Is There a Role for Platelet Flowcytometry in the Diagnosis of Severe Thrombocytopenia in Pregnancy?

Minodora Onisâi[1], Ana-Maria Vlădăreanu[1], Horia Bumbea[1], Sânziana Radeşi[1], Anca Nicolescu[1], Irina Voican[1], M. Ciorăscu[2], C. Pop[2], D. Câmpean[3], F. Anca[3], I. Badea[3], Valerica Horhoianu[3], R. Vlădăreanu[2]

1. Emergency University Hospital Bucharest, Hematology Department
2. "Elias" University Emergency Hospital Bucharest, Obstetrics-Gynecology Department
3. Emergency University Hospital Bucharest, Obstetrics-Gynecology Department

Correspondence:
Radu Vlădăreanu
e-mail: vladareanu@gmail.com

Acknowledgements:
The current presentation is a part of a larger, ongoing study for the PhD thesis of MO, concerning platelet functional study through flowcytometry in preeclampsia; the intermediary data obtained so far were particularly useful in the differential diagnosis of the presented case. The case was mostly challenging and it required teamwork between hematologists and obstetricians; the contribution of each author was important for the favorable outcome.

Abstract

We discuss the case of a 16 years-old woman, approximately seven months pregnant (unmonitored pregnancy), that presented to the Emergency Department with intense hemorrhagic syndrome on skin (disseminated petechial lesions) and mucosa (including nose bleed). Emergency laboratory tests reveal severe thrombocytopenia (less than 10000 thrombocytes/mmc), slight anemia, leucocytosis with neutrophilia, liver cytolysis and signs of slight hemolysis. The first suspicion of diagnosis was preeclampsia/HELLP syndrome. The differential diagnosis is discussed, starting from a presentation of the causes of thrombocytopenia in pregnancy.
We highlight the value of platelet functional study - immunophenotyping through flowcytometry, because it particularly oriented us to the positive diagnostic.
Keywords: *pregnancy, thrombocytopenia, flowcytometry, preeclampsia/HELLP syndrome, platelet antibodies*

Patient TN, female, 16 years old, rrom ethnic, pregnant; approximate age of gestation: 7 months (unmonitored pregnancy), first parity, first gestation, affirmatively without personal pathological or family antecedents, initially arrives to an ENT Department because of an epistaxis episode in the nearly past; ENT physician observes beyond nasal bleeding (which stops spontaneously), the occurrence of intense disseminated purpuric lesions on teguments and mucosa, so he sends the patient to the Emergency Universitary Bucharest Hospital.

Clinical examination at admission revealed a good general status, without fever, with slight pallor, disseminated purpura (thorax, abdomen, inferior members) and mucosa (oral cavity, lips) - the patient affirms they had appeared 3-4 days before (figure 1 a, b), oral ulcerations, face with hyper-pigmentation especially on the nose and cheeks (figure 1c), without any disorders of cardio-pulmonary system (no anomalies at the pulmonary examination, blood pressure at the time of admission into the hospital was 100/60 mmHg, ventricular allure was 90 beats/minute, rhythmical), normal distension of the abdomen due to the presence of the pregnant uterus, with spontaneous pain especially in the anterior pelvic region. The liver and spleen could not be assessed by clinical examination because of the pregnant uterus.

Emergency laboratory tests revealed:

- **Complete blood count:** slight normochromic normocytic regenerative anemia (Hb 9.7 g/dl, reticulocytes 12%), severe thrombocytopenia – 20000 thrombocytes/mmc (confirmed on peripheral blood smear – PBS, where the platelet count was assessed to only 5000/mmc), medium platelet volume at inferior limit (6.1 fl), leucocytosis 13100/mmc; blood type BIII, positive Rh.
- **PBS showed:** left shift up to myeloblast: Blast 1%, Promyelocytes 3%, Myelocytes 5%, Metamyelocytes 2%, Bands 1%, Granulocytes 59%, Eosinophils 2%, Basophils 1%, Lymphocytes 19%, Monocytes 7%; Erythroblasts 6/100 elements (erythroblasts with basophilic stippling and binucleated erythroblasts), Plasmocytes 1/100 elements; other observed anomalies: erythrocytic anisocytosis (microcytes, macrocytes) and poikilocytosis (rare fragmented erythrocytes), few hyper-granular granulocytes, polymorphic stimulated lymphocytes, stimulated monocytes with vacuoles.
- **Biochemistry exam** showed liver cytolysis (ALT 115U/l, AST 141U/l) with cholestasis (alkaline phosphates 265 U/l), LDH increased (419 U/l - twice the normal value), total bilirubin slightly raised (1.8 mg/dl) due to direct fraction (DB 1.45 mg/dl, IB 0.36 mg/dl); renal tests were normal, normal ionogram, slight hypocalcaemia, serum

