THE CORRELATION BETWEEN MARKERS OF SYSTEMIC INFLAMMATION AND ANGIOGENIC MARKERS IN PRE-ECLAMPSIA

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THE CORRELATION BETWEEN MARKERS OF SYSTEMIC INFLAMMATION AND ANGIOGENIC MARKERS IN PRE-ECLAMPSIA (Abstract): Pre-eclampsia is a severe multi-systemic syndrome that represents a major cause of maternal, foetal and neonatal mortality and morbidity. In the study we conducted it stood out the significant modifications of angiogenic markers in pregnant women suffering from pre-eclampsia and the existence of a correlation between C-reactive protein (CRP) and SBP, DBP and average BP. Material and method: The group included in the study consisted of 138 pregnant women hospitalized at the “Cuza-Voda” Clinical Hospital of Obstetrics and Gynaecology of Iasi, between 2012-2014, with over 20 weeks gestational age and which gave their free consent to take part in the study. Results: It is confirmed the importance of determining the markers for diagnosing and monitoring hypertensive pregnant women and at the same time it was pointed out that the sFlt-1/PlGF ratio represents a good pre-eclampsia predictor. Conclusions: The results of our study confirm the importance of determining sFlt-1 and PlGF as markers for diagnosing and monitoring pregnant women with HBP as well as the sFlt-1/PlGF ratio which represents a good pre-eclampsia predictor. Keywords: MOLECULAR MARKERS, PRE-ECLAMPSIA, PLACENTAL DYSFUNCTIONS, ENDOTHELIAL DYSFUNCTION, HELP SYNDROME.

Preeclampsia represents a pathologic condition specific to the pregnancy period, with an incidence of 3-5%, characterized by de novo hypertension and significant proteinuria (>300mg/24h), that frequently begins after the 20th gestational week. It is a systemic vascular disease that can affect numerous organs leading to severe complications for both the mother and the fetus. Although the exact causes that determine the apparition of pre-eclampsia are not known, it was noticed that placental dysfunctions play a key role in its apparition, being correlated with the subsequent maternal endothelial dysfunction (1-5).

The low vascularization of the uterus leads to the deficitary development of the fetus, with consequences on its further development (6, 7, 8). The clinical manifestations can vary from mild to severe forms, reaching its peak with the HELLP syndrome (haemolysis, growth of hepatic enzymes and
thrombocytopenia) and eclampsia (apparition of convulsive crises) (6, 9).

In Romania, the annual incidence of preeclampsia cases is between 6-14%, varying between 10 and 14% at primiparas and 5.7-7.3% at multiparas with a significantly increased incidence of preeclampsia cases in women with gemelar / multiple pregnancy, in the one with pre-eclampsia at previous pregnancies, in primiparas under 20 and over 35 years (20).

Considering the prevalence of this disorder, especially in developed countries, the existence of a validated biological marker allows a more careful supervision of asymptomatic patients so to anticipate complications with lethal risk. An ideal biological marker should have: high sensitivity and specificity level, predictive capacity for the prognostic and the evolution of the disorder as well as for the response to treatment, low accessibility and invasive level for collecting; its testing should be simple, effective and cheap.

C-reactive protein (CRP) is considered a sensitive marker of endothelial dysfunction and inflammation (10), but there are divergent opinions when referring to the value of CRP as a pre-eclampsia marker (11).

This study focused on assessing the clinical and progressive aspects of preeclampsia, by checking the correlation between inflammatory parameters, represented by CRP and blood pressure, in a group of pregnant women with pre-eclampsia and PIH compared to a group of normal pregnant women. 138 pregnant women hospitalized at the “Cuza-Vodă” Clinical Hospital of Obstetrics and Gynecology of Iasi were selected, between 2012-2014, with over 20 of weeks gestational age, which requested a specialized consultation and gave their free consent to take part in the study. At the same time, the study received the approval from the hospital’s ethics commission. These patients were collected biological samples to determine their CRP not being the case of an infectious situation or spontaneous premature rupture of membranes. At the same time, there have also been determined the PlGF and sFlt-1 biomarkers and it was calculated the sFlt-1/ PlGF ratio, as a prognosis instrument for the patient with pre-eclampsia, depending of the influence of the cumulative risk factors.

