ, Bach R, Guha A, Zhou XD, Miller WA, Nemerson Y. Amniotic fluid contains tissue factor, a potent initiator of coagulation. *American Journal of Obstetrics and Gynecology* 1991; **165**: 1335–41.
- 6 Hastwell GB. Accelerated clotting time: an amniotic fluid thromboplastic activity index of fetal maturity. *American Journal of Obstetrics and Gynecology* 1978; **131**: 650–4.
- 7 Yaffe H, Eldor A, Hornshtein E, Sadovsky E. Thromboplastic activity in amniotic fluid during pregnancy. *Obstetrics and Gynecology* 1977; **50**: 454–6.
- 8 Kaufmann CR, Dwyer KM, Crews JD, Dols SJ, Trask AL. Usefulness of thromboelastography in assessment of trauma patient coagulation. *Journal of Trauma* 1997; **42**: 716–22.
- 9 Kang YG, Martin DJ, Marquez J, *et al.* Intraoperative changes in blood coagulation and thromboelastographic monitoring in liver transplantation. *Anesthesia and Analgesia* 1985; **64**: 888–96.
- 10 Tuman KJ, Spiess BD, McCarthy RJ, Ivankovich AD. Comparison of viscoelastic measures of coagulation after cardiopulmonary bypass. *Anesthesia and Analgesia* 1989; **69**: 69–75.
- 11 Liu E, Shailaja S, Koh S, Lee T. An assessment of the effects on coagulation of midtrimester and final-trimester amniotic

- fluid on whole blood by thromboelastograph analysis. *Anesthesia and Analgesia* 2000; **90**: 333–6.
- 12 Scott JR, Beer AE, Guy LR, Liesch M, Elbert G. Pathogenesis of Rh immunization in primigravidas. Fetomaternal versus maternofetal bleeding. *Obstetrics and Gynecology* 1977; **49**: 9–14.
  - 13 Sharma SK, Philip J, Wiley J. Thromboelastographic changes in healthy parturients and postpartum women. *Anesthesia and Analgesia* 1997; **85**: 94–8.
  - 14 Koh SCL, Anandakumar C, Arulkumaran S, et al. Amniotic fluid plasminogen activators and inhibitors and TAT-complex levels during 2nd trimester pregnancy and labour. *Fibrinolysis* 1995; **9**: 121–6.
  - 15 Ratnoff OD, Vosburgh GH. Observations on the clotting defect in amniotic fluid embolism. *New England Journal of Medicine* 1952; **247**: 970.
  - 16 Estelles A, Gilabert J, Andres C, Espana F, Aznar J. Plasminogen activator inhibitors type 1 and type 2 and plasminogen activators in amniotic fluid during pregnancy. *Thrombosis and Haemostasis* 1990; **64**: 281–5.
  - 17 Stuart M, Wu M, Sunderji S, Ganley C. Effect of amniotic fluid on platelet thromboxane production. *Journal of Pediatrics* 1987; **110**: 289–92.
  - 18 Salem HH, Walters WA, Perkin JL, Handley CJ, Firkin BG. Aggregation of human platelets by amniotic fluid. *British Journal of Obstetrics and Gynaecology* 1982; **89**: 733–7.
  - 19 Uzynski M, Zekanowska E, Uzynski W, Kuczynski J. Tissue factor (TF) and tissue factor pathway inhibitor (TFPI) in amniotic fluid and blood plasma: implications for the mechanism of amniotic fluid embolism. *European Journal of Obstetrics, Gynecology and Reproductive Biology* 2001; **95**: 163–6.
  - 20 Osterud B, Bjorklid E. The tissue factor pathway in disseminated intravascular coagulation. *Seminars in Thrombosis and Hemostasis* 2001; **27**: 605–17.