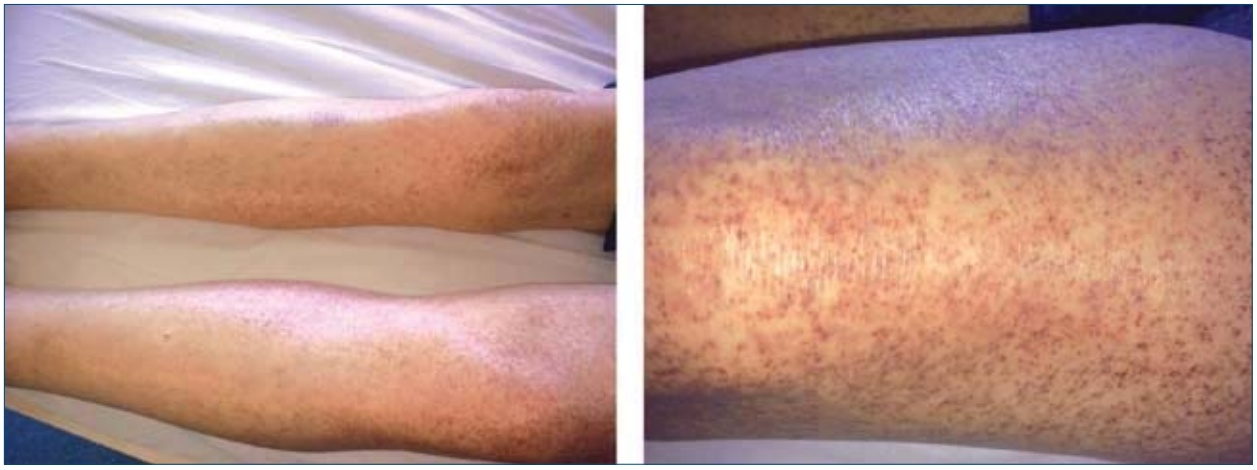

Figure 1 a, b. Disseminated petechial lesions on skin (thorax, abdomen, inferior members) and mucosa (oral cavity, lips)

iron slightly increased. Also, important inflammatory syndrome was present: ESR 80 mm/h, fibrinogen 629 mg/dl. Coagulogram - within normal range.

■ **Urine sample** - macrohematuria, leucocyturia (100 leucocytes/mmc), proteinuria (500 mg/dl).

After admission, we also performed:

■ **Abdominal ultrasound** - spleen with longitudinal diameter of 120 mm, without significant modifications.

■ **Pregnancy ultrasound** - unique pregnancy in evolution, cranial presentation, FCB present, rhythmical 146 /min; amniotic fluid bag intact with diameters of AP 42 mm; anterior placenta grade II-III, inserted at distance from internal orifice of cervix; estimated gestational age of 37 weeks + 2 days; estimated weight - 3,000 grams; cervix was severely shortened.

■ **Non-stress fetal test** - fetal heart rate (FHR) present, 140-150 /minute, uterus contractility was observed, rare and with low intensity.

■ **Emergency gynecological examination** - recommended the administration of anti-spastic perfusion and raised the suspicion of HELLP syndrome based on laboratory results (HELLP syndrome – acronym from words HELL and HELP, which is characterized by hemolysis, elevated liver enzymes and low platelet count)[1].

■ **Eye examination** - without hemorrhages; without pathological modification.

Positive and differential diagnosis: thrombocytopenia may frequently be encountered in pregnancy; it may be specifically related to pregnancy or appearing to a pregnant woman without any relationship to the pregnancy itself[2,3]. Based on the facts presented above, there were some suspicions and discussions of diagnostic, presented in table 1, together with the logical algorithm, with the arguments for and against each suspicion, and with the supplementary tests recommended.

