

Hemostatic Function in Healthy Pregnant and Preeclamptic Women: An Assessment Using the Platelet Function Analyzer (PFA-100®) and Thromboelastograph®

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BACKGROUND: The PFA-100® is a point-of-care platelet function analyzer which measures the speed of formation of a platelet plug *in vitro*, expressed as closure time (CT) in seconds. This device could potentially be used to assess primary hemostasis prior to regional anesthesia. In this prospective, observational study we sought to establish 95% reference intervals for PFA-100 and Thromboelastograph® (TEG®) values for our normal pregnant population, before comparing the PFA and TEG in measuring platelet function in preeclamptic and healthy pregnant women at term, using confidence interval analysis and analysis of variance.

METHODS: Routine hematologic and coagulation tests were performed along with von Willebrand Factor, CT, and TEG measurements. Results are expressed as mean (SD).

RESULTS: Increased severity of preeclampsia was associated with increasing prolongation of CT, even in the presence of normal platelet counts. In severe preeclampsia, the PFA-100 CT (mean (SD): 155 (65) s) exceeded the 95% reference interval of the control group (70–139 s). In contrast, TEG maximum amplitude (MA) in severe preeclampsia (mean (SD): 71 (8) mm) remained within the 95% reference interval for MA in normal pregnancy (64–82 mm).

CONCLUSION: We conclude that impairment of primary hemostatic function with increasing severity of preeclampsia was recorded by the PFA-100 but not the TEG.

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The ability to arrest bleeding, whether from a small puncture or a blood vessel, is largely a function of primary hemostasis, the formation of a platelet plug; although other mechanisms are involved as part of the global hemostatic process. The process is dependent on sufficient numbers of functional platelets. Normal pregnancy may be associated with a lower mean platelet count than nonpregnancy (1,2), and approximately 30%–50% of cases of severe preeclampsia are associated with thrombocytopenia (platelet count $<150,000/\text{mm}^3$) (3,4). Excessive platelet activation by dysfunctional endothelium, as a result of abnormal nitric oxide, prostaglandin and endothelin release and metabolism, accounts for increased platelet turnover, and ultimately, reduced numbers. Work with flow cytometry, using a variety of activation markers, confirms that this abnormal activation is associated specifically with preeclampsia, as opposed to normal

pregnancy or essential hypertension during pregnancy (5). The existence of intrinsic platelet dysfunction in preeclampsia, independent of number, is supported by aggregometry studies demonstrating reduced aggregation in severe preeclampsia compared with normal pregnancy, perhaps because of degranulation as a result of the excessive activation (6).

Regional techniques are popular in obstetric anesthetic practice, but may be withheld from thrombocytopenic women so as to avoid intraspinal bleeding. Anesthesiologists administering regional anesthesia tend to follow local policies on safe lower limits of platelet count. One review suggests a platelet count of $75,000/\text{mm}^3$ as a realistic value above which regional anesthesia may be safely performed (7). Limited data preclude a precise threshold, although most institutions would recommend between $50,000$ and $100,000/\text{mm}^3$. A survey of United Kingdom anesthesiologists' records reported that about 70% would withhold an epidural with platelet counts $<80,000/\text{mm}^3$ and only about 5% would administer one at counts of $<50,000/\text{mm}^3$ (8).

The Thromboelastograph® (TEG®) (Hemoscope, Skokie, IL), shown to be useful in assessing perioperative coagulation in liver and cardiac surgery (9), has been used to propose platelet count guidelines based on the TEG variables observed in preeclamptic thrombocytopenia (4,10). However, an editorial comment questions the value of TEG as an assessment of primary

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hemostasis before obstetric regional blockade (11,12), since standard TEG parameters are not specific for platelet activity.

The PFA-100® (Dade-Behring, Marburg, Germany) is a point-of-care platelet function analyzer which simulates primary hemostatic capacity (PHC) by recording the time taken (closure time, CT) for a platelet plug to occlude a 150- μ m aperture in a collagen membrane coated with platelet activator (adenosine diphosphate or epinephrine). CT has been shown to be dependent on platelet count, hematocrit, von Willebrand Factor concentration and activity, shear, and glycoprotein Ib and glycoprotein IIb/IIIa function (13). It is coagulation and fibrinogen independent, since hemophilia A and B, Factor VII deficiency, and afibrinogenemia all produce normal CTs (14).

