

# Assessing Platelet and Fibrinogen Contribution to Clot Strength Using Modified Thromboelastography in Pregnant Women

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The monoclonal antibody fragment c7E3 Fab (ReoPro<sup>®</sup>), by binding to platelet surface fibrinogen receptors (glycoprotein, GPIIb/IIIa), inhibits platelet aggregation and its interaction with fibrinogen. In this study, we used thromboelastography with ReoPro<sup>®</sup> to evaluate the independent contribution of fibrinogen and platelets to clot strength. Thromboelastography was performed in 21 healthy, term parturients scheduled for elective cesarean delivery with 360  $\mu$ L of celite-activated whole blood and with 5  $\mu$ L of (2 mg/mL) ReoPro<sup>®</sup> added to 355  $\mu$ L of celite-activated whole blood. The contribution of platelets to clot strength (MA<sub>plt</sub>) was derived by subtracting MA<sub>fib</sub> (maximal amplitude with ReoPro<sup>®</sup>) from MA<sub>wb</sub> (maximal amplitude with whole blood). Thus, MA<sub>wb</sub> –

MA<sub>fib</sub> = MA<sub>plt</sub>. The value for MA<sub>wb</sub> (mean  $\pm$  SD) was  $73 \pm 4$  mm, for MA<sub>fib</sub> it was  $33 \pm 5$  mm, and for MA<sub>plt</sub> it was  $40 \pm 3$  mm. The contribution of fibrinogen and platelets to the MA<sub>plt</sub> was 45% and 55%, respectively. Modified thromboelastography using ReoPro<sup>®</sup> in healthy parturients can be used to determine the contribution of fibrinogen and platelets to blood clot strength. **Implications:** Determining the independent contribution of platelets and fibrinogen to the maximal amplitude of thromboelastography using c7E3 Fab may further improve the use of thromboelastography in detecting and treating coagulation defects.

(Anesth Analg 1999;89:1453–5)

**T**hromboelastography is useful for evaluating hemostasis and managing clotting dysfunction during major surgical procedures, and its use has been associated with a reduced and judicious use of blood products (1). The use of thromboelastography to evaluate and manage coagulation dysfunction has recently been extended to obstetric practice also (2,3).

The maximal amplitude (MA) of thromboelastography using whole blood (MA<sub>wb</sub>), measures clot strength and represents the collective contribution of both fibrinogen and platelets (4). However, MA<sub>wb</sub> provides no information about the independent contribution of platelets and fibrinogen to clot strength. Such information could be used for targeted blood component therapy in the management of coagulation dysfunction. The monoclonal antibody fragment c7E3 Fab (ReoPro<sup>®</sup>), by binding to platelet surface fibrinogen receptors (glycoprotein, GPIIb/IIIa), inhibits platelet aggregation and its in-

teraction with fibrinogen. Thromboelastography with ReoPro<sup>®</sup> (modified thromboelastography) has been used to evaluate platelet function (5,6).

We used modified thromboelastography to evaluate the independent contribution of fibrinogen and platelets to clot strength in healthy, term parturients.

## Methods

After institutional review board approval and informed consent, 21 healthy, term parturients scheduled for elective cesarean delivery were included in this study. Women with a history of coagulation disorders, preeclampsia, hemorrhage, and women receiving magnesium sulfate, aspirin, or heparin therapy were excluded. Ten mL of fresh blood was drawn from each patient from a peripheral vein via an 18-gauge needle using a two-syringe technique. The first sample was discarded to avoid tissue contamination of blood, while the second sample was used for thromboelastographic measurements, complete blood counts, and plasma fibrinogen levels. The plasma fibrinogen concentration was measured by the Clauss quantitative fibrinogen assay using thrombin derived from bovine plasma (Ortho Diagnostic System Inc., Raritan, NJ).

Presented in part at the annual meeting of the American Society of Anesthesiologists, Orlando, FL, October, 1998.

Accepted for publication August 4, 1999.

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**Table 1.** Thromboelastographic Variables

	Whole blood <i>n</i> = 21	c7E3 Fab (ReoPro®) <i>n</i> = 21
r (mm)	8.80 ± 2.71 (3.0–13.0)	9.27 ± 2.73 (3.5–13.5)*
k (mm)	3.12 ± 0.66 (2.0–4.5)	7.87 ± 3.94 (3.5–22.5)*
α angle (°)	70.37 ± 3.78 (64.0–76.5)	54.20 ± 8.92 (31.5–74.0)*
MA (mm)	72.57 ± 3.57 (66.0–79.0)	33.17 ± 4.93 (24.5–41.5)*
CI	2.88 ± 1.96 (–2.5–+6.9)	–3.87 ± 2.43 (–9.0–+1.90)*

Values are mean ± SD (range).

r = reaction time (normal range, 3–11 mm), k = clot formation time (normal range, 2–5 mm), α angle = clot formation rate (normal range 60°–80°), MA = maximal amplitude (normal range, 55–80 mm), and CI = coagulation index (normal range, –3 to 3).