We mention that we have to consider the severe gravity of the case and the necessity to establish quickly a therapeutic conduit, only based on the results available at that moment - even if these results were incomplete. Therefore, having in mind that the most severe problem remained thrombocytopenia, with possible vital implications both on mother and

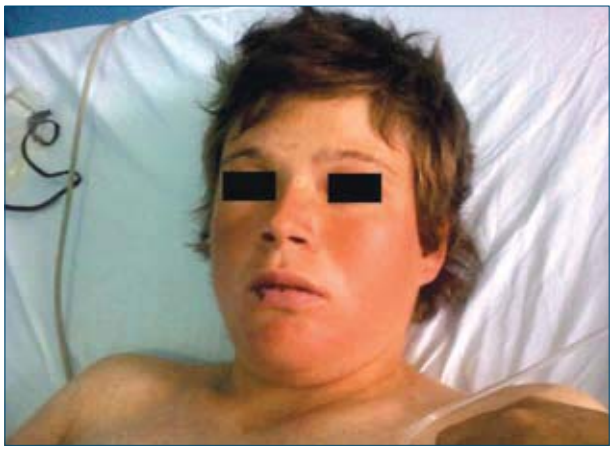

Figure 1 c. Oral ulcerations, face with hyperpigmentation especially on the nose and cheeks

on fetus, associated or not with an adjacent pathology, such as SEL or even HELLP syndrome, it was immediately initiated high dose emergency cortico-therapy, respectively Dexamethasone 16 mg/day with gastric protection (because of the benefic effect also on thrombocytopenia and on the fetal pulmonary maturation), continuing - in the mean time - the investigations; corticotherapy is also treatment for TTP.

In the same evening of admission, before dexamethasone treatment was initiated, we also drew blood for flowcytometry exam in order to evaluate platelet function.

Theoretical data on flowcytometry technique

On the membrane surface, the thrombocytes express a series of receptors - glycoproteins that have specifies roles corresponding to thrombocyte functions - respectively the implication into three major processes: inter-platelet aggregation, adhesion to endothelial surface, platelet activation. The level of expression of these studied may be detected by flowcytometry, a technique with high sensitivity and sensibility. The data is digitally stored and analyzed, allowing the analysis of the density of receptors on membrane surface. Flowcytometry[9,10] is a very useful investigation in platelet study, allowing a study which is very close to in vivo status.

Table 1 Differential diagnosis of thrombocytopenia in the presented case; elements that are in favor or in opposition to each diagnostic possibility

PROs	CONs	Supplementary tests recommended
1. Thrombocytopenic immune purpura:		
Severe thrombocytopenia Young age, female gender	Exclusion diagnostic - a certain disapproval of all possible causes of thrombocytopenia is necessary	Necessity of other investigation in order to exclude other causes. Marrow exam (in ITP: megakaryocytic lineage increased) Serum anti-platelet antibodies Recent viral infection? Recent administration of legal/illegal drugs?
2. Evans syndrome - immune thrombocytopenia with autoimmune haemolytic anaemia		
Thrombocytopenia Regenerative normochromic normocytic anemia (high Reticulocytes)	Exclusion diagnostic Very rare condition associating to pregnancy[4]	Anti-platelet antibodies Coombs' tests
3. Thrombocytopenic thrombotic purpura (TTP)		
There 3 criteria present (74% of patients with TTP have 3 criteria; the full pentad is present in only 40%[5]) ■ Thrombocytopenia ■ Microangiopathic hemolytic anemia (Regenerative normochromic normocytic anemia, fragmented erythrocytes, negative Coombs tests), normal coagulation tests ■ Renal (abnormal urine test) Pregnancy - increased risk for TTP[5-7]	LDH and bilirubin only slightly increased.	Presence of abnormal vWF multimers (agarose gel electrophoresis) - inaccessible
4. Disseminated intravascular coagulation (DIC)		
Thrombocytopenia, microangiopathic hemolytic anemia, fragmented erythrocytes	Normal coagulation tests	Search for adjacent causes of DIC
5. HELLP syndrome		
Thrombocytopenia, microangiopathic hemolytic anemia, fragmented erythrocytes Proteinuria Liver cytolysis Risks factors for preeclampsia/HELLP syndrome: nulliparity, age under 18 years-old	Normal blood pressure!!!	Supplementary investigations of proteinuria - associated urinary infection?? - urine culture must be performed!
6. Marrow insufficiency		
Thrombocytopenia, anemia Left shift up to myeloblast, presence of erythroblasts and plasmocytes at PBS - acute leukemia? Rapid proliferation with hematopoietic dislocation? Medullar fibrosis? Medullar aplasia?		Marrow evaluation - aspirate + biopsy - investigations which can not be performed at the respective moment mainly because of severe thrombocytopenia
7. Gestational thrombocytopenia (by dilution)		
Advanced gestational age	Exclusion diagnostic (appears in about 5% of pregnancy, but 75% of pregnancy thrombocytopenia is gestational) The thrombocytopenia is too severe!!	Necessity of other investigation in order to exclude other causes. CBC in postpartum and CBC of newborn (must be normal)
8. Immune pathology - Systemic erythematosus lupus		
3 criteria present[8]: ■ Hematological anomalies: Thrombocytopenia and regenerative anemia (possible immune) ■ Nose and cheeks hyperpigmented rash ■ Oral ulcerations		ANA count, lupic cell, anti Sm antibodies. Reumathological examination