The PFA-100 has been validated against clinical and laboratory tests and found to be as sensitive and specific as platelet aggregometry the current "gold standard" (15). It has been proposed as a replacement for template bleeding time as first-line screening of platelet dysfunction (13). The ability of the PFA-100 to determine preoperatively the risk of postoperative bleeding has not been assessed, although it has been demonstrated to identify individuals with impaired hemostasis preoperatively (16).

The aim of this study was first to establish a reference range for the PFA-100 for healthy parturients, and second, to compare the performance of the PFA-100 and TEG in measuring platelet function in preeclamptics and healthy parturients.

METHODS

After ethics committee approval and written informed consent, 93 healthy term women (presenting for induction of labor or elective cesarean delivery) and 50 preeclamptic women admitted to the delivery suite were recruited between April 2000 and February 2001. The preeclamptic women were subdivided on presentation into mild or severe, in accordance with American College of Obstetrics and Gynecology guidelines (17) and criteria established in comparable studies (3,10). Mild preeclampsia was defined as systolic blood pressure >140 mm Hg and diastolic blood pressure >90 mm Hg on two separate readings 4 h apart, and proteinuria (1+/2+) on dipstick measurements. Severe preeclampsia was diagnosed on the presence of a systolic blood pressure >160 mm Hg or a diastolic blood pressure >110 mm Hg on two readings and proteinuria. Patients with a known hemorrhagic diathesis, or who had taken nonsteroidal antiinflammatory drugs in the previous 7 days, were excluded.

Twenty milliliters blood samples were taken through a 16-gauge sheath at venous cannulation on admission to the labor suite for the preeclamptic women and induction controls, and immediately preoperatively for the elective cesarean delivery controls. Routine

laboratory tests performed for controls and preeclamptic women included hematocrit and platelet count. Three 105- μ mol 3.2% buffered citrate blood sample tubes were filled: one for a standard clotting screen (prothrombin time, activated partial thromboplastin time [APTT] international normalized ratio) and fibrinogen concentration, one for von Willebrand Factor antigen and activity measurement and one for use on the PFA. For TEG, 360 μ L of 1% celite-activated blood was pipetted into a prewarmed (37°C) plastic cuvette within a TEG series 3000 and measurement started within 4 min of blood collection. The parameters measured included reaction time, clot formation time, clot formation rate, and maximum amplitude (MA). For PFA-100 analysis, 800 μ L of citrated blood was pipetted into a test cartridge containing epinephrine as a platelet activator, and the CT measured. The TEG and PFA tests were performed by two trained operators (JD and RF). At the time this study was conducted, magnesium sulfate was not routinely used for seizure prophylaxis in our institution; when administered, the patients were included and the magnesium levels were recorded.

All results are expressed as mean \pm SD, and where appropriate, differences between sample means are presented with 95% confidence intervals (CI). Comparisons were also made using analysis of variance (ANOVA). *Post hoc* analyses were performed using the Tukey Kramer multiple comparisons test. Linear regression analysis was used to evaluate any relationship between platelet count and CT. A *P* value of <0.05 was considered significant.

Ninety-five percent reference intervals for CT and MA in normal pregnant subjects were established using the mean \pm 1.96SD after confirming a normal distribution. Ninety-five percent CI for the upper and lower limits of the reference interval for both CT and MA were calculated from the limit \pm SE of the percentile (18).

Statistical analysis was performed using Statview 4.5 (Abacus Concepts, Berkeley, CA) and the Confidence Interval Analysis package provided with Statistics with Confidence 2nd Edition (Altman D, et al., 2000, BMJ Books).

RESULTS

Ninety-three healthy controls were recruited for the study. These were used to establish a 95% reference interval with 95% CI for CT and TEG MA (Table 1) for our local healthy pregnant population.

