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CASE REPORTS

A study of the hematological picture and of platelet function in preeclampsia – report of a series of cases

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ABSTRACT

Introduction: *Preeclampsia – pregnancy specific condition associating pregnancy induced hypertension and proteinuria – may present diverse hematological features, variating from normal laboratory tests to severe thrombocytopenia (due to platelet activation and consumption), and/or anemia.*

Objectives: *a theoretical and practical presentation of hematological picture that may appear in preeclampsia.*

Materials and methods: *We studied a number of 10 patients with preeclamptic pregnancies compared to 10 females with normal pregnancies, from Obstetrics-Gynecology Departments of Emergency University Hospital Bucharest, Elias University Emergency Hospital and "Prof. Dr. Panait-Sarbu" Clinical Hospital. For both cases and controls we studied clinical and laboratory parameters – especially hematological aspects. We particularly performed a complete study of the platelet surface markers by flowcytometry, in order to establish the functional status.*

Results and Discussions: *Regarding the laboratory parameters, the CBC showed significant differences between the two studied groups only concerning platelet count – preeclamptic pregnancies presented slight thrombocytopenia, but with a significantly higher medium platelet volume; there were no differences in white blood count (all had slight leucocytosis with neutrophilia) or haemoglobin and erythrocytic parameters. The biochemistry showed only increased liver enzymes in patients (4 patients had HELLP syndrome); all patients had proteinuria, with a medium of 205mg/dl; the coagulogram didn't show any variations. The flowcytometric study of platelet surface markers revealed increased medium values for all studied parameters – markers of adhesion (CD42b / CD42a, CD31 – PECAM), aggregation (CD41 / CD61), activation (CD63, CD62P); for 5 out of 7 markers, the differences were statistically significant.*

Conclusions: *Preeclampsia is associated with a degree of thrombocytopenia (which may become severe) versus normotensive pregnancies, but with younger, regenerative platelets present. Platelets of preeclamptic patients express significantly higher levels of activation markers (CD63, but especially CD62P – $p<0.05$), and higher levels of adhesion markers (CD42a, CD42b, and especially CD31, consistent with a higher level of endothelial-platelet interactions). The research is continuing.*

Key words: preeclampsia, platelet, p-selectin, granulophysin

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INTRODUCTION – A FEW GENERAL FACTS ON PREECLAMPSIA

Arterial hypertension (High Blood Pressure – HBP) represents one of the most frequent complications in pregnancy, affecting about 5% of all pregnancies. Pregnancy induced hypertension includes Preeclampsia and Eclampsia, according to the Classification proposed by the American College of Obstetrics and Gynecology. Preeclampsia is a pregnancy specific syndrome, actually a multisystemic disorder, diagnosed when the patient is presenting HBP (> 140/90 mmHg) appearing for the first time after week 20 of gestation and proteinuria over 300mg proteins/24h or over 30mg proteins/dl (1+) persistent; edema – although not a diagnostic criteria – is frequently present (1, 2). Eclampsia is defined when seizures appear in a woman that meets the criteria for preeclampsia; the seizures are not due to any concomitant neurological disorder. HELLP syndrome is a particular complication of preeclampsia, defined by hemolysis, elevated liver enzymes, low platelet count. It is interesting to notice that preeclampsia, eclampsia and HELLP syndrome may also develop for the first time their characteristic features after delivery, in post-partum period. (3-5).

The importance of the problem is linked to the significant morbidity and mortality potential of pregnancy induced hypertension – the mother may develop: disseminated intravascular coagulation, acute renal failure, stroke (ischemic, due to vasospasm and microthrombosis or even hemorrhagic due to severe thrombocytopenia), acute pulmonary edema, cerebral edema, placental abruption, liver hemorrhage/rupture, transformation in chronic hypertension, or even maternal death (preeclampsia is the second cause of maternal death linked to pregnancy) (2,6); the fetal sufferance seems to be due exclusively to the placental insufficiency and may include: pregnancy loss, fetal death in utero, intrauterine growth restriction, premature labor (6). On a long term, a woman with a history of preeclampsia has a chance to repeat it at a future pregnancy (7) and also a higher cardiovascular risk (6,8): a 2.5 times higher risk of dying by ischemic cardiovascular disease (9).

Considering the risk factors defined for preeclampsia, there are several conditions with a higher association with the disease (1,10-22): age (under 18 / over 35 years old), nulliparous women, multiple pregnancy, African-American race; environment factors – high altitude; deficitary economical status, familial history of pregnancy induced hypertension, increased body mass index, preeclampsia at an anterior pregnancy, chronic renal disease, chronic arterial hypertension, thrombophilia and antiphospholipid syndrome, vascular/conjunctive tissue pathology, diabetes mellitus (resistance to insulin).