The criteria for including them into the study group were: gestational age > 20 weeks; high blood pressure and proteinuria; the exclusion criteria were: gestational age < 20 weeks; high blood pressure pre-existent to pregnancy; spontaneous rupture of membranes; associated infectious pathology (chorioamnionitis, urinary infections, respiratory infections), seropositivity to HIV.

**RESULTS AND DISCUSSION**

**Lab analyses.** Hematological and biological investigations were conducted in the laboratory of medical analyses from the
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“Cuza Voda” Maternity of Iasi, while the immunological ones were conducted within the Immunology laboratory from the “Sf. Spiridon” University Hospital of Iasi. For the hematological investigations total blood was used, collected on K2EDTA type anticoagulant using an automatic hematology analyzer (Celltac MEK - 6318K). The biochemical investigations used serum obtained after collecting the blood into “clot activator” type test tubes and spinning it at 4000 rpm, for 10 minutes. In determining the biochemical parameters it was used an automatic analyzer of Clinical chemistry, RX Imola model, along with calibrators and control serums. The immunological investigations were made for the C reactive protein using the chemiluminescence technique and a IMMULITE 2000 type automat, while for the PIGF and sFlt-1 markers, it was used the chemiluminescence technique with the COBAS E411 ROCHE analyzer. The detection limits for PIGF-1 were between 1-1.500 pg/ml, and for sFlt-1 were between 100-30,000 pg/ml. Proteinuria was determined from the urine samples collected upon hospitalization, using the VITROS 950 dry chemistry automatic analyzer and diluting the samples accordingly.

A clinical picture was made for each woman. It was recorded the height of the pregnant woman and the evolution of the weight during the pregnancy period. The dates and the hours of the results of the collected proteinuria were recorded in 24 hours, where appropriate. The markers of the inflammatory process were determined (CRP). At the same time, there were registered the dates and the hours when the highest blood pressure values were recorded during the pregnancy. By monitoring the blood pressure value, in the previous chapter, there were pointed out the correlations between the markers of systemic inflammation and cardiovascular risk.

Laboratory tests were conducted on all pregnant women, the blood tests being focused on the parameters of the hepatic function (TGO, TGP, bilirubin, LDH) and renal function (urea, creatinine, uric acid), hematological parameters (thrombocytes, white globules), coagulation indexes (INR, Quick time, and APTT), of the inflammatory process (CRP, fibrinogen) and determining the serum concentrations of angiogenic markers (sFLT-1 and P1GF), by calculating their ratio (sFlt-1/P1GF) and establishing the appropriate statistical correlations.

The data were processed and assessed using the statistic functions from SPSS 19, at the significance threshold of 95% (p<0.05). The statistical analysis used the F (ANOVA) test, $\chi^2$ test, the Spearman correlation coefficient, the coefficient of determination ($R^2$) and the sFlt-1/P1GF factor.

Considering the clinical picture: HBP associated to proteinuria and/or edemas 3 study groups were created: PE group - 54 pregnant women (39.13%) with pre-eclampsia (PE), aged between 19 - 41 years; pregnancy induced hypertension group (PIH) - 34 pregnant women (24.63%) with high blood pressure values and/or insignificant proteinuria (<300 mg/24h) and/or edemas, aged between 22 and 44 years; normal pregnant women group (NG) - 50 pregnant women (36.24%) aged between 16 and 41 years.

From the 138 pregnant women included in the study, most of them were in the age group of 25-34 years (51.45%), the frequency peak being somewhere around the age of 29 years old. Depending of their gestational age, in the PE group the patients were generally in 33-36 weeks (37.5%), the patients in the PIH group had
a homogenous distribution for the gestational age intervals of 33-36 weeks and 37-40 weeks (29.4%), compared to the witness group (NG) where the patients were mostly over 37 weeks (76%).

