Platelet function during pregnancy: an evaluation using the PFA-100 analyser

A. Vincelot, N. Nathan*, D. Collet, Y. Mehaddi, P. Grandchamp and A. Julia

Department of Anaesthesia and Intensive Care, Service d'Hématologie, CHU Dupuytren, 2 avenue Martin Luther King, F-87042 Limoges cédex, France

**Corresponding author*

In clinical practice, the only tests of platelet function are bleeding time and platelet number. Bleeding time lacks sensitivity and specificity but the PFA-100, an *in vitro* analyser of platelet function may be of value. This study aimed to evaluate any correlation between platelet number and function using the PFA-100 in pregnant women. During a 21-month period, platelet function was evaluated in whole blood as part of the pre-anaesthetic coagulation testing screen with the PFA-100 using collagen and epinephrine (PFA-EPI) or ADP (PFA-ADP) as platelet agonists. Thrombocytopenia was defined as a platelet number less than150 G litre^{-1}. The patients were divided into four groups: Group I (n=110) normal pregnancy; Group II (n=38) thrombocytopenia of pregnancy; Group III (n=13) women with pre-eclampsia without thrombocytopenia; Group IV (n=19) women with pre-eclampsia and thrombocytopenia. Results are expressed as mean (SD). Platelet count was not statistically different between Groups II and IV (111.1 (23.1) *vs* 99.5 (28.0) G litre^{-1}). PFA-EPI was statistically increased in Group II (124.0 (26.3) s), Group III (128.3 (17.9) s), and Group IV (143.6 (47. 7) s) compared with normal pregnant patients (114.6 (27.3) s, P<0.05, Mann–Whitney U-test). PFA-ADP was statistically increased only in Group II compared with normal pregnant patients (90.5 (18.9) *vs* 80.2 (11.2) s, P<0.05). PFA values were increased above normal laboratory values in (four of 38) Group II patients and (six of 19) Group IV patients but in no patients in Group III. PFA-ADP results were correlated with platelet count only in Group IV (r=–0.74, P=0.0003). The increased PFA values and the correlation between PFA-ADP and platelet number in hypertensive thrombocytopenic women confirms that platelet function may be decreased in such patients. In patients with pregnancy-induced thrombocytopenia, platelet function may be preserved when the platelet count is as low as 60 G litre^{-1}.

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Until recently, only two tests could be performed to evaluate platelet function in a routine clinical setting. Bleeding time, which is highly operator-dependent, lacks sensitivity and specificity and has a poor diagnostic value even when platelet function is altered.[1 2] Platelet aggregation tests cannot be used in usual practice and like the bleeding time, cannot predict the risk of haemorrhage. However, during pregnancy, knowledge of platelet function may be of critical importance before performing epidural anaesthesia. Indeed, platelet count may be decreased at the end of the third term of normal pregnancy in about 0.3% of women despite an increased platelet aggregability.[3] In contrast, 28% of women with pre-eclampsia may have platelet counts of less than 150 G litre^{-1}.[4] Thrombocytopenia less than 100 G litre^{-1} is only observed in severe pre-eclampsia.[4] In such patients, regional anaesthesia has been performed without any subsequent neurological complications,[5–8] even when decreased platelet function has been documented from both platelet aggregation tests and bleeding times.[9 10] In pre-eclampsia patients, the platelet count above which epidural anaesthesia may be performed safely is not known. This may explain why there is still controversy regarding the value of measuring the platelet count at the end of pregnancy. The recent report of a spinal haematoma following epidural anaesthesia in an eclamptic woman with thrombocytopenia reinforces the need for a reprodu-

Table 1 Physical characteristics of patients. Mean (SD), *$P<0.05$ compared with Group I, †$P<0.05$ compared with Group II. Group I, normal patients; Group II, pregnancy-induced thrombocytopenia; Group III, PIH women without thrombocytopenia; and Group IV, PIH women with thrombocytopenia

	Group I (n=110)	Group II (n=38)	Group III (n=13)	Group IV (n=19)
Age (yr)	28.7 (16–41)	28.9 (19–41)	27.9 (19–38)	28.6 (21–37)
Age of pregnancy at sampling time (weeks)	38.2 (1.3)	38.4 (2.0)	36.0 (3.3)*†	34.1 (4.5)*†
Time from sampling to delivery (days)	11.9 (8.3)	11.6 (10.3)	13.7 (12.6)	6.0 (9.1)*†
Regional anaesthesia	66/110	21/38	10/13	8/19
Caesarean section	18/110	7/38	7/13	15/19

