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ABSTRACT

DIAGNOSTIC AND PROGNOSTIC MARKERS IN PREECLAMPSIA

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Abbreviations:

HELLP - Hemolysis, Elevated Liver enzymes, Low Platelet count

VEGF – Vascular Endothelial Growth Factor

PlGF – Placental Growth Factor

sVEGFR – soluble Vascular Endothelial Growth Factor Receptor

sFlt-1 - soluble Fms-like tyrosin kinase 1

CSF – cerebrospinal fluid

INR – international normalised ratio

BMI – body mass index

AST – aspartat aminotransferase

ALT – alanin aminotransferase

SBP – Systolic Blood Pressure

DBP – Diastolic Blood Pressure

MAP – Mean Blood Pressure

ROC – receiver operating curve

AUC – area under curve

PRES - posterior reversible encefalopaty syndrome

INTRODUCTION

Preeclampsia is a pregnancy associated medical condition defined by hypertension and proteinuria.

Preeclampsia and its complications, eclampsia and HELLP sindrome (Hemolysis, Elevated Liver enzymes, Low Platelet count), are the most common maternal and fetal pregnancy related morbidities worldwide. Despite thorough research efforts, the etiopathogeny of preeclampsia is still unclear, with numerous research studies not being able so far to identify any widely accepted theory. Recent studies suggest that the imbalance between pro-angiogenic factors (VEGF – vascular endothelial growth factor and PlGF – placental growth factor) and anti-angiogenic factors (sFlt-1 - soluble Fms-like tyrosin kinase 1 and sEng - soluble endoglin) may be causally related to preeclampsia.

PART I – OVERVIEW

Pregnancy related hypertension is defined as new onset rise of systolic blood pressure above 140 mm Hg and dyastolic blood pressure above 90 mm Hg after 20 weeks of gestation in a previously normotensive pregnant woman[1].

Preeclampsia is a pregnancy related syndrome defined by hypertension and proteinuria and is associated with at least one of the following symptoms: edema, visual disturbances, headache or epigastric pain. Proteinuria is defined as the presence of more than 0.3 g of protein in 24 hour collected urine[1].

There are two forms of preeclampsia: mild and severe[3]. The mild form is defined by the rise of the systolic blood pressure up to 160 mm Hg and dyastolic blood pressure up to 110 mm Hg as well as proteinuria above 0.3 g /24 h and below 5 g/24 h. The severe form of preeclampsia is diagnosed by: systolic blood pressure above 160 mm hg or dyastolic blood pressure above 110 mm Hg at two meassurments 6 hours apart with the patient at bedrest, proteinuria above 5 g/24 h, oliguria, visual disturbances, epigastric pain, nausea, vomiting, pulmonary edema, liver dysfunction, thrombocytopenia.

Preeclampsia occurs in 5-7% of previously healty nuliparous pregnant women. Realted to severity, the incidence of the two forms is among preeclamptic women is: 75% mild preeclampsia and 25% severe preeclampsia[4].

Risk factors for preeclampsia are medical conditions associated with microvascular disease (diabetes mellitus, chronic arterial hypertension, vascular or connective tissue disease), antiphospholipidic syndrome, protein C, S and antitrombin defficiency and nephropathies. Other risk factors are associated with mother, father or fetus[2].

Currently, the etiology of preeclampsia is stil unknown. A series of theories have been suggested, but they remain incompletely proved. Preeclampsia pathogeny pathways are only partially known and they seem to involve uteroplacentar ischemia due to increased vascular resistence and utero-placentar blood flow decrease, immunologic imbalance as a result of endothelial injury or prostaglandin tromboxan ballance shift towards prostaciclin with subsequent vasoconstriction an platellet aggregation[5]. Recent theories suggest that a significant contribution to the onset of preeclampsia may be owed to the imbalance between pro-angiogenic factors (VEGF – vascular endothelial growth factor and PlGF – placental growth factor) and anti-angiogenic factors (sFlt-1 - soluble Fms-like tyrosin kinase 1 and sEng - soluble endoglin)[6].