In addition to the 93 controls, we enrolled 50 preeclamptic women (23 with mild preeclampsia [mild PET]; 27 with severe preeclampsia [severe PET]) producing three different study groups from 143 subjects. Age, weight, and height data were similar across the three groups (Table 2). Both PET groups were delivered at a significantly lower mean gestational age than the controls, reflecting the obstetric

Table 1. Ninety-five % Reference Interval and 95% Confidence Limits for PFA-100® Closure Time and Thromboelastograph® Maximum Amplitude in Healthy Pregnant Patients

	Closure time (s) (n = 93)	Maximum amplitude (mm) (n = 93)
Mean (sd)	105 (18)	73 (5)
95% Reference interval	70–139	64–83
95% CI for lower; upper limits	64–76; 133–145	63–66; 81–84

Table 2. Patient Characteristics of the Control and Preeclamptic (PET) Groups

	Controls (n = 93)	Mild PET (n = 23)	Severe PET (n = 27)
Age (yr)	33 (4)	33 (5)	33 (5)
Weight (kg)	79 (16)	80 (12)	79 (11)
Height (cm)	162 (7)	162 (8)	161 (5)
Gestational age (wk)	38 (2)*	37 (3)	36 (3)

* P < 0.05 versus mild and severe PET groups.

All values mean (±sd).

management of the condition. Routine hematological test results (Table 3) show significantly reduced mean platelet counts in the severe PET women compared with the controls: 6 of the 93 controls (6.4%), 3 of the 23 mild PET women (13%), and 10 of the 27 severe PET women (37%) had platelet counts <150,000/mm³. APTT and fibrinogen values in the severe PET subjects remained within the reference ranges for our institution (28–38 s for APTT; 1.5–4.0 g/L for fibrinogen). In all groups, values for von Willebrand Factor activity and antigen exceeded the normal ranges established by our laboratories (50–150 IU) and indeed were more than double the upper limit of the normal reference range. There were higher values in the PET groups, but the differences were only statistically significant for antigen.

Of the four TEG variables, no significant differences were observed between the control and mild PET groups (Table 4). MA did not differ among the groups, and indeed, was hypercoagulable in relation to the nonpregnant normal range supplied by the TEG manufacturers (59–68 mm). The mean CT of the PFA-100 (Table 4), the time taken to form an effective platelet plug, was significantly prolonged in severe PET compared with both the controls and mild PET women, and exceeded the 95% CI for the upper limit of the normal range. In six severe PET women, the CT was at least double the mean value for pregnancy. In summary, severe preeclampsia was associated with CT values exceeding the normal range for pregnancy, but with MA values normal for pregnancy.

With three severe PET women, but no mild PET women or controls, the CT values exceeded the PFA-100 test time limit of 300 s (registered as >300 by the machine). For the purposes of data analysis, they have

Table 3. Hematological and Clotting Variables

	Controls (n = 93)	Mild PET (n = 23)	Severe PET (n = 27)
Hct (%)	35 (4)	34 (4)	33 (5)
Platelet count (×1000/mm ³)	257 (89)	230 (83)	177 (81)*
PT (s)	13.2 (0.9)	13.2 (0.9)	13.3 (1.2)
APTT (s)	28.7 (3.0)	30.2 (4.3)	31.3 (3.4)†
Fibrinogen (g/L)	4.4 (1.0)	4.1 (1.0)	4.0 (0.9)
VWF activity (IU/L)	312 (97)	335 (119)	360 (93)
VWF antigen (IU/L)	349 (105)	371 (122)	409 (110)‡

Hct = hematocrit; PT = prothrombin time; APTT = activated partial thromboplastin time; VWF = von Willebrand Factor; PET = preeclamptic.

Data are mean (±sd).

* P < 0.001 (control versus severe PET).

† P < 0.01 (control versus severe PET).

‡ P < 0.05 (control versus severe PET).

Table 4. Thromboelastography and PFA-100® Results

	Controls (n = 93)	Mild PET (n = 23)	Severe PET (n = 27)
r (mm)	4.5 (1.8)	4.9 (1.9)	5.2 (2.5)
K (mm)	1.4 (0.5)	1.4 (0.4)	2.0 (1.7)*
MA (mm)	73 (5)	73 (5)	71 (8)
α-angle (°)	70 (9)	71 (6)	66 (11)
PFA-100® CT (s)	105 (18)	115 (22)	155 (65)†

r = reaction time; K = clot formation time; α-angle = clot formation rate; MA = maximum amplitude (clot strength); CT = closure time.