Table 2 Laboratory data at the time of sampling. Mean (SD), *$P<0.05$ *vs* Group I, †$P<0.05$ *vs* Group II. Group I, normal patients; Group II, pregnancy-induced thrombocytopenia; Group III, PIH women without thrombocytopenia; and Group IV, PIH women with thrombocytopenia

	Group I (n=110)	Group II (n=38)	Group III (n=13)	Group IV (n=19)
Platelet count (G litre^{-1})	218.9 (51.3)	111.1 (23.1)*	281.4 (63.8)*†	99.5 (28.0)*
Haematocrit (%)	35.9 (3.0)	36.4 (3.3)	35.8 (2.5)	37.2 (3.6)
PFA–Epi (s)	114.6 (27.3)	124.0 (26.3)*	128.3 (17.9)*	143.6 (47.7)*
PFA-ADP (s)	80.2 (11.2)	90.5 (18.9)*	85.0 (11.2)	89.3 (26.5)
APTT ratio	0.94 (0.08)	0.97 (0.07)	0.95 (0.07)	1.11 (0.25)*
PT INR (%)	95.8 (11.1)	99.2 (13.6)	95.1 (8.1)	104.7 (11.9)*
Fibrinogen (g litre^{-1})	4.8 (0.8)	4.6 (0.5)*	5.2 (1.0)†	5.1 (1.1)†

cible and accurate test to evaluate platelet function in this clinical setting.[11]

The PFA-100 platelet function analyser may be of value in such circumstances. The PFA-100 evaluates *in vitro* primary haemostasis by measuring the time required for whole blood to occlude an aperture in the membrane of a test cartridge, which is coated with platelet agonists. A sample (500 μl) of citrated blood is placed in the reservoir of the test cartridge, which is maintained at 37°C. It is aspirated under steady vacuum into the stainless steel capillaries, through which there is a central aperture cut into the membrane covered with collagen and the platelet agonist, epinephrine (PFA-EPI), or adenosine diphosphate (PFA-ADP). Platelet activation and aggregation occurs on the membrane leading to occlusion of the aperture and the interruption of blood flow.[12] This test is easy to perform and gives reproducible results, measured in seconds and similar to *in vitro* aggregation tests, within a few minutes.[13] Moreover, as this test is performed on whole blood, it can evaluate platelet function in its natural environment. This study in pregnant patients aimed to determine if there is a correlation between platelet count and platelet function as assessed by the PFA-100 analyser.

Methods

After local ethical committee approval and informed consent, platelet function was assessed at the end of pregnancy during the anaesthetic visit on the first 110 normal pregnant patients studied and each time a patient presented with thrombocytopenia or pregnancy-induced hypertension (PIH) over a 21-month period. Platelet count, activated partial thromboplastin times (APTT), prothrombin times (PT) and fibrinogen levels were also performed. PFA-100s (Dade Behring, Mannheim, Germany) were carried out by one of the authors with 2 ml of whole citrated blood within 1 h of sampling. Two types of cartridge containing porous membranes covered with collagen and either ADP (PFA-ADP) or epinephrine (PFA-EPI) were used. According to the recommendations of the manufacturer, the PFA-100 results were considered abnormal when the occlusion time was higher than 160 s for PFA-EPI and 120 s for PFA-ADP. Patients were defined as normal (Group I) when they had no thrombocytopenia, hypertension, or past history of clinical bleeding, and clinical examination revealed no bleeding tendency. The other patients were divided into three groups according to the presence of thrombocytopenia (platelet count <150 G litre^{-1}) and/or PIH: Group II, normal pregnancy with thrombocytopenia; Group III, hypertension of pregnancy without thrombocytopenia; and Group IV, hypertension with thrombocytopenia.

Results are reported as mean (SD) and compared between groups using two tailed *t*-tests or Mann–Whitney *U* tests according to the homogeneity of variance (Bartlett tests). Barnet Woolf and Fisher exact tests were used for non-parametric data. Correlations between biological tests were performed in each group by regression analysis. A $P<0.05$ was considered significant.