\* *P* < 0.05.

Thromboelastography was performed with 360 μL of 1% celite-activated whole blood and with 5 μL of (2 mg/mL) ReoPro® added to 355 μL of 1% celite-activated whole blood using disposable plastic cups and pins. The test was performed within 4 min of blood collection on two separate channels of a preheated Thromboelastograph (TEG®; Hemoscope Corp, Skokie, IL). Thromboelastographic variables collected included reaction time (r), clot formation time (k), MA (maximal amplitude-clot strength), and clot formation rate (α angle). These were obtained from respective thromboelastographic tracings for both celite-activated whole blood and that with ReoPro®. The contribution of platelets to clot strength (MA<sub>plt</sub>) was derived by subtracting MA<sub>fib</sub> (maximal amplitude with ReoPro®) from MA<sub>wb</sub> (MA<sub>wb</sub> – MA<sub>fib</sub> = MA<sub>plt</sub>). A thromboelastographic coagulation index (TEG CI), which reflects overall blood coagulability, was derived from a linear equation that combines all thromboelastographic variables (TEG CI = –[0.3258]r + [0.1886]k + [0.1224]MA + [0.0759]α – 7.7922, normal range for nonpregnant women = 2 to –2) (7,8).

Data were analyzed using SPSS statistical software (SPSS for windows version 8.0, Chicago, IL). Analysis of parametric data was performed using paired Student's *t*-test, and a *P* value ≤ 0.05 was considered significant. Linear regression analysis was used to examine the correlation between plasma fibrinogen levels and MA<sub>fib</sub> and platelet counts and MA<sub>plt</sub>.

## Results

Demographic and laboratory data are as follows (mean ± SD), age: 29.0 ± 7 yr, height: 158 ± 11 cm, weight: 79 ± 11 kg, gestational age: 39 ± 1 wk, hematocrit: 35 ± 2 (%), platelet count: 195 ± 63 × 10,000/mm<sup>3</sup>, and fibrinogen level: 468 ± 68 mg/dL. The amount of blood loss for all patients was within the normal range (750–1000 mL).

Thromboelastographic data for these women are shown in Table 1. The r and k values were significantly prolonged, whereas α angle, MA, and TEG CI

were significantly reduced with ReoPro® compared with whole blood thromboelastography without ReoPro®. The value for MA<sub>wb</sub> [mean ± SD (range)] was 73 ± 4 mm (66–79 mm), that for MA<sub>fib</sub> (MA with ReoPro®) was 33 ± 5 mm (25–42 mm), and that for MA<sub>plt</sub> (MA<sub>wb</sub> – MA<sub>fib</sub>) was 40 ± 3 mm (34–45 mm). The contribution of fibrinogen and that of platelets to the MA<sub>wb</sub> was 45% (range 35%–55%) and 55% (range 45%–65%), respectively.

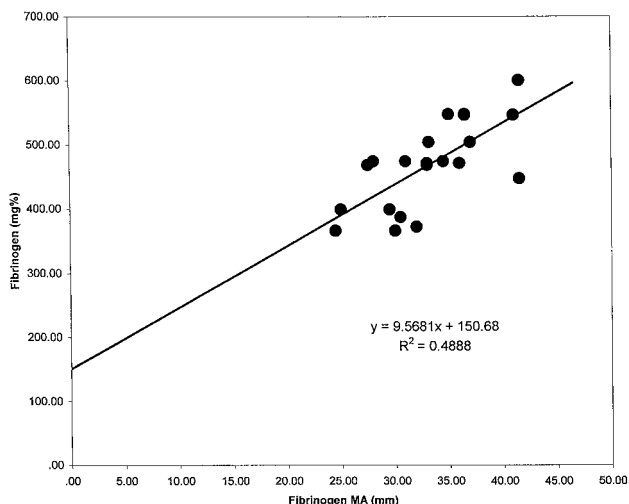
Linear regression analysis revealed MA<sub>fib</sub> as a significant predictor of the plasma fibrinogen level, with an adjusted (calculated through the origin) *r*<sup>2</sup> value of 0.49, and a slope of *y* = 9.56*x* + 150.68 (Figure 1). However, there was no correlation between MA<sub>plt</sub> and the platelet count (*r*<sup>2</sup> value of 3E-05, and a slope of *y* = –0.104*x* + 199.4) (Figure 2).