The pathophysiology of preeclampsia is still discussed and there are several theories attempting to explain it. The most plausible refers to altered placentation – the second step of placentation is incomplete: the spiral miometrial arteries are not correctly invaded by the trophoblast, the transformation of the vessels does not appear, and they remain small caliber vessel, capable of vasospasm, reactive, even with increased vascular reactivity (6,9,10,15,23-28). The result is placental ischemia, followed by the release of a number of vasoactive factors that alter the endothelial function, the platelet function, all conquering to change the balance between vasoconstriction and vasodilatation (1,29); among these, the most important features refer to:

- Increased thromboxane/prostacyclin ratio (9,30), by increased placental and platelet release of thromboxane A_2 (9,31) and decreased placental prostacyclin (PgI_2) and prostaglandin (PgE_2) release (6, 32-35). The level of TxA_2 is correlated with the severity of vasoconstriction and of preeclampsia (9,31,36).
- Increased endothelin-1 release – highly vasoconstrictive (1,6,9,37-39).
- Decreased synthesis of nitric oxide (NO) – natural vaso-dilatator – due to decreased expression of its endothelial synthetase (1,6,40).

The final consequence is endothelial dysfunction, generalized constriction with consequent hypertension, with signs and symptoms of preeclampsia (3,6,41). In fact, there are two major cellular elements involved in vicious circle, acting in a positive feedback loop – the endothelial cell and the platelet, both highly active, releasing several active substances that stimulate and maintain the endothelial and platelet dysfunction.

The **hematological changes** that appear in preeclamptic pregnancy are divided into 3 major groups:

A) Numerical and functional **platelet** anomalies: platelet dysfunction and thrombocytopenia. Up to 50% of preeclampsias associate thrombocytopenia which is generally proportional to the severity and may precede clinical manifestations (10,11); thrombocytopenia may be severe, potentially life threatening. The major role played by the thrombocyte in the pathophysiology of preeclampsia is related to the release of thromboxan A_2, with subsequent increase of thromboxane/prostacyclin ratio (9,30). Thromboxane A_2 promotes vasospasm, induces supplementary platelet aggregation and endothelial damage, which add an important contribution to maintaining platelet dysfunction and promoting platelet consumption (activation, aggregation, microangiopathic hemolysis induced by severe vasospasm), resulting in thrombocytopenia, which is an important **sign of severe/aggravating preeclampsia.** Therefore, excessive platelet activation is associated with endothelial dysfunction, thrombosis in microcirculation, end organ degenerative necrosis, placental infarction (IUGR). The response of a normal bone marrow is followed by the release of young elements, with increased MPV; studies show that MPV is significantly increased in women with preeclampsia (42) and it is even linked to the cases with altered uterine Doppler velocymetry predictive for preeclampsia (43). Studies also advocate a more extensive platelet activation in preeclamptic pregnancies, suggested by an increased expression of P-selectine, CD63 and PECAM – platelet surface glycoproteins, markers of platelet activation (44,45).

B) Alterations of **hemoglobin and erythrocytic parameters:** most frequently – hemoconcentration manifested with increased hematocrit (46) – due to increased endothelial permeability; anemia may also be present in rare cases. The anemia that is strictly connected to preeclampsia (and not in the context of the pregnancy, such as due to hemodilution, bleeding, deficient iron balance) is most frequently associated with HELLP syndrome and it is due to microangiopathic intravascular hemolysis – physical destruction of erythrocytes in the microcirculation affected by disseminated microthrombosis; the anemia will be slight/medium, normochromic, normocytic, with a hemolytic pattern (increased bilirubin – unconjugated fraction, increased LDH, increased reticulocyte count), fragmented erythrocytes and microspherocytes or peripheral blood smear (1), and, in severe forms, hemoglobinuria and hemoglobinemia.

C) Considering the **coagulation changes**, it is known that normal pregnancy is a procoagulant status and that this tendency is increasing during the development of the pregnancy with the end-point of minimizing the blood loss intrapartum.

In preeclamptic pregnancies, the coagulation cascade is generally activated (1, 47, 48) – preeclampsia being by itself a highly thrombotic and procoagulant state, with platelet activation and consumption, promoting of thrombin formation, promoting of fibrin formation and destruction. In spite of these changes, in most cases of preeclampsia the coagulation anomalies do not have major clinical significance (1, 10), and the usual coagulation investigations are not modified. About 20% of patients have altered coagulation (49). The exceptions are for the situations when a severe complication is added, such as abruptio placentae, massive hemorrhage, liver infarction, when intravascular disseminated coagulation is frequently present.

Still, a complete investigation of the coagulation in patients with preeclampsia in the compensated step without major clinical effect, using complicated and less accessible tests, may reveal that preeclampsia compared to normal pregnancy is associated with significantly increased levels of thrombin-antithrombin III (TAT) complexes (31,50,51) and PAI-1, while fibrinogen, antithrombin III and PAI-2 are significantly reduced (52); preeclampsia is also associated with a decrease of natural anticoagulants – protein C and S (17) and antithrombin III (49). The level of D-Dimers as markers for Disseminated Intravascular Coagulation is increased only in severe forms and fibrin monomers are not increased in preeclampsia (52). More than that, clear signs of DIC are noted to be associated with a higher risk of intrauterine growth restriction (10, 52), and the presence of IUGR is independently associated with a higher level of PAI-1 and a lower level of PAI-2 (52). Preeclampsia is also associated with increased level of factor VIII antigen and of thrombomodulin – as markers of endothelial injury (31, 53-55) – the complex thrombin-thrombomodulin physiologically initiates the synthesis of protein C, directly inhibits fibrin formation, platelet aggregation and the activity of factor V (31). ❑

OBJECTIVES

We aimed to clarify particular ethio-pathogenical aspects of preeclampsia, by performing a multi-disciplinary approach (obstetrics, hematology, flowcytometry) – combining the cellular expression of the pathophysiological events with the clinical features of preeclampsia.