Results

The physical characteristics of the patients are presented in Table 1. None of the patients were in labour at the time of sampling. The laboratory data of the patients are shown in Table 2. In Group II (38 women), the platelet count was less than 100 G litre^{-1} in 14 samples, and between 100 and 125 G litre^{-1} in 13 samples. In the 19 patients in Group IV, platelet count was less than 100 G litre^{-1} in 11 samples, less than 125 G litre^{-1} in four patients, and less than 150 G litre^{-1} in four patients. This distribution was not statistically different

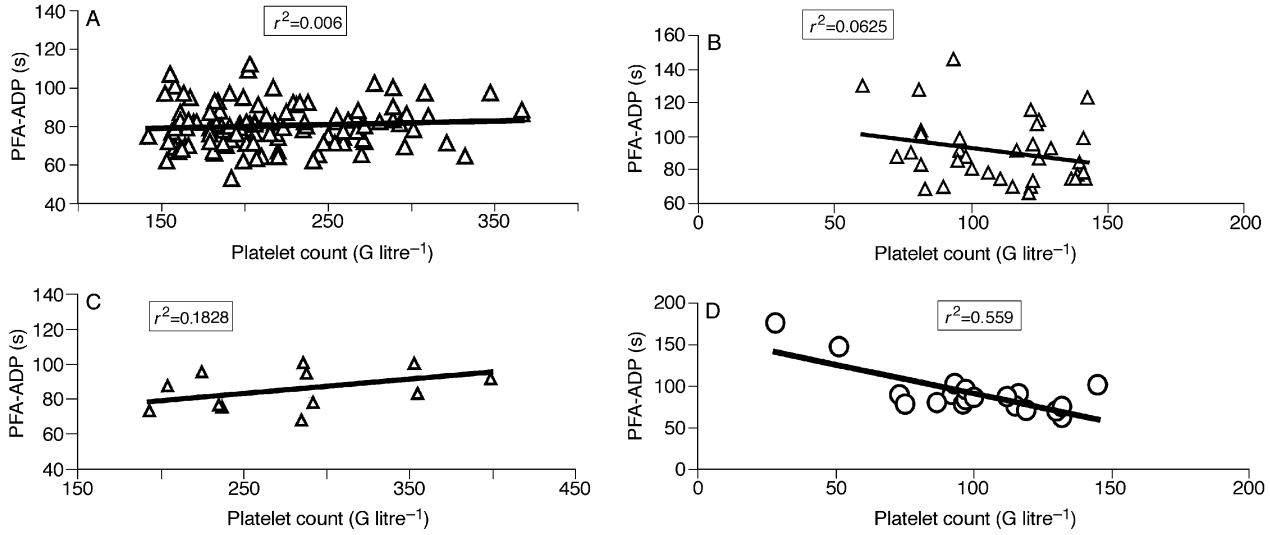

Fig 1 PFA-100 results obtained with collagen and ADP agonists compared with the corresponding platelet count in Group I, normal patients (A); Group II, pregnancy-induced thrombocytopenia (B); Group III, hypertensive women without thrombocytopenia (C); and Group IV, hypertensive women with thrombocytopenia (D). A significant correlation was only found in Group IV.

between Groups II and IV using Barnet Woolf tests (P>0.05).

PFA-EPI was statistically increased in Groups II, III, and IV compared with Group I (Table 2) whereas PFA-ADP was only increased in Group II compared with Group I (Table 2). When looking at individual results, PFA-ADP levels were within normal limits in all the patients from Groups I and III but higher in four patients from Group II and two from Group IV (P<0.01 by Barnet Woolf test). PFA-EPI was slightly increased above normal values in six samples from Group I, three samples from Group II, four from Group IV, and none from Group III (P>0.05 between groups by Barnet Woolf test; P=0.04 between Groups 1 and II or IV by Fischer exact test). Four out of 38 samples in Group II, and six of 19 in Group IV had platelet function abnormality as measured by PFA-100 whereas none in Group III had platelet function abnormality (P<0.01 by Barnet Woolf test). No correlation was observed between platelet count and PFA-EPI or PFA-ADP in any group except in Group IV where PFA-ADP was correlated with platelet count (P=0.0003, r=–0.74) (Fig. 1A–D). When patients with the lowest platelet values were excluded, this correlation disappeared. PFA values were not correlated with haemoglobin except in Group II for PFA-EPI (P=0.011, r=–0.41). No other correlation was observed between PFA results and coagulation data.