All data are mean (±sd).

* P < 0.01 (control versus severe PET); P < 0.05 (mild PET versus severe PET).

† P < 0.001 (severe PET versus both mild PET and controls).

been ascribed values of 300 s. Their corresponding MA values were 40, 61.5, and 70 mm. Two of these three preeclamptic women admitted to the delivery suite with a clinical diagnosis of preeclampsia went on to develop HELLP syndrome and recorded the lowest platelet counts (46,000 and 51,000/mm³) of all subjects in the study. One HELLP patient had impaired clotting (APTT [40.8 s] and MA [40 mm]); and relatively reduced fibrinogen [2.5 g · L⁻¹], but these variables returned to within normal ranges after administration of 15 mL · kg⁻¹ fresh frozen plasma (FFP). No platelets were transfused. The CT, however, did not return to normal after the FFP, and continued to exceed 300 s.

The relationship between CT and platelet count in the three study groups is presented graphically in Figure 1. This demonstrates that in the control and mild PET groups CT was not a function of platelet count (control group R² = 0.004, P = 0.55; mild PET group R² = 0.015, P = 0.57), and that there was a tendency to higher CT values in the severe PET group at normal as well as low platelet counts (R² = 0.37, P < 0.001).

In *post hoc* analysis performed to determine the degree to which the lengthening CT could be attributed to low platelet counts, patients with platelet

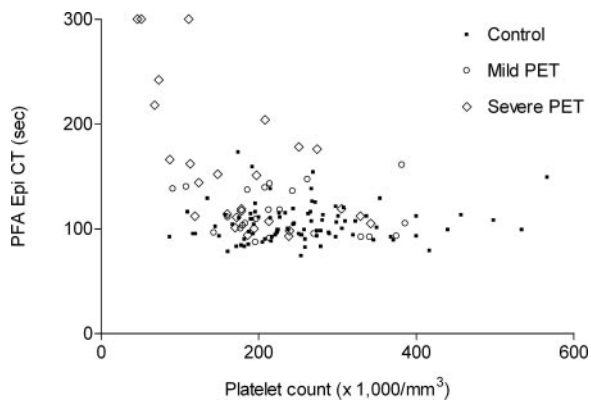


Figure 1. Platelet count versus closure time (CT) by subject group (control, mild PET, severe PET). Data are mean values for all subjects in each group. Linear regression analysis: Control $R^2 = 0.004$, $P = 0.55$; mild PET $R^2 = 0.015$, $P = 0.57$; severe PET $R^2 = 0.37$, $P < 0.001$. Control = healthy pregnant control patients; mild PET = mild preeclampsia; severe PET = severe preeclampsia.

counts lower than $100,000/\text{mm}^3$ were excluded and the resultant groups reanalyzed. Of these patients with a platelet count more than $100,000/\text{mm}^3$, the controls ($n = 92$) had a mean (SD) CT of 105 (18) s, the mild PET subjects ($n = 22$) had a mean (SD) of 114 (22) s, and the severe PET subjects ($n = 22$) had a mean (SD) of 135 (48) s. The severe PET result was significantly different from the control group ($P < 0.0001$), confirming a reduction of hemostatic function independent of platelet number.

Magnesium was administered to four severe and three mild PET patients. Magnesium blood levels were performed on the same blood samples used to measure TEG and PFA values. Five of these patients had levels in excess of the normal physiological range. If excluded from the data as a potential confounding variable, there were no significant differences among the groups in terms of MA, whereas there were significant differences in CT between severe PET and control patients. The mean (SD) for MA was 74 (5) and 71 (8) for the mild PET and severe PET groups, respectively. The mean (SD) for CT was 116 (22) and 147 (66) for the mild PET and severe PET groups ($P < 0.001$, severe PET versus control).