We accomplished a clinical and laboratory study of the patients diagnosed with preeclampsia, with a special accent on outlining the hematological aspects and on platelet dysfunction, as the platelet is one of the major elements in the pathophysiological process. ❑

METHODS

We present a transversal study on 10 pregnant females diagnosed with preeclampsia, compared to 10 females with uncomplicated pregnancy. We report preliminary data on a series of cases, the study is currently continuing on a higher number of patients. The cases were selected from the Obstetrics Departments of Emergency University Hospital Bucharest and Elias Emergency University Hospital and from "Prof. Dr. Panait Sarbu" Hospital. We studied the clinical and the laboratory parameters, with a particular accent on the platelet function. In order to characterize platelet functional defects, the study of the three major processes accomplished by the platelet is performed: inter-platelet aggregation, adhesion to endothelial surface, platelet activation. There are two major methods of investigation: „miming" these processes in vitro – using aggregometry, and platelet study in vivo – using flowcytometry. The latter is of particular interest; it uses monoclonal antibodies against platelet surface glycoproteins, as markers for platelet functional processes; it has several valorous advantages (56, 57): a) The study is performed in the natural environment of platelets (with leucocytes and erythrocytes, both of which affect cell activation); b) There is a minimal manipulation in vitro that prevents accidental activation and loss of platelet subpopulations; c) The results are not influenced by the platelet number; d) Several markers may be used at the same time; e) The technique may detect specific changes in platelet activation status and it may distinguish even small populations of activated platelets (1%). There are of course disadvantages – sample preparation is complicated, the technique and the agents used are expensive and in order to avoid ex vivo activation, the sample must be processed as soon as possible.

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

The fundamental platelet receptors, with large number and clear function are presented in table 1.

In our study, the platelet function was assessed by studying the medium level of expression of platelet surface glycoproteins using flowcytometry techniques; we used the following antibodies:

- CD61 FITC conjugated – directed against Gp IIIa (β3), sub-unit of Gp IIb-IIIa – platelet aggregation marker
- CD41 PE conjugated – (always non-covalently associated with the CD61) reacts with (GpIIbα) in the intact complex Gp IIb-IIIa, but not with the GpIIb or GpIIIa separately – fibrinogen receptor – platelet aggregation marker
- CD42a – reacts with Gp Ib-IX (but not with Gp Ib or Gp IX separately) – von

Glycoprotein	Family	HLDA classif.	Function	Observations
GP Ib-IX-V	Leucine rich Gp	CD42b/CD42a	Receptor for von Willebrand Factor	Deficit: Bernard-Soulier Syndrome
GP IIb-IIIa	αIIb β3 integrin	CD41/CD61	Receptor for Fibrinogen and von Willebrand Factor	Deficit: Glanzmann thrombasthenia
GP 53	4 domain trans-mb protein	CD63	Platelet secretion marker	Also on endothelial cells, neutrophils, monocytes
GMP-140 (P-selectin)	Selectins	CD62P	Platelet activation marker	Also on the endothelial cells
GP IIa' (PECAM)	Ig	CD31	Heparin receptor	Adhesion to endothelium

TABLE 1. Fundamental platelet receptors (modified after 58)

Willebrand factor receptor – platelet adhesion marker
- CD42b – binds to Gp Ib-α, from Gp Ib-IX – platelet adhesion marker
- CD31 – PECAM = Platelet endothelial cell adhesion marker – adhesion marker
- CD62P – specific for P-selectin (which appears on the surface of the activated platelet) – platelet activation marker
- CD63 – recognizes Gp53 (granulophysin), translocated on cell surface after platelet activation and secretion – platelet activation marker.

Preparation of the blood samples: Blood samples (3 ml peripheral blood) from patients (n=5) and volunteers (n=10) were used. The samples were collected by venous punction from antecubital vein with a minimum of stasis in Beckton-Dickinson vacutainers with anticoagulant sodium citrate. The analysis was performed within 4-6 hours from the venous punction.

The protocol for the platelet immunophenotyping is described below: the blood samples were centrifuged at 20°C and 200g for 10 min and the platelet-rich plasma obtained was removed and centrifuged at 20°C and 800g for 10 min to prepare the platelet pellet. The platelet pellet was washed three times with phosphate buffer saline (PBS) containing EDTA (0.009 mol/L Na2EDTA; 0.01 mol/ L Na2HPO4; 0.0018 mol/L KH2PO4; 0.17 mol/L NaCl and 0.0033 mol/L KCl) and then fixed by incubation for 10 min at room temperature with 2% paraformaldehyde in PBS-EDTA. The fixed platelets were washed twice with PBS-EDTA and adjusted to a concentration of 5x109/L. Aliquots (200 μL) of the platelet suspension were added to 12x75 mm polystyrene tubes previously coated with 30 μL of 5% bovine albumin. The platelets were incubated for 15 minutes with the above described monoclonal antibodies conjugated with fluorocromes for surface receptors. The analyses were performed on a FACS Calibur BD four channels flowcytometer, using the CellQuest Software. ❑

RESULTS

The medium age of patients was 29.8 years old [limits between 16-41], compared to 27.0 in controls; 6/10 patients were primiparas, primigravidas. The average body mass index (BMI) was 24.26 [limits between 18.72-32.46] (at the upper normal limit) for patients versus 21.30 for controls; 4 patients had BMI over 25 kg/m^2.