Discussion

PFA-100, a global test of platelet function, is capable of diagnosing von Willebrand disease and various congenital platelet defects such as Bernard–Soulier syndrome or Glanzmann's thrombasthenia with higher sensitivity and specificity than the bleeding time.[13] It is, however, similar in its sensitivity and specificity to platelet aggregation tests.[13] PFA-100 is a whole blood test able to measure the ability of platelets to occlude a vascular breach whereas a platelet aggregation test evaluates only platelet function in plasma. In this study, all the PFA-ADP values were normal in otherwise healthy patients whereas PFA-EPI was increased in six patients. This suggests that PFA-EPI may either give false positive values, probably a result of technical problems, or may not be clinically relevant in this setting. However, we cannot exclude oral intake of drugs or food interfering with platelet function without inducing clinical bleeding in these patients. Moreover, such a discrepancy between PFA-ADP and PFA-EPI has already been shown in patients given aspirin suggesting that PFA-EPI may be more sensitive or less relevant than PFA-ADP.[14 15] As we did not carry out platelet aggregation tests in this study, however, we cannot recommend performing only PFA-ADP without performing PFA-EPI simultaneously.

In women with pregnancy-induced thrombocytopenia, mean values of PFA were increased compared with normal pregnant patients suggesting that despite a pregnancy-induced increased platelet reactivity, platelet function is lowered. However, despite platelet values lower than 100 G litre^{-1} in approximately half of the patients, only four had PFA values slightly increased above normal, with PFA-ADP and PFA-EPI both increased in three of them. This demonstrates that overall platelet function remains within physiological values in most of the patients with pregnancy-induced thrombocytopenia despite a platelet count below 100 G litre^{-1}. In normal pregnant women, platelet reactivity is known to be increased, if platelet agonists such as ADP are still present, counterbalancing the decreased platelet number.[3] Despite such reassuring results, it must be noted that in only one patient from Group II was the platelet count

lower than 70 G litre^{-1} (namely 60 G litre^{-1}) and thus these results cannot be extrapolated to normal pregnant patients with even lower platelet counts. As no platelet aggregation test was performed in our patients, we cannot explain the increased PFA values in women with a low platelet count but without pre-eclampsia. It is striking, however, that in pregnancy-induced thrombocytopenia, the PFA results were not correlated with platelet count but with the haemoglobin level, suggesting that overall platelet function in whole blood was preserved despite the low platelet number, unless anaemia was present. Erythrocytes act as physiological ADP donors in whole blood and it has already been observed that anaemia may influence PFA results.[16] The correlations between PFA-EPI and haemoglobin levels suggest that, in patients with pregnancy-induced thrombocytopenia, whole blood platelet function depends on erythrocytes, and that one must be especially cautious about an increased bleeding risk when low platelet and erythrocyte counts coexist. These results may also explain the increased bleeding risk associated with thrombocytopenia in the presence of anaemia.[17]

In patients with pre-eclampsia-induced thrombocytopenia, the increase in PFA-EPI and the correlation observed between platelet number and PFA-ADP values demonstrates that a decrease in platelets interferes with platelet function. The absence of a statistical increase in PFA-ADP values in this group may result from a lower sensitivity of this test and/or an insufficient number of patients, particularly if the platelet count is lower than 50 G litre^{-1}. In pre-eclamptic patients, lower platelet reactivity related to depletion of granules stored in the platelets has been demonstrated by decreased platelet aggregation and increased bleeding time.[3 10] Because of its low sensitivity and specificity, the bleeding time has been criticized and other tests, such as the Thrombelastograph® (TEG®), have been proposed to evaluate platelet function in whole blood. In pre-eclamptic patients, it has been shown from whole blood thrombelastography, that a correlation exists between platelet count and reduced platelet function especially in severe cases.[18] Neither TEG® nor platelet aggregation tests were studied in our patients. However, the correlation between platelet number and function assessed by PFA-100 paralleled the results obtained by others with TEG® or bleeding time.[10 18 19] The higher reliability and ease of use of the PFA-100, especially with ADP as the agonist, suggests that this test might be an alternative method of evaluating platelet function in pre-eclamptic patients with low platelet counts. This remains to be demonstrated.

This is the first study reporting platelet function assessed by the PFA-100 in pregnant women. PFA-100 is not correlated with platelet count in women exhibiting pregnancy-induced thrombocytopenia suggesting that platelet function is well preserved in most of these patients at least when the platelet count is over 70 G litre^{-1}, unless anaemia is present. In women with pre-eclampsia, PFA-100 values remained near normal until the platelet count decreases to around 50 G litre^{-1}. The clinical value of PFA-100 in such patients remains to be demonstrated.

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