DISCUSSION

This study provides a normal range of PFA CT and TEG MA for our healthy pregnant populations. It demonstrates diminishing PHC with increased PET severity registered by CT but not MA. In severe cases of PET accompanied by low platelet counts, the PFA has recorded failure of PHC where TEG values remained normal. It also provides evidence of a reduction in platelet function associated with preeclampsia.

The study confirms the hypercoagulable profile of normal term pregnancy on standard coagulation parameters and TEG, with the mean MA of 73 mm approximating the value of 72 mm presented by Sharma et al. (19). The mean CT of the normal

pregnant population (105 s) is lower than that of the nonpregnant population, as established by Bock et al. (110 s) (20), suggestive of enhanced primary hemostatic function. A reduction of CT during pregnancy has been demonstrated (21), but our study is the first to generate reference intervals for pregnancy.

Fifty PET women, divided at presentation on the labor suite into mild and severe, were compared with the 93 controls using a number of laboratory and point-of-care tests. The results broadly agree with similar studies in many respects, notably in the incidence of thrombocytopenia within the three groups. Von Willebrand Factor levels increase in pregnancy (22) and activity and antigen values exceeded the normal ranges for our laboratory (50–150 IU) in all groups, but agreed with other studies in showing no significant difference in von Willebrand Factor activity between controls and PET women (23). The TEG results of the mild PET subjects remained similar to the controls. The mean MA of the severe and mild PET women did not differ from controls and were hypercoagulable when compared with previously established normal ranges for the nonpregnant population. In contrast, the PFA CT lengthened with preeclampsia, and to a greater degree with severe preeclampsia.

At platelet counts $<80,000/\text{mm}^3$, platelet number begins to influence CT, causing it to lengthen (13). If patients with counts below $100,000/\text{mm}^3$ are excluded from the analysis, such that the platelet number is no longer a determining factor of CT, there is a significant difference between the controls and the severe PET women and a borderline difference between the controls and mild PET women. This suggests the presence of a primary hemostatic defect independent of platelet number. One previous study (24) has shown a lengthening of CT associated with hypertension in pregnancy, although the patients involved had no other features of preeclampsia, and platelet numbers were not quoted. Another study has shown increased CT in preeclampsia complicated by thrombocytopenia (25).

As mentioned earlier, the magnitude of the CT changes in preeclampsia are more marked than the TEG MA. The assessment of platelet function with the TEG in pregnancy may be complicated by pregnancy-related changes in blood composition, such as increased fibrinogen levels. Since the MA is a composite parameter derived from fibrinogen and platelet activity, it is possible that the relatively high fibrinogen values associated with pregnancy may compensate for deficiencies of PHC. Three CT values more than 300 s were associated with normal or near-normal MA values of 70, 63, and 62 mm and fibrinogen values of 4.2, 3.6, and 5.9 g/L, respectively. By the same token, as Whitten and Greilich (11) have suggested, a reduced MA may be related either to altered platelet function or fibrinogen deficiency. Of the two patients in our study with HELLP syndrome, one developed a global clotting deficit suggestive of disseminated intravascular coagulation, reflected in a TEG MA of 40

mm. After administration of fibrinogen-containing FFP (but no platelets) the platelet count remained essentially unchanged ($50,000/\text{mm}^3$) and the CT still registered >300 s. However, repeated TEG measurement at that time returned a MA of 63 mm. This suggests that, in the absence of a global coagulopathy, or when such a coagulopathy has been corrected, celite-activated TEG registers a normal MA even in those with severe PET and marked thrombocytopenia.

The PFA results do not establish a platelet count threshold above which it is safe to conduct regional anesthesia, nor a CT above which an intraspinal bleed becomes an appreciable hazard. These shortcomings are shared with other available tests of hemostatic function, and given the rarity of intraspinal hematomas, may be insurmountable. However, this study provides evidence of an intrinsic platelet dysfunction in preeclampsia, with the implication that platelet count may not, in itself, be a sufficient criterion of PHC. Platelet count thresholds for regional anesthesia established for other causes of thrombocytopenia, therefore, may not be equally applicable in preeclampsia. Furthermore, platelet count thresholds established by TEG studies for use in preeclampsia may be too low if the TEG is not sufficiently sensitive to detect impairment of primary hemostasis.

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