Considering the history, 2/10 patients (no 3, no 4) had a family history of high blood pressure (outside of pregnancy). Regarding personal medical history, we observed that 3/10 patients had been hypotensive before the pregnancy; 2/10 patients had gestational diabetes; and 1 patient had antiphospholipid syndrome.

The controls were healthy pregnant females, without any significant problems with the current or the previous pregnancies; 4/10 controls were primiparas, primigravidas.

The main clinical complaints that brought to the hospital the patients with preeclampsia were: altered general condition, headache, and edema (more severe in the last days/hours previous to presentation); 3/10 cases had generalized edema; 3/10 patients presented bleeding of skin and mucosa.

All patients had blood pressure over 140/90mmHg:
- Systolic BP was between 140-200mmHg, with a medium of 164mmHg
- Diastolic BP was between 90-110mmHg, with a medium of 98mmHg.

All patients presented proteinuria – over 30mg/dl at repeated urine tests, therefore all 10/10 cases were diagnosed with preeclampsia, and 9/10 presented a severe form, based on the presence of at least one of the criteria for a severe form (BP over 160/110mmHg, proteinuria over 2000mg/24h or over 2+ persistent or signs of organ failure: creatinine over 1.2mg/dl, increased AST, ALT; epigastric or right upper quadrant pain (likely resulting from hepatocellular necrosis, ischemia, and edema that stretches Glisson's capsule), persistent headache or other cerebral or visual anomalies, and hematological disorder: platelet count under 100.000/mmc and/or signs of microangiopathic hemolysis).

All control cases had normal blood pressure, and no proteinuria.

The medium age of gestation at the diagnosis of preeclampsia was 32weeks of gestation [limits between 25-37 – case number 8 presented a very early onset on disease, at 25 weeks]. Among the controls, 2/10 were in the first trimester of pregnancy, 2/10 were in the second trimester of pregnancy and 6/10 in the third trimester, with a general medium of 26 weeks of gestation.

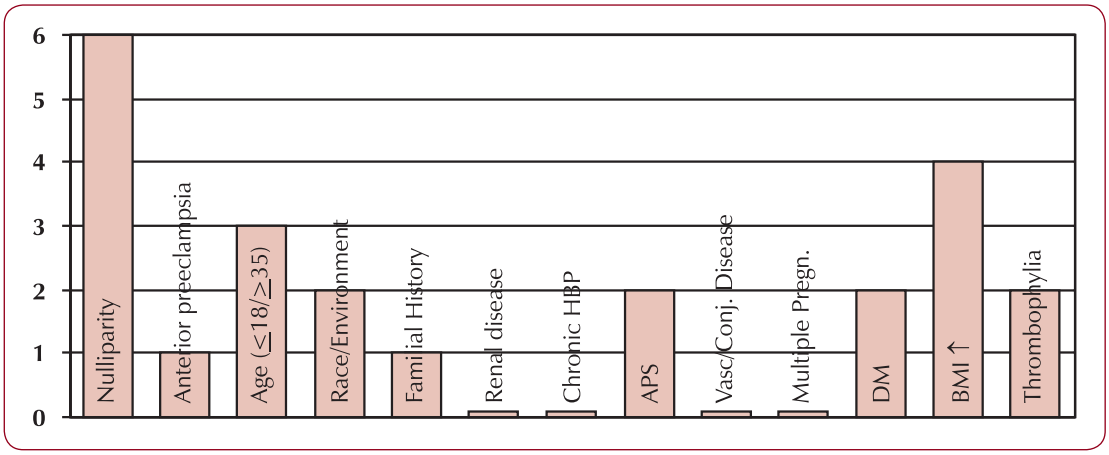

FIGURE 1. The frequency of various risk factors in patients with preeclampsia – the number of patients that presented the selected risk factor: nuliparity was the most frequent factor, encountered in 6/10 cases.

At pregnancy ultrasound, 7/10 cases were complicated with intrauterine growth restriction.

Considering the risk factors for preeclampsia, 9/10 patients presented at least one risk factor; 3/9 patients had 4 risk factors present at the same time; increased BMI in 4/10 cases. In the figure 1, the frequency of the risk factors is detailed – the most common risk factor was nulliparity, present in 6/10 cases.

The treatment plan was based of Dopegyt (Metil DOPA) in 8/10 cases, up to 1500mg/day, completed in 2/8 patients with calcium channel blockers (Amlodipine/Nifedipine); in 2/10 cases, emergency cesarean section was performed as treatment for preeclampsia. In fact, all cases ended with Cesarean-section, which was performed at a medium of 2.6 weeks from the onset on preeclampsia [limits 0-6], hence stressing the emergency nature of this pathology. In the two cases mentioned above, the C-section was imposed because of fetal sufferance. None of the mothers had further medical problems after the termination of pregnancy; the hypertension disappeared in early post-partum (maximum 5 days after surgery).

Considering the newborns, nine live newborns resulted from the 10 preeclamptic pregnancies – 1 case of preeclampsia was complicated with intrauterine fetal death.