PART II – PERSONAL RESEARCH

Study objectives

The study named ***Diagnostic and Prognostic Markers in Preeclampsia*** aims to evaluate the diagnostic role of the angiogenic factors (PlGF and sFlt1) in preeclampsia and assessing their prognostic value related to preeclampsia severity. Considering the risk of eclamptic convulsions and sometimes the need for emergency cesarean section our secondary objective was the analisis of the markers in the cerebrospinal fluid (CSF) in patients with preeclampsia and the possible correlation with their serum levels as well as the correlation with the severity of preeclampsia (blood pressure, proteinuria, urine output, etc.)

Patients and method

The study was designed as a prospective case-control study and included patients recruited following admission to the Obstetrics and Gynecology Department and/or Anesthesiology and Intensive Care Department of the in Craiova Emergency Hospital from January 2009 to December 2012. We included in the study a total of

92 pregnant women of which 65 were diagnosed with preeclampsia and 27 were normotensive pregnant women. Patients were assigned to three study groups based on the classification of preeclampsia: group A – Mild Preeclampsia - 44 patients, group B - Severe Preeclampsia - 21 patients and group C - Pregnant normotensive - 27 patients (control group).

Several inclusion criteria were used: pregnant women aged between 16 and 40 years who have indication for delivery by cesarean operation, patients who were informed about the study, accepted voluntary participation and signed an informed consent, pregnancy of 20 weeks or more in the absence of other pregnancy complications, no history of preeclampsia, the absence from personal history of chronic pathology that could influence the study parameters: gynecological history, hypertension (primitive/secondary), chronic kidney disease, hepatic failure, coagulopathy, thrombocytopenia, haematological or neurological disorders.

We used a standardized diagnostic protocol for the initial evaluation of all patients that included collection of demographic, clinical, laboratory and obstetrical data and sampling of blood and CSF (at the time of spinal anesthesia) for dosing PlGF and sFlt1. ELISA was used for dosing both serum and cerebrospinal fluid PlGF and sFlt1 (R&D Systems Quantikine PlGF Kit ® and SVEGF1/FLT1 Quantikine Kit®).

The research was conducted with the rules of ethics in research and medical ethics, without harming the environment, falling within environmental standards.

Results

As the selection of patients in the control group C was done respecting age distribution in the two study groups (A and B), there was no significant difference of mean patient age between the three groups ($p = 0.864$). As expected, all other clinical data (MAP, diuresis, BMI) and obstetric (parity, gestational age, fetal weight, prematurity) were significantly altered both in patients with mild preeclampsia and severe compared with normotensive pregnant women in the control group ($p<0.05$).

We also recorded significant increases ($p<0.001$) of proteinuria of transaminases (ALT, AST) and a significant decrease ($p<0.001$) in platellet count in patients in groups A and B respectively compared with those in group C. The measured values for hemoglobin, hematocrit, INR, fibrinogen, and creatinine did not change significantly in patients with preeclampsia.

It is known that PlGF levels are generally elevated in pregnancy, so that its values in our study were above normal (N <20 pg/ml) in all cases ranging between 46 and 315 pg/ml, with an overall average of 138.7 ± 56.6 pg/ml. The levels of PlGF were significantly higher for normotensive pregnant women (195.6 ± 60.9 pg/ml) than both patients with mild preeclampsia (126.6 ± 29.9 pg/ml) and severe preeclampsia (138.7 ± 56.6 pg/ml) which confirms the hypothesis that PlGF levels significantly lower in preeclampsia ($p<0.001$).