Platelet activation status (as a marker of in vivo pro-thrombotic activity) may be determined in several pathological conditions, such as pregnancy induced hypertension.

There are several platelet surface glycoproteins that can be „measured" using flowcytometry. Table 2 presents the main receptors and their function.

In order to establish the level of expression for selected markers, we used platelet rich plasma (PRP) obtained through centrifugation from venous blood on anticoagulant sodium citrate. 6 test tubes were prepared: 1 for control, 2 for activation markers (CD62P, CD63), and 3 for adhesion markers (CD42a, CD42b, and CD31 - PECAM); each of the test tubes contained one of the aggregation markers (CD 41, CD61), because they represent markers normally expressed on platelet surface, therefore used to identify the platelets. The results obtained from the initial test are presented in figure 2.

We observed the complete lack of expression for all platelet surface markers. We mention that the tests were prepared and retested repeatedly by qualified employees, using also control sample for comparative evaluation (from healthy volunteer), initially considering a human or technical error. No such errors in the preparation of tests or acquiring the data were discovered, and the results for healthy volunteer were normal (high level of aggregation and adhesive markers and low level of activation markers). Also, the tests were repeated the next day, with similar results. Therefore, we consider as a possible explanation for the obtained results, the presence of a large numbers of antibodies on platelet surface, which simply, by steric mechanism, inhibit the connection between fluorocrome-marked antibodies to their specific receptors - expressed receptors, but un-identifiable. This hypothesis suggests an immune pathogeny of thrombocytopenia.

Initial evolution of the patient under corticoid-therapy was not suitable. Therefore, due to the severe thrombocytopenia, persisting - medical emergency - although not accompanied by active hemorrhage, and considering gestational age as advanced and weight suitable, we decided, in agreement with the Obstetrics-Gynecology team, that the best course of action in this context would be the termination of pregnancy. Therefore, after 24 hours from admission, a caesarian-section was performed, with the support of platelet transfusion, haemostatic perfusion, Novoseven (recombinant activated factor VII - for prevention of severe hemorrhage), and under an increased dose of corticoid-therapy - both Solumedrol and Dexamethasone. The intervention was without accidents or incidents, without any uncontrolled bleeding; the newborn was in good condition - a male new-born was extracted: Apgar Index - 9, weight 2,850 grams, length 47 cm.

The patient's evolution after surgery was of particular interest: 12 hours postpartum, she developed high blood pressure, with the highest value 166/79 mmHg, with oliguria - 400 ml/24 hours. She received diuretics, but without significant effect. In the next 24 hours, she developed BP 174/101 mmHg, with tachycardia, but without oliguria (urine output - 2,300 ml/24 hours). Another important milestone was the appearance of generalized seizures – at 24 hours after the C-section. A cerebral CT-scan was performed, but there was no evidence of a cerebral lesion that could cause the seizures. She received therapy with Depakine Chrono 500 mg at 12 hours. The blood pressure slowly decreased after treatment in the next day; there was only one episode of high BP (150/100 mmHg) in day 3 after surgery.

The development of these new elements inclines the diagnostic balance possibly towards preeclampsia/eclampsia with HELLP syndrome, which in this case would develop all the suggestive elements postpartum! There is current data in the literature sustaining that HELLP syndrome may be diagnosed postpartum - in as much as 30% of cases[13-16], and that, even though normally it is associated with severe preeclampsia, HELLP syndrome may also appear in the absence on this condition[13].