The live newborns were mostly females (M/F ratio – 3/6), born at a medium age of gestation of 35.1 weeks [31-37]. The Apgar Score at 1 minute was between 7 and 9. The medium weight on newborns was 1980 grams [limits 1500-2850]: 4 newborns were premature, 3 were small for the respective gestational age, and 2/9 were normally developed (normal weight for gestational age); 2 newborns had a degree of perinatal hypoxia, but without any subsequent apparent sufferance.

By comparison, in the control group, 4 patients were subjected to Cesarean section and 6 patients had a normal delivery; the medium age of gestation at pregnancy termination was 38.11weeks and the medium weight of newborns (all healthy and normally developed) was 2900grams.

Considering the laboratory parameters, we obtained the following results:

- Complete Blood Count:
  - WBC 12111/mmc in patients vs 11935/mmc in controls.
    - The differential didn't show significant changes – both patients and controls presented neutrophilia (73.% in patients and 73.1% in controls).
  - No variations in Hb parameters:
    - Hb was 11.6g/dl in patients and 11.6g/dl in controls.
    - Ht: 34.1% in patients and 34.3% in controls.
    - MCV: 86.4fl in patients and 86.7fl in controls.
    - RDW: 15.6% in patients and 15.2% in controls.
    - Nr ery: $4.0x10^6$ in patients and $3.9x10^6$ in controls.
    - No variations in erythrocytic parameters.
  - Platelet count:
    - Medium platelet count was significantly lower in patients versus controls: 154900/mmc [6000 – 338000] in patients vs 267800/mmc [150000 – 516000] in controls (p=0.02117).

- 5 patients presented thrombocytopenia, with a minimum level of 6000 platelets/mmc – a patient with severe preeclampsia and HELLP syndrome with platelet consumption. We remember that only 3 patients had clinical signs of thrombocytopenia – bleeding of skin and/or mucosa. Platelet count was very low in 2 of these cases (respectively under 20000/mmc), but one patient had epistaxis and normal platelet count (242000/mmc) – the bleeding was probably due to the hypertension alone.
- Patients with preeclampsia presented a significantly higher medium platelet volume – 12.1fl [10.8 – 15.4] vs 8.7fl [4.1 – 11.4] – p=0.00073.
- The biochemistry showed only increased levels of the liver enzymes at the patients: medium level AST 86.4U/l in patients and 22.6U/l in controls; medium level ALT 101.3 in patients and 24.7U/l. Two patients had HELLP syndrome associated, but we encountered increased liver enzymes in a total of 4 cases, out of which two did not present thrombocytopenia.
- The usual coagulogram (TQ, aPTT, AP, fibrinogen) didn't show any variations, except for a higher fibrinogen level in patients: 589mg/dl, versus 498mg/dl. Unfortunately, a complete investigation of coagulation was not available.
- Urine exam – as stated before, all patients had proteinuria (with a medium of 200mg/dl); in 4/10 cases, 24h-protein-

Platelet marker	CD42a	CD42b	CD31	CD41	CD61	CD63	CD62P
Medium level of expression – PATIENTS	93.11%	94.86%	94.28%	84.78%	98.66%	19.88%	75.00%
Medium level of expression – CONTROLS	86.22%	59.62%	44.83%	70.42%	98.24%	5.50%	5.98%
p-value	*0.143*	*0.002*	*0.004*	*0.045*	*0.235*	*0.028*	*<0.001*

TABLE 2. The medium level of expression for platelet surface markers in patients with preeclampsia and controls (normal evolving pregnancy), and the p-value – statistical significance.