Edemas were present in 50% of the pregnant women with PE and in 29.4% of the ones with PIH, the difference in percent being significantly higher compared to the witness group (p=0.001). Proteinuria was found in almost 87.5% of the patients in the PE group and 64.7% in the one with PIH. Pathological urinanalysis was observed in 57.1% of the pregnant women with severe pre-eclampsia and in 44.7% of the pregnant women with milder types of pre-eclampsia, frequency distributions that were not statistically significant (p=0.833).

HBP and proteinuria are not invariably present; HBP may appear late in the evolution of PE and proteinuria can be absent in case of milder types of pre-eclampsia (12). On the other hand, there have also been cases of HBP during pregnancy that did not necessarily lead to pre-eclampsia, as it is the case of gestational hypertension and chronic hypertension. For the groups studied, the Systolic blood pressure (SBP) was significantly higher in the case of pregnant women with PE (p<0.001), with variations from 120 to 180 mmHg, the group mean being of 153.44±18.95 mmHg. Most pregnant women in the group with PE recorded values of systolic blood pressure between 143-164 mmHg (37%), but 24% of the pregnant women with PE recorded values higher than 160 mmHg. The pregnant women in the PIH group recorded values of SBP between 120-200 mmHg, with a group mean of 148.65±21.03 mmHg. In normal pregnant women, the systolic blood pressure varied between 100-165 mmHg, the mean value of the group being of 120.40±11.99 mmHg. The diastolic blood pressure (DBP) was significantly higher in the pregnant women from the group with PE (p<0.001), with variations from 70 to 150 mmHg, the group mean being of 92.50±14.83 mmHg. Most pregnant women from the PE group recorded values of DBP between 85-100 mmHg (35%), but 26% of the pregnant women with PE recorded values of higher than 120 mmHg. The pregnant women from the PIH group recorded values of DBP between 60 and 120 mmHg, with a group mean of 88.80±14.17 mmHg. In normal pregnant women, the DBP varied between 55 and 115 mmHg, the mean value of the group being of 72.56±11.09 mmHg. Depending of the growth of DBP, PE was grouped in two distinct clinical categories: mild (<110 mmHg) and severe (>110 mmHg). In the cases studied, it resulted 13% of pregnant women with severe pre-eclampsia and 87% pregnant women with mild pre-eclampsia. The severe types of PE were identified in the patients around the age of 37 years, while the mild types of pre-eclampsia were associated to the ages of 28-29 years (p=0.020).

The mean values obtained for the body mass index (BMI) indicated significant over weight (BMI=30.52 kg/m²) in the patients from the PE group (p=0.001) and morbid obesity in 11.8% of the patients in PIH group compared to the witness group (NG). Depending of the BMI variation during pregnancy it was noticed that pregnant women with PE recorded body weight growth by more than 32%, while the pregnant women with PIH, the BMI growth was of almost 18%. Gestation and parity did not point out significant differences between the monitored groups (p>0.05).

In our study it was noticed that the women at their first pregnancy, teenagers and the women who remained pregnant after the age of 40 years presented a higher
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The risk of being affected by pre-eclampsia. The values of the biological markers indicated significantly higher differences for the PE group compared to the normal group (NG) for CRP (p=0.001), TGO (p=0.001), uric acid (p=0.001), serum urea (p=0.005) and proteinuric (p=0.001). In the cases when the pregnant women developed PE, the mean value of PIGF was significantly lower (p=0.003) and sFlt-1 was significantly higher (p=0.001) compared to the NG group. At the same time, the angiogenic factors were statistically significantly correlated with the mean values of BP. Thus, PIGF was significantly correlated with the age of the pregnant woman and with high mean BP (r=0.831; p=0.01). The sFlt-1 factor was significantly correlated with age, the mean BP and the high levels of LDH and TGO (r=0.784; p=0.046), being a better pre-eclampsia predictor than PIGF. The sFlt-1/PIGF ratio was significantly higher at the group of pregnant women with PE (p=0.001) compared to the NG. The plurality of risk factors: age, mean blood pressure, LDH, TGO, INR, TQ and CRP were significantly correlated with the sFlt-1/PIGF ratio.