CSF values of PlGF were between 7.3 and 27.2 pg/ml, with an overall average of 14.6 ± 5.5 pg/ml. In contrast with the serum levels PlGF - CSF was significantly higher in patients with preeclampsia (16.9 ± 5.0 pg/ml) compared to normotensive pregnant women (9.3 ± 1.7 pg/ml) ($p<0.001$). This discrepancy is confirmed by evaluating the serum PlGF/PlGF-CSF ratio that was 9:1 for patients with mild preeclampsia and 5.5:1 in those with severe preeclampsia, while normotensive pregnant women had a significantly higher ratio of 22:1 ($p<0.05$). This finding indicates a 4 fold increase the blood-brain barrier permeability for PlGF in patients with preeclampsia that is likely to be directly involved in the pathogenesis of preeclampsia and its severe neurological complications (eclampsia), given that one of the main properties of PlGF in addition to angiogenesis is increased microvascular permeability.

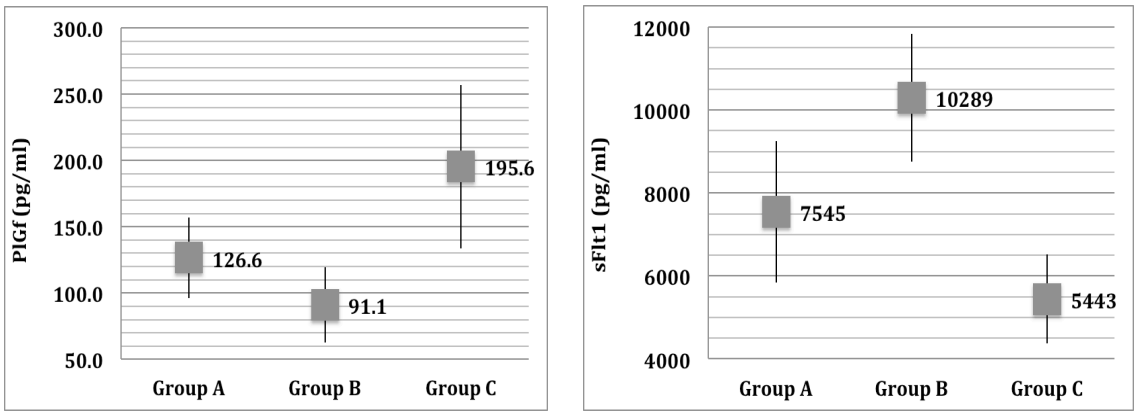

Fig. 1 The serum PlGF and sFlt1 levels in patients with preeclampsia

Measured values of serum sFlt1 ranged from 3311 pg/ml to 15422 pg/ml, with an average of 8432 ± 2075 pg/ml. Comparing the study groups, we observed significant increases of serum sFlt1 both in patients with severe preeclampsia (10289 ± 1523 pg/ml) and those with mild preeclampsia (7545 ± 1686 pg/ml)

compared to normotensive pregnant women (5443 ± 1047 pg/ml) confirming the hypothesis that sFlt1 is also increased in women with preeclampsia (p<0.001). sFlt1-CSF ranged between 19.8 and 48.9 pg/ml, average 34.9 ± 4.3 pg/ml. In patients with preeclampsia sFlt1-CSF values were similar (34.6 ± 5.5 pg/ml) with those of normotensive pregnant women (33.4 ± 4.8 pg/ml) (p = 0.3092).

Serum sFlt1/sFlt1-CSF ratio had higher values in patients with severe preeclampsia (317:1) compared to those with mild preeclampsia (220:1) and normotensive pregnant women (166:1) (p <0.05).

As in most cases there were significant changes of the values of both markers between patients with mild and severe preeclampsia we subsequently evaluated the correlations between the two markers and severity of preeclampsia. The analysis started with the evaluation of the correlations between serum PlGF and sFlt1. Linear regression analysis indicated a significant negative correlation between the two markers (R = - 0.6413, p < 0.001) indicating a possible link between the serum levels and their functional relationship (factor-receptor). Analysis of the correlation between CSF PlGF and sFlt1 levels did not reveal a significant correlation between the two markers (R = - 0.1882, p = 0.1745), suggesting that differences between the values of the two markers in CSF are not due to a causal link between them.