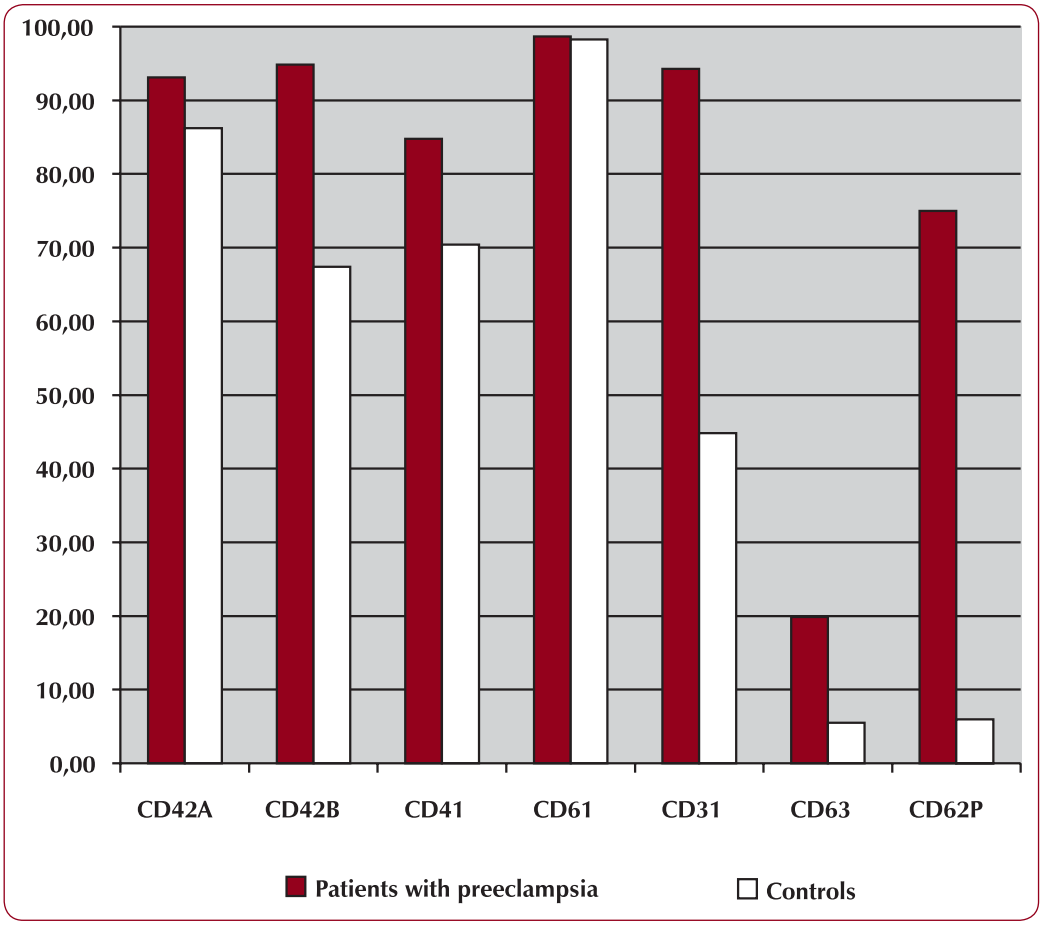

FIGURE 2. Comparison between the level of expression of platelet surface markers in patients with preeclampsia (red) and controls (white).

uria was measured, giving a very high medium value – 6g/24h.

According to the current data in the literature, that suggests an important role of the thrombocyte in the pathophysiology, platelet function was assessed via flowcytometry both for the patients and the controls.

The medium level of expression for the adhesion, aggregation and activation markers is presented in the table 2 and the figure 2, along with the p-value, in order to establish the statistical significance of the differences between the two groups. We mention that for CD41 and CD61, the presented values are averages obtained from 2/3 measurements, since the two markers served to identify the platelets in each test tube. For both patients with preeclampsia and with normal pregnancies we used control test in order to correctly establish the positive and negative level for each test.

We also present an example of results from flowcytometric study of platelet surface receptors for a patient with preeclampsia and a patient with normal pregnancy – uni- and biparametric histograms (figure 3 and figure 4).

We observed that all platelet markers presented a higher value in patients with preeclampsia compared to the value for normal pregnancies and the differences are statistically significant for 5 markers out of 7, respectively: CD42b, CD31, CD41, CD63, CD62P. These results suggest that preeclamptic status is associated with a high functional platelet status, reflected primarily in enhanced activation status (suggested by higher levels of expression for P-selectin – CD62P and granulophysin – CD63), generating enhanced adhesion – both to other platelets and to the endothelial cell – (based on the differences of expression for CD42a, CD42b and CD31) and aggregation (higher CD41 and CD61).