Meanwhile, we continued completing the diagnostic tests:

- **Coombs' tests** - direct and indirect, in order to see weather autoimmune anemia is present; the direct test was slightly positive (one level +) both for IgG and for IgG & C3. The indirect test was negative. The conclusion of the test is that there is a low number of antibodies present on the surface of erythrocytes, but not in the serum. Evans syndrome would be unlikely.
- We also **tested for anti-platelet antibodies** (but a few days later) - the result was highly positive! The result confirmes the hypotheses of auto-immune cause of thrombocytopenia - ITP or SEL.
- **Complete coagulogram**, which revealed: low C protein (discreetly), low S protein (discreetly), quantitative D-dimers - positive, fibrin degradation products - positive, fibrinogen level 800mg/dl, lupus anticoagulant: 53s – low positive value. The presence of anti-platelet antibodies was also confirmed. This way, the suspicion of auto-immu-

Table 2 Platelet surface markers (Modified after[5,11,12])

Glycoprotein	HLDA classif.	Function	Observations
Fundamental receptors - large number, clear function			
GP Ib-IX-V	CD42b/CD42a	Receptor for Von Willebrand Factor	Deficit - Bernard-Soulier Syndrome
GP IIb-IIIa	CD41/CD61	Receptor for Fibrinogen and von Willebrand Factor	Deficit - Glanzmann thrombasthenia
GP 53	CD63	Platelet secretion marker	Also on the endothelial cells, neutrophils, monocytes
GMP-140 (P-selectin)	CD62P	Platelet activation marker	Also on the endothelial cells
GP IIa' (PECAM)	CD31	Heparin receptor	Platelet/endothelial adhesion

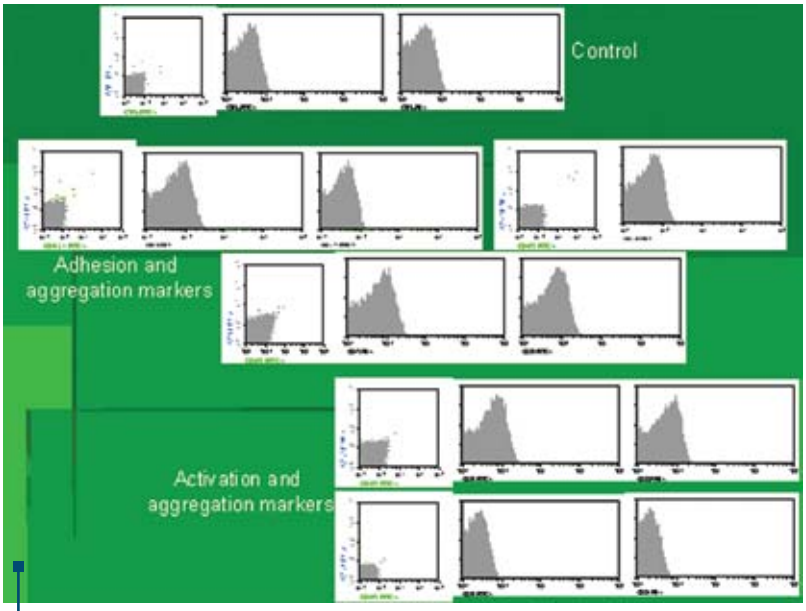

Figure 2. Results for platelet surface markers; out of the whole population of platelets, there is a very small population (under 1% - coloured in green) that bind surface markers; view explanations in text. BD Facs - Calibur Flowcytometer, CellQuest Software.

ne thrombocytopenia is highly probable and there is still a discussion on associated thrombophilia (which can be confirmed only after the end of pregnancy and childbed, due to the known fact that C and S protein can be physiological decreased in this period[17-19]).

- **Urine-culture** - urinary infection with Proteus spp, thus providing a possible explanation for proteinuria, other than in the context of HELLP syndrome (proteinuria being accompanied by other abnormality of urinary sample). Aimed antibiotic treatment was commenced.
- **Lupic cells present** - strong argument for SEL and a 4th criteria, thus establishing the diagnosis!!
- **The CBC of the newborn** showed thrombocytopenia - 20000 thrombocytes/mmc, pleading for an immune pathogeny of mother's thrombocytopenia (antibodies crossing the placenta). We mention that corticotherapy was immediately started in the newborn, with a very good response of platelet count - increased to 180000/mmc in 48 hours.
- **Other investigations:** serum immunogram - normal; B12 vitamin dosage - normal value