## Discussion

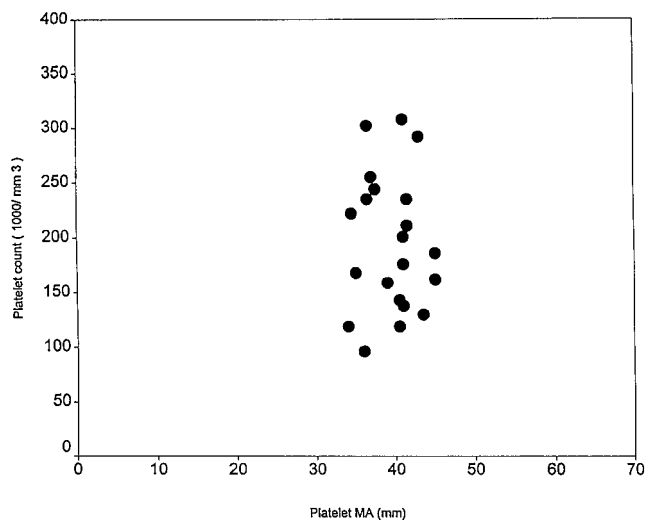
Modified thromboelastography enabled us to determine the independent contribution of fibrinogen and platelets to the clot strength of whole blood in healthy pregnant women at term, which was 45% and 55%, respectively. MA in the presence of ReoPro® has a strong correlation with plasma fibrinogen concentrations and, hence, may be used to predict the plasma fibrinogen concentration.

Thromboelastography measures whole blood coagulation by delineating the interaction between platelets, fibrinogen, and other clotting factors (4). Individual thromboelastographic variables include r, k, α angle, and MA, which is an indication of clot strength. Both platelets and fibrinogen contribute to the development of MA (4). Therefore, in a patient with a coagulopathy, the MA of whole blood alone does not indicate whether the coagulopathy is caused by platelet dysfunction or hypofibrinogenemia.

With modified thromboelastography, a monoclonal antibody fragment ReoPro®, which binds to platelet surface fibrinogen receptors (glycoprotein, GP IIb/IIIa), is used to inhibit platelet aggregation and its interaction with fibrinogen. Therefore, by excluding the platelet contribution, MA in the presence of



**Figure 1.** A Linear regression slope indicating the relation between maximal amplitude using c7E3 fab (ReoPro<sup>®</sup>) (MA) and plasma fibrinogen concentration. The line was drawn using the least square method.



**Figure 2.** Scattergram of platelet maximal amplitude (MA) versus platelet count. There was no correlation between the two variables.

ReoPro<sup>®</sup> gives information about the fibrinogen contribution (MAfib) to clot strength. The platelet contribution to clot strength (MAplt), can thereby be derived by subtracting MAfib from MAwb (MAwb – MAfib = MAplt). In this way, we determined the independent contribution of platelets and fibrinogen to whole blood MA. Such information about the platelet and fibrinogen contribution to clot strength can also be obtained by comparing thromboelastography with platelet-rich and platelet-poor plasma (5).

Modified thromboelastography was associated with a significant reduction in all thromboelastographic variables of coagulation compared with thromboelastographic variables with whole blood alone. This was the

result of the exclusion of the effect of platelets on coagulation by ReoPro<sup>®</sup>. More importantly, the use of ReoPro<sup>®</sup> in thromboelastography allowed us to determine the independent contribution of fibrinogen and platelets to clot strength (MA). This information can be used to determine precise defects in hemostasis and can thus allow targeted blood component therapy. There was a strong correlation between plasma fibrinogen concentrations and MAfib. Therefore, the plasma fibrinogen concentration can be extrapolated from the regression equation. However, no such correlation was noticed between platelet counts and MAplt. This suggests that the quality of platelet function, and not the platelet count alone, is responsible for clot strength.

In conclusion, modified thromboelastography using ReoPro<sup>®</sup> in healthy parturients can be used to determine the contribution of fibrinogen and platelets to clot strength. Furthermore, plasma fibrinogen concentrations from MAfib can also be predicted. Further studies are warranted to determine whether modified thromboelastography using ReoPro<sup>®</sup> can be used in targeted blood component therapy and in determining the adequacy of platelet function in pregnant women with thrombocytopenia who request regional analgesia/anesthesia.

The authors thank Jackie Wiley, RN, for assistance in data collection and Vladislav Markin, PhD, for assistance in statistical analysis.

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