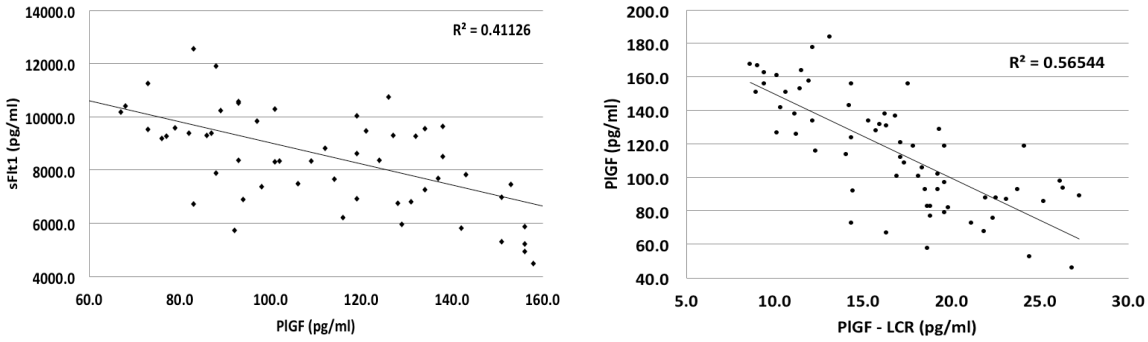

Fig. 2 Correlations between serum levels of sFlt1 and PlGF and between PlGF and PlGF-CSF

For PlGF, we discovered a strong negative correlation between serum and CSF values, with a correlation coefficient R = - 0.7519 (p <0.001), while no correlation was identified between serum and CSF sFlt1 values, R = - 0.0409 (p = 0.567). These results confirm that the differences between the values of PlGF and sFlt1 between the serum and CSF are not due to the interactions between them, but to

changes in microvascular and blood-brain barrier permeability that favors PlGF with a molecular weight (16 kD) 10 times lower than that of sFlt1 (180 kD).