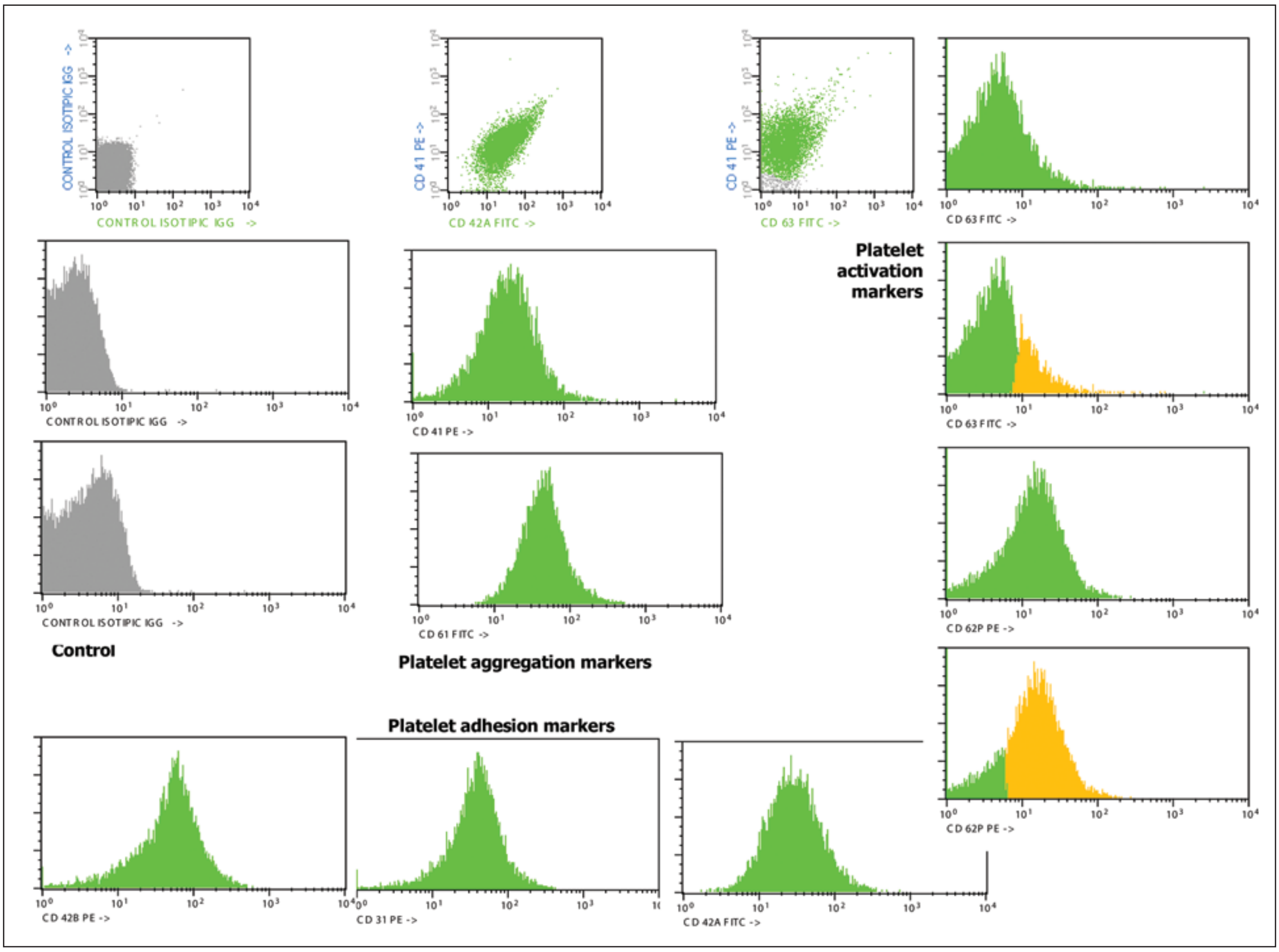

FIGURE 3. Examples of dot – plot histograms from a patient with preeclampsia: platelet population is colored in green; negative control on the right of the picture; the population of activated platelet is highlighted in yellow. We observe high levels of expression for all markers, especially for CD62P. (BD Facs – Calibur, Software CellQuest version 3.3)