CRP varied from values under 6 up to 192 mg/l recording a mean value significantly higher in the group of pregnant women with pre-eclampsia compared to the group of pregnant women with PIH (35.89 vs 6.72 mg/l) (p=0.001)

The individual values of CRP in the group of pregnant women with PE varied from 6 to 192 mg/l, most pregnant women recording values in the reliability interval (RI 95%):26.18-45.60 mg/l (42.6%), but 14.9% of the pregnant women with PE recorded values significantly higher of this parameter. In the case of pregnant women with PIH, CRP presented some values over the reference limit (=6 mg/l), but most of them presented values < 6 mg/l (82.1) (tab.1).

<table>
<thead>
<tr>
<th>TABLE I</th>
<th>The values of some statistical indicators depending on the severity of pre-eclampsia</th>
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<tbody>
<tr>
<td>STUDY GROUP</td>
<td>N</td>
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<tr>
<td>Mean blood pressure (mmHg)</td>
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<tr>
<td>Mild (moderate)</td>
<td>47</td>
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<tr>
<td>Severe</td>
<td>7</td>
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<tr>
<td>Total</td>
<td>54</td>
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<tr>
<td>C-reactive protein (mg/L)</td>
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<tr>
<td>Mild (moderate)</td>
<td>47</td>
</tr>
<tr>
<td>Severe</td>
<td>7</td>
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<tr>
<td>Total</td>
<td>54</td>
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</table>

In the group of pregnant women with PE the work hypothesis was confirmed, the plasmatic level of CRP is in direct, statistically significant correlation with the blood
pressure. The values of CRP were directly correlated, having statistical significance, with both SBP (r=0.460; p=0.001) and DBP (r=0.614; p=0.001) as well as with the mean calculated pressure (r=0.612; p=0.001).

The mean values of CRP were significantly higher in the case of pregnant women with severe PE (89.14±51.31mg/L) while the pregnant women with mild PE the mean value was of 22.86±21.35 mg/L. In the cases studied, the mean values of Hb indicated a mild anemia in the group of pregnant women with PE (p=0.138) without a significant correlation with its severity (p=0.451). The hematocrit varied from 32.7 to 43.5% in the group of pregnant women with PE, recording a mean value of 37.25%±2.63 that is not significantly higher if compared to the values of the hematocrit recorded in the group of pregnant women with PIH 36.88% ±3.49 (p=0.102). In the cases of severe PE, the mean values of the hematocrit were slightly lower (33.93%±2.92) compared to the ones recorded in mild cases (34.87%±6.11) (p=0.847).

It was also observed that the plasmatic level of CRP was directly correlated also with the individual values of hemoglobin or hematocrit, but without any statistical significance (13).

In the group of pregnant women with PE the individual values of PIGF recorded a variation between 16.37-942 pg/mL with a mean value (119.3 pg/mL) significantly lower if compared to the mean value recorded in the witness group (327.57 pg/mL) or the PIH group (129.13 pg/mL) (p=0.003).

It was observed that the patients under 20 years of age had the PIGF median of 200 pg/mL, and at the patients aged between 20 and 34 years the median slightly surpassed the value of 100 pg/mL; in the pregnant women aged over 35 years the median of PIGF values was below 100 pg/mL. Depending of the gestational age the mean value of PIGF was significantly higher in the gestational weeks 33-36 (245 pg/mL) and 37-40 (254.4 pg/mL) (p=0.049). The correlations between the individual values of PIGF and the maximum values of blood pressure recorded were indirect, of moderate intensity. The higher values of PIGF were accompanied by lower values of systolic blood pressure (r= -0.359; R²=0.1771, p=0.001) or diastolic (r= -0.320; R²=0.1331, p=0.003). The correlation between the individual values of PIGF with BMI was not statistically significant (r= -0.126; R²=0.016, p=0.255).