Final diagnosis and discussion

We consider the final diagnosis in this case was systemic erythematosus lupus with severe thrombocytopenia; the diagnosis is sustained by the presence of 4 criteria[8] (the diagnostic criteria for lupus during pregnancy are the same as the general diagnostic criteria for SEL, since they can be reasonably separated from the normal symptoms and signs of pregnancy [20]): facial hyper-pigmentation on nose and cheeks - rash, oral ulcerations, presence of lupic cells and blood anomalies: severe thrombocytopenia with obviously immune substrate, anemia with a hemolytic component. Besides, there are at least 4 other facts that support the diagnosis of SEL: **a)** the results from the flowcytometry, that suggest a very large number of antibodies present on cell surface; **b)** the newborn also presented thrombocytopenia, suggesting the passage of anti-platelet antibodies through the placenta; **c)** lupus anticoagulant was present, suggesting a possible antiphospholipid syndrome associated; **d)** very slow remission of the thrombocytopenia after delivery (see below); the remission was obtained only after prolonged and high doses of corticotherapy.

The other major diagnostic possibility would have been HELLP syndrome, based on the presence of all the criteria in the definition, and on the development of high blood pressure and seizures (after delivery). Although there are cited cases of preeclampsia/HELLP syndrome after delivery, we consider this is not the case. The arguments are: LES may develop in the immediate postpartum major decompensation with cerebral vasculitis, renal failure; the patient was persistent hypotensive during the days she was in the hospital before the delivery (BP 100/60mmHg); the increase in BP and the seizures may be linked to SEL vasculitis, perioperative stress or corticotherapy; the child also presented thrombocytopenia which was responsive to corticotherapy; proteinuria may be due to urinary infection with Proteus spp.!!

The onset of SEL during pregnancy is a very rare occurrence - a Chinese group recently reported a series of 4 cases; in the study, the onset seems to be mainly during the second trimester[20]. In fact, it is a well known fact that SEL is a risk factor for preeclampsia (to cite only a few recent studies[21-24]). Although the diagnosis should not be very difficult, the implications may be major - to name only preeclampsia (up to 20-25% of pregnant women with lupus may develop preeclampsia![24]) or prematurity or even fetal death - up to 29%[20]. Therefore, it is very important to apply an aggressive treatment in order to control the disease activity - symptoms and signs and pathophysiological changes. The recommendations for treatment include aggressive immunosuppression rather than termination of pregnancy[20]. As immunosupresive drugs, the safe options include mainly hydroxychloroquine or azathioprine, either of them combined with corticotherapy[20,25-28]. Delivery is recommended only is life-threatening complications develop - for mother or fetus[28].

Treatment and evolution: we continued the corticoid therapy in the mother - with the same high doses: Solumedrol 250mg/day and Dexamethasone 24mg/day, antibiotherapy protection, antiseizure medication. The evolution was very slowly favorable: the platelet count began to increase at 6 days after the surgery and reached 100000 plateles/mmc at 9 days after the C-section. The biochemistry levels also normalized at about Day 9. The blood pressure was normal (without any medication) and the seizures did not repeat. The patient was discharged from our Department (at her request) and referred to a Rheumatology Clinic for establishing the long term treatment course.

Considering the initial flowcytometry results, we decided to repeat the tests for platelet surface markers when the platelet count started to increase (day 7). The results are presented in figure 3:

As the figure shows, there is a population of platelets (aro-

und 20% of all platelets) where the markers binded on their surface receptors. This suggests that new young platelets appear, which are not covered with antibodies and that normally express their markers. We can observe that the level of expression is normal (high) for aggregation and adhesion markers, respectively: CD41 - 99.00%, CD61 - 100%, CD42A - 96.14%, CD42B - 95.14%, CD31 - 94.43%. Activation marker CD62P is also highly expressed on a part of the platelets (97.01%), while activation marker CD63 in lower (33.54%); this suggests platelet membrane activation is present (which may be due to a number of causes, including the pathology itself, prolonged and complex treatment, perioperative stress, etc), but platelet secretion is not enhanced (CD63 low).