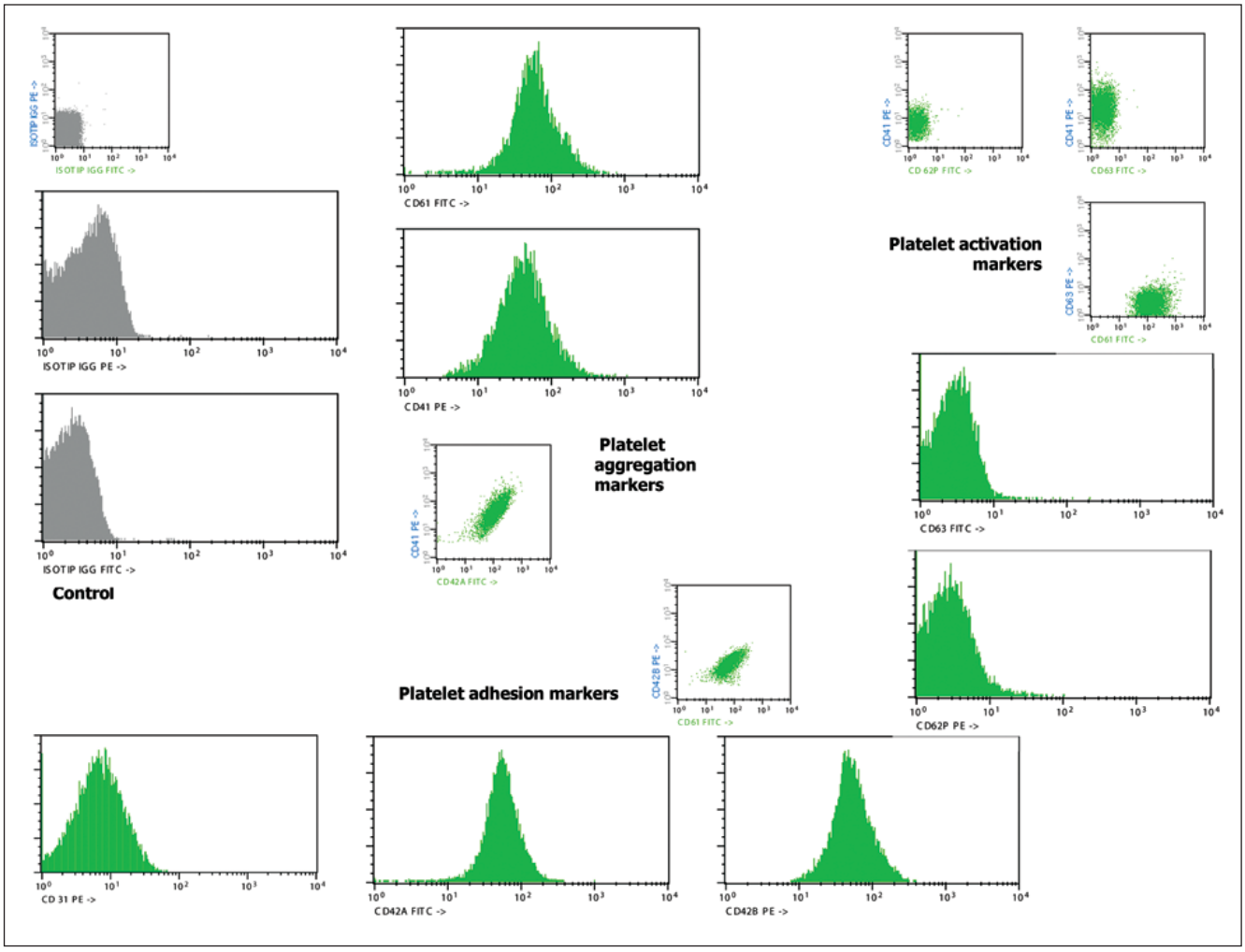

FIGURE 4. Examples of dot - plot histograms from a patient with normal developing pregnancy: platelet population is colored in green; negative control on the right of the picture. We observe low levels of expression for activation markers (CD62P, CD63) and also for CD31. (BD Facs - Calibur, Software CellQuest version 3.3)

Another observation is that the differences are very high for three markers: CD31, CD63, and especially CD62P, respectively markers of platelet activation and of platelet-endothelial adhesion, suggesting important increases in these particular thrombocyte processes in pre-eclampsia; the activation level is more extensive on cell membrane – CD62P higher than CD63.

We also made an attempt to find whether there are any correlations between the platelet parameters and their level of expression, and we obtained the following results (for patients with preeclampsia and controls with normal evolving pregnancy):

- Correlation coefficient between platelet count and platelet volume for patients was -0,4679
- Correlation coefficient between platelet count and platelet volume for controls was -0,1825
- Correlation coefficient between platelet count and the level of expression of CD62P for patients was -0,3794
- Correlation coefficient between platelet count and the level of expression of CD62P for controls was -0,1670
- Correlation coefficient between platelet count and the level of expression of CD63 for patients was -0,4037
- Correlation coefficient between platelet count and the level of expression of CD63 for controls was -0,2308
- Correlation coefficient between platelet volume and the level of expression of CD62P for patients was -0,0151
- Correlation coefficient between platelet volume and the level of expression of CD62P for controls was 0,3187
- Correlation coefficient between platelet volume and the level of expression of

CD63 for patients was 0.7165
- Correlation coefficient between platelet volume and the level of expression of CD63 for controls was -0,1794

We did not notice any degree of correlation between the studied parameters with the one exception: for patients with preeclampsia there is a degree of positive correlation between the platelet volume and the surface expression of CD63 – activation marker.

We also tried to find if there were any correlations between the platelet count or platelet activation markers (CD63 and CD62P) and other maternal parameters, such as age, number of pregnancies, number of risk factors, gestation week of onset for preeclampsia or the highest BP. We present below the most representative of these correlation indices:

- An inverse correlation between platelet count and number of risk factors: correlation index was -0,6575, suggesting that patients with a higher number of risk factors have a lower platelet count.
- A degree of positive correlation between platelet count and the number of pregnancies: correlation index was 0,5315; actually, all the 5 thrombocytopenic patients were primiparas.
- There was also a positive correlation between the level of expression of CD63 and the maximum systolic BP, with a coefficient of 0,6214, suggesting the level of platelet secretion is proportional to the systolic blood pressure.

At this point, we cannot discuss whether there is a correlation between the severity of preeclampsia and the level of platelet activation or thrombocytopenia, because 9/10 patients had a severe form. The current study in ongoing, with a larger number of patients. ❑

CONCLUSION

- Preeclampsia (pregnancy induced hypertension) represents an important pathology in pregnancy, which may present vital prognosis and which may be complicated with prematurity (4/10 cases in our study), dismaturity – small newborns for gestational age (3/10 cases in our study), intrauterine growth restriction (7/10 cases in our study), or even fetal death (1 case in this study). In our study there were no major complications for the mothers (except of course for the clinical manifestations presented).
- Platelets play a central role in the pathophysiology of preeclampsia, and current data suggest there is an altered functional status.
- Patients with preeclampsia present thrombocytopenia, which is also a feature of severe preeclampsia – in our study, half of those patients had low platelet count; in average, preeclampsia was associated with slight thrombocytopenia (statistically significant), but with signs of medullar regeneration: younger platelets present (higher MPV – also statistically significant); no significant differences in WBC, differential, hemoglobin levels or erythrocyte parameters.
- We observed increased platelet aggregation (CD41, CD61), adhesion (CD42a, CD42b) and activation (CD63, CD62P) in preeclampsia patients – statistically significant, reflecting a very active platelet status in preeclampsia.
- We especially notice the prominent levels of CD62P ($p<0.001$), CD63 ($p<0.028$) and CD31 ($p<0.004$) expressed on platelets from patients, consistent with a high activation status (especially at the membrane level – CD62P level higher than CD63 level of expression) and high level of endothelial-platelet interactions.
- It is interesting to notice a degree of positive correlation between the level of expression of CD63 and the platelet volume, suggesting younger platelets are presenting a higher level of activation.
- Other correlation studies suggested that:
  - Patients with preeclampsia with a higher number of risk factors have a lower platelet count.
  - Preeclamptic primiparas tend to have a lower platelet count.
  - The level of platelet secretion (based on the expression of CD63) is proportional to the systolic blood pressure.
- The low number of cases doesn't allow a statistically significant conclusion; research is continuing in order to validate the results on a higher number of patients. ❑

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