The values obtained for the sFlt-1 factor varied from 1152 to 32708 pg/mL with a variation of more than 92%, recording in the PE group the highest mean value (14365±6464), significantly higher if compared to the other study groups (p=0.001). Correlated to the age group, the mean values of sFlt-1 did not indicate statistically significant differences (p=0.342); nonetheless there have been noticed higher mean values at the age group of over 35 years. Depending of the gestational age, the mean value of sFlt-1 was significantly higher in the gestational weeks 25-28 (13867 pg/mL) and 29-32 (13033 pg/mL) (p=0.001). The correlations between the individual values of sFlt-1 and the maximum values of blood pressure recorded were direct, of moderate intensity, and statistically significant. The higher values of sFlt-1 were accompanied by high values of systolic blood pressure (r= +0.397; R²=0.3445, p=0.001) or diastolic (r= +0.430; R²=0.2755, p=0.001). There were not any significant correlations between the individual values of sFlt-1 and BMI (r= -0.136; R²=0.0184, p=0.222) (Fig.1).
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Fig 1. Mean values of PI GF, sFlt-1 and the sFlt-1/PIGF ratio on study groups
The values of the sFlt-1/PIGF ratio indicated significant growth for the PE group compared to the witness group NG or PIH (p=0.001). In the patients with pre-eclampsia, it was observed a direct correlation, of moderate intensity, between the systolic blood pressure (SBP) and sFlt-1/PIGF (r= +0.555; p=0.026), but the high values of diastolic blood pressure (DBP) are associated with high values of sFlt-1/PIGF only in 18.5% of the patients (r=+0.185; p=0.493). At the patients from the PIH group, the correlation between SBP (r=+0.014; p=0.937) and DBP (r=+0.105; p=0.555) and the sFlt-1/PIGF ratio were not statistically significant.

The high values of CRP were accompanied by high values of sFlt-1/PIGF ratio only in 13.8% of the patients with PE (r=+0.138; p=0.724) and in 48% of the patients with PIH (r=+0.480; p=0.015). Correlating the values of PIGF with the pre-eclampsia risk factors it was noticed that the logistic model indicated an important cumulative risk depending of the age of the pregnant woman and the presence of high mean blood pressure (p=0.01 - Model 2) (fig. 2).

It was noticed that PE was characterized by high levels of sFlt-1 associated with HBP, proteinuria and high values of CRP (p=0.049 - Model 8).

Correlating the values of sFlt-1 with the risk factors for PE it was noticed that the logistic model indicated an important cumulative risk depending of the age of the pregnant woman, the presence of high mean blood pressure and high LDH values (p=0.047 - Model 3) and the pregnant woman’s age, the presence of high mean blood pressure and high LDH and TGO values (p=0.046 - Model 4) (fig. 3).

The logistic model indicated that a group of risk factors as age, blood pressure, LDH, TGO, INR, TQ and CRP were significantly correlated with the sFlt-1/PIGF ratio (R=0.663; p=0.04).

Nowadays there isn’t any single objective test to identify PE or to quantifying the risk of certain severe complications, the diagnosis relying only on the clinical lab parameters evaluated in dynamics. Experience shows that early identification and the monitoring of the mother and fetus are use-
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...ful and hence the prediction of PE becomes very important. Early identification of PE, not only based on the inventory of risk factors, but also with the use of some markers might indicate better the moment suitable for beginning the treatment, its action and the moment to remove the child. Because of the fact that these modifications appear a long time before the apparition of the clinical signs of the disease, recent research focus on the endothelial dysfunction as a physiopathological mechanism of PE.

Many researchers (14, 15) noticed that the inflammatory maternal answer in PE and especially in severe PE, being known that there is an exaggerated systemic maternal inflammatory answer (16). In this context, it is considered that PE, at a given moment in its evolution, borrows a link of the systemic inflammatory answer that combines with the endothelial dysfunction. The increase of CRP indicates the presence of an inflammatory, destructive, infectious or non-infectious process but not specific.