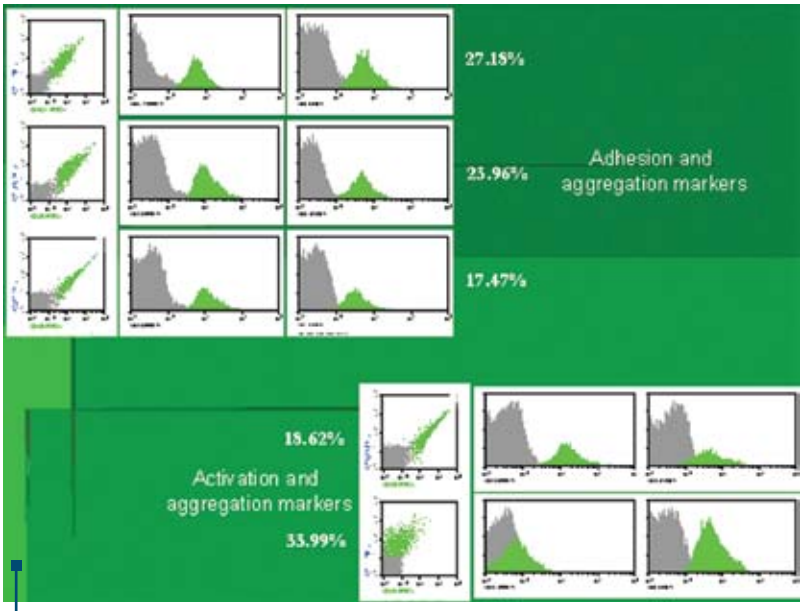

Figure 3. Results for platelet surface markers of adhesion, aggregation and activation; we notice there are 2 populations of platelets seen in each test; the percentage near each line represents the platelets that bind surface markers; see text for explanations. BD Facs - Calibur Flowcytometer, CellQuest Software

Conclusions

The case was chosen for presentation due to the difficulty of diagnosis; it represents a major obstetrical emergency, with potentially vital complications for both mother and fetus and emergency action was taken, based of the available data. The positive diagnosis was established in fact after the delivery, when the immediate danger had passed. We would like to underline that, although the cesarean-section was performed at a very low platelet count (under 20000/mmc, in spite of transfusional support), no complications appeared and there was no excessive hemorrhage.

We conclude that, in the workup of thrombocytopenia in pregnancy, flowcytometry may play a very important role, although it is not accessible as an usual investigation. It represented a strong and rapid argument for immune pathology and against HELLP syndrome (with the aid of dynamic measurement and taking into account the patient evolution). Of course that it is possible to have an association of both pathologies, because SEL in pregnancy is indeed associated with a higher incidence of preeclampsia/HELLP syndrome (as shown above) - even with an atypical onset, in postpartum. However, in the case of preeclampsia, platelet surface markers are normally or highly expressed[11,12,29-31] from the very beginning, long before clinical manifestations appear. ■