In this study we have demonstrated that the plasmatic level of CRP is significantly and directly correlated with SBP and DBP. Thus, Can (17) use the mean arterial pressure as a severity indicator and he demonstrated the direct association with the inflammatory reaction. This result joins the studies that support the affirmation that CRP is an effective marker for the apparition of PE and it correlates significantly with the severity of the disorder.

Carl et al. (18) demonstrated that a value of more than 3 mg/L is a good predictor for cardiovascular and inflammatory risk in women with pre-eclampsia / eclampsia antecedents. At the same time, Mihu D et al. (10), state that CRP is a marker for PE’s severity and for the new-born’s birth weight. A prospective study, initiated by Behboudi et al. (20) on a group of 778 pregnant women, established a reference value of 4.5 mg/dL for CRP in the first trimester of pregnancy as a predictive factor for PE, and Bita (12) with a study on 400 pregnant women established the threshold over which PE can be predicted.

Fig. 3. sFlt-1 correlation with age and mean blood pressure
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In the first trimester of pregnancy of more than 5 mg/L. In order to establish reference values for CRP in normal pregnant women and pregnant women with PE, Hwang et al. (19) demonstrated also the possibility of using CRP as a severity marker in PE.

Other authors pointed out the relationship between CRP and the clinical and biochemical parameters from PE; the high levels of hemoglobin, creatinine, TGO, TGP, LDH, blood urea and proteinuria were associated with high levels of CRP.

Nowadays, more and more studies confirm the fact that the CRP (10) marker can help in differentiating the pregnancy associated HBP whose etiology is not PE. Other studies, on the other hand, could not establish a real relationship between CRP and PE (13). In recent studies, Stefanovic (21) focused on the endothelial dysfunction as an anomaly in PE and concluded that CRP as a marker of the inflammation does not have high values and it is not associated with the severity of PE.

The high CRP values are a useful parameter in assessing the severity risk of PE in pregnant women with high body mass index in the third trimester of pregnancy (13). In our case it was noticed a risk of 6.71 times higher of severe PE in obese patients.

Considering the importance of carefully monitoring the mother and the fetus and the therapeutic intervention at the best moment in the process of PE, to avoid fontal and maternal complications, in this study it was assessed the sensitivity and the specificity of some laboratory tests, including the usual ones, that focus on assessing various angiogenesis markers in pre-eclampsia diagnosis and management. PIGF and sFlt-1/PIGF ratio were correlated with the clinical and laboratory signs of the cases included in the study. The higher the sFlt-1/PIGF ratio the better can it assess the risk of premature birth and it represents a potential prognosis parameter in PE monitoring.

It was noticed that PIGF and sFlt-1 levels are simultaneously affected in PE (especially in early pre-eclampsia); thus, the sFlt-1/PIGF ratio represents now a predictor element superior to the individual analysis of these markers. Another approach in assessing these markers might consist in the sequential determination of serum levels, an approach that has provided better predictive results than the punctual assessment in of the pregnancy a certain moment. The sensitivity of sequential determinations of the sFlt-1/PIGF ratio is of 100% and its specificity is of 98-99% (3, 5).

It was noticed that sFlt-1 levels and sFlt-1/PIGF ratio increase while PIGF levels decrease significantly in patients who develop PE, all this compared to the levels measured in normal pregnancies or in patients with chronic kidney disease (CKD). sFlt-1 levels increase and PIGF levels decrease even more in women who simultaneously associate CKD or chronic hypertension during pregnancy.

CONCLUSIONS

In our study it resulted that the mean value for the CRP marker correlated with SBP, DBP and mean BP, but it did not correlate with hemoglobin and hematocrit.

It was pointed out that CRP is an inflammation marker but in PE it was not proved that it can be used in the clinical practice on a daily basis.

The results of our study confirm the importance of establishing sFlt-1 and PIGF as markers for the diagnosis and the monitoring of pregnant women with HBP as well as the sFlt-1/PIGF ratio which represents a good PE predictor.
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