References

1. Sibai BM. Diagnosis, controversies, and management of the syndrome of hemolysis, elevated liver enzymes, and low platelet count. Obstet Gynecol. 2004 May;103(5 Pt 1):981-91.
2. Ajzenberg N, Dreyfus M, Kaplan C. Pregnancy-Associated Thrombocytopenia Revisited: Assessment and Follow-Up of 50 Cases; Blood, 1998, 92: 4573-4580.
3. Levy J, Murphy L. Thrombocytopenia in Pregnancy; J Am Board Fam Pract 2002;15:290 .
4. Lefkou E, Nelson-Piercy C, Hunt BJ. Evans' syndrome in pregnancy: A systematic literature review and two new cases. Eur J Obstet Gynecol Reprod Biol. 2009 Dec 21. [Epub ahead of print].
5. Greer J, Foerster J, Lukens J, Rodgers G, Paraskevas F, Glader B. Wintrobe's Clinical Hematology, 12th Edition, Lippincott Williams & Wilkins, 2009.
6. Ezra Y, Rose M, Eldor A. Therapy and prevention of thrombotic thrombocytopenic purpura during pregnancy: a clinical study of 16 pregnancies. Am J Hematol 1996;51:1-6.
7. Esplin MS, Branch DW. Diagnosis and management of thrombotic microangiopathies during pregnancy. Clin Obstet Gynecol 1999;42:360–367.
8. Hochberg MC. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum. 1997 Sep;40(9):1725.
9. Orfao A, Ruiz-Arguelles A, Lacombe F, Ault K, Basso G, Danova M. Flow Cytometry: Its Applications In Hematology. Haematologica 1995; 80:69-81.
10. Michelson AD. Flow cytometry: a clinical test of platelet function. Blood 1996; 87(12):4925-36.
11. Vlădăreanu AM, Andrei C, Onisâi M, Vasilache V, H Bumbea, R Vlădăreanu, The endothelial-platelet dysfunction in preeclampsia, Maedica - A Journal of Clinical Medicine, Vol 2, No 3, 2007, pg 214 - 221.
12. Onisai M, Vladareanu AM, BumbeaH, Ciorascu M, Pop C, Andrei C, Nicolescu A, Voican I, Vasilescu S, Visan L, Adrian I, Horhoianu V, Marinescu B, Vladareanu R. A study of the hematological picture and of platelet function in preeclampsia - report of a series of cases. Maedica - a Journal of Clinical Medicine, Vol 4, No 4/2009, pg 326-337.
13. Mihu D, Costin N, Mihu CM, Seicean A, Ciortea R. HELLP syndrome - a multisystemic disorder. J Gastrointest Liver Dis, 2007; vol 16, no 4, pg 419-424.
14. Rahman TM, Wendon J. Severe hepatic dysfunction in pregnancy. QJM 2002; 95: 343-357.
15. Reddy A, Suri S, Sargent IL, Redman CW, Muttukrishna S. Maternal circulating levels of activin A, inhibin A, sFlt-1 and endoglin at parturition in normal pregnancy and pre-eclampsia. PLoS One. 2009;4:e4453.
16. Matthys LA, Coppage KH, Lambers DS, Barton JR, Sibai BM. Delayed postpartum preeclampsia: an experience of 151 cases. Am J Obstet Gynecol. 2004;190:1464–6.
17. Franchini M. Haemostasis and pregnancy. Thromb Haemost. 2006 Mar;95(3):401-13.
18. Brenner B. Haemostatic changes in pregnancy. Thromb Res. 2004;114(5-6):409-14.
19. Bremme KA. Haemostatic changes in pregnancy. Best Pract Res Clin Haematol. 2003 Jun;16(2):153-68.
20. Yang M-J, Cheng M-H, Lin H-Y. Onset of Systemic Lupus Erythematosus During Pregnancy. J Chin Med Assoc 2006;69(3): 130–133.
21. Buyon JP. Updates on lupus and pregnancy. Bull NYU Hosp Jt Dis. 2009;67(3):271-5.
22. Ruiz-Irastorza G, Khamashta MA. Managing lupus patients during pregnancy. Best Pract Res Clin Rheumatol. 2009 Aug;23(4):575-82.
23. Mirza FG, Cleary KL. Pre-eclampsia and the kidney. Semin Perinatol. 2009 Jun;33(3):173-8.
24. Teh CL, Wong JS, Ngeh NK, Loh WL. Systemic lupus erythematosus pregnancies: a case series from a tertiary, East Malaysian hospital. Lupus. 2009 Mar;18(3):278-82.
25. Borden MB, Parke AL. Antimalarial drugs in systemic lupus erythematosus. Use in pregnancy. Drug Safety 2001;24:1055-63.
26. Levy RA, Vilela VS, Cataldo MJ, Ramos RC, Duarte JL, Tura BR, Albuquerque EM, et al. Hydroxychloroquine (HCQ) in lupus pregnancy: double blind and placebo-controlled study. Lupus 2001;10:410-4
27. Abu-Shakra M, Shoenfeld Y. Azathioprine therapy for patients with systemic lupus erythematosus. Lupus 2001;10:152-3
28. Branch DW, Porter TF. Autoimmune disease. In: James DK, Steer PJ, Weiner CP, Gonik B, eds. High Risk Pregnancy. Management Options, 2nd ed. London: WB Saunders, 1999: 853-84
29. Holthe MR, Staff AC, Berge LN, Lyberg T Different levels of platelet activation in preeclamptic, normotensive pregnant, and nonpregnant women. Am J Obstet Gynecol. 2004 Apr;190(4):1128-34.
30. Janes SL, Goodall AH. Flow cytometric detection of circulating activated platelets and platelet hyper-responsiveness in pre-eclampsia and pregnancy Clin Sci (Lond). 1994 Jun;86(6):731-9.
31. Konijnenberg A, Stokkers E, van der Post J, et al. - Extensive platelet activation in preeclampsia compared with normal pregnancy: Enhanced expression of cell adhesion molecules. Am J Obstet Gynecol, 1997; 176(2):461-469.