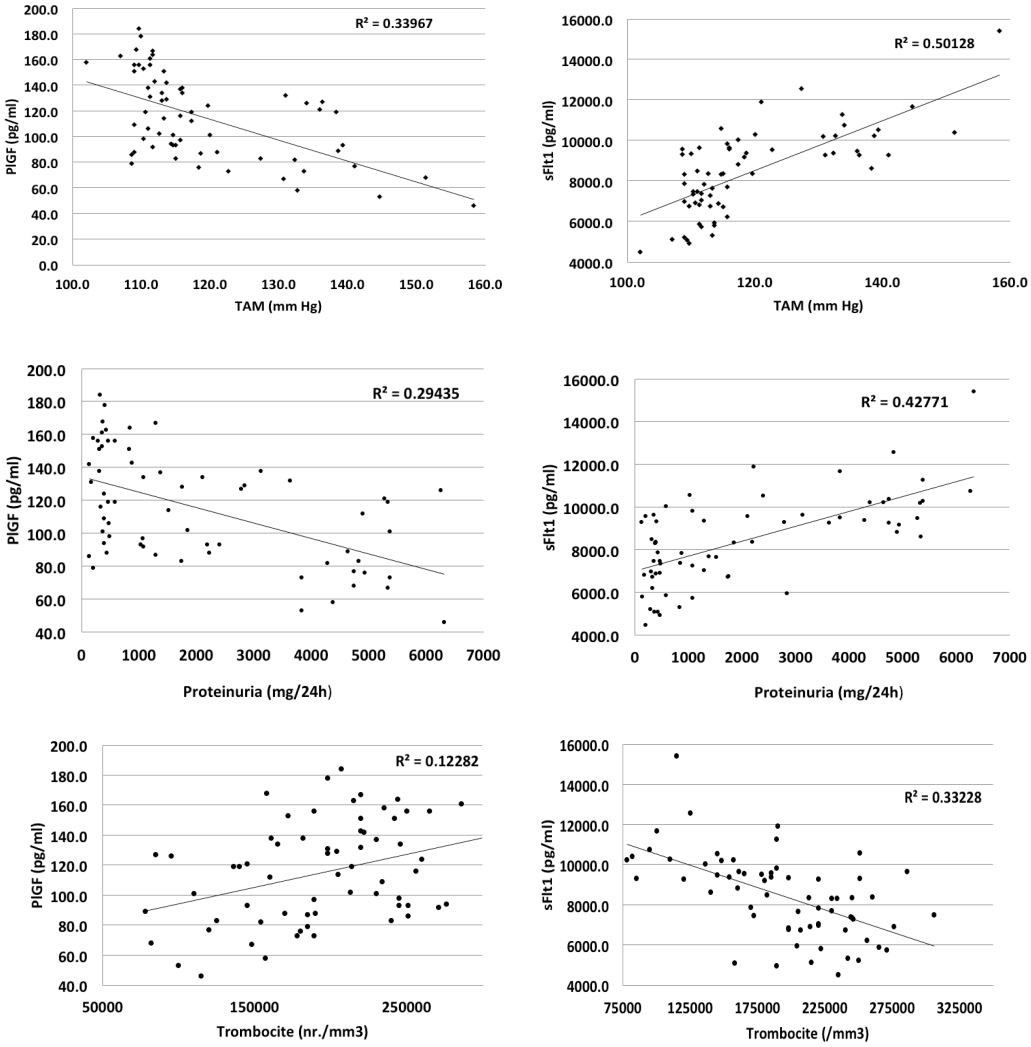

Fig. 3. Correlations between PlGF, sFlt1 and the most significant parameters of preeclampsia patients in the study

We subsequently analysed the correlations between each marker and the severity of preeclampsia. For serum PlGF we found significant negative correlations with: MAP ($R = -0.5832$, $p < 0.001$), proteinuria ($R = -0.5425$, $p < 0.001$) and AST ($R = -0.3620$, $p < 0.05$) and a significant positive correlation with platellets number ($R = 0.3504$, $p < 0.05$). No significant correlations were found between PlGF and diuresis, creatinine and ALT. Serum sFlt1 had significant positive correlations with: MAP ($R = 0.7080$, $p < 0.001$), proteinuria ($R = 0.6539$, $p < 0.001$) and AST ($R = 0.4897$, $p < 0.001$) and negative correlations with platellets ($R = -0.5764$ $p < 0.001$) and diuresis ($R = -0.4803$, $p < 0.05$) while no significant correlations were found

with creatinine or ALT . PlGF - CSF significantly correlated with : MAP (R = 0.3925, p < 0.05), AST (R = 0.3472, p < 0.05) and proteinuria (R = 0.2975, p < 0.05). No correlations were found for PlGF-CSF with diuresis, creatinine, platelets and ALT .

We may therefore state that our analysis proves significant correlations between PlGF, sFlt1 and PlGF-CSF with most severity parameters of preeclampsia.

Given the significant correlations mentioned above we further assessed the potential prognostic role of each angiogenic factor in patients with preeclampsia. The evaluation was done by analyzing the performance indicators of PlGF and sFlt1 in achieving discrimination between mild and severe preeclampsia using ROC curve analysis (receiver operating curve) and AUC (area under curve).

Since MAP and proteinuria are the diagnostic criteria used in the allocation of preeclampsia patients groups, we obtained a very high predictive value for both the cut-off value of 115 mm Hg MAP (Sn 100%, Sp 89%, AUC = 0.987) and the cut-off value of 2.8 g/24h for proteinuria (Sn 91%, Sp 93%, AUC 0.975), but with no statistically significant difference between them (p = 0.671). For the other parameters performance indicators were significantly lower than those of MAP or proteinuria (p <0.05): platelets (Sn 91%, Sp 82%, AUC = 0.884) and ALT (Sn 71%, Sp 86%, AUC = 0.853), while lower values obtained for AST (Sn = 62%, Sp = 75%, AUC = 0.626) were not statistically significant (p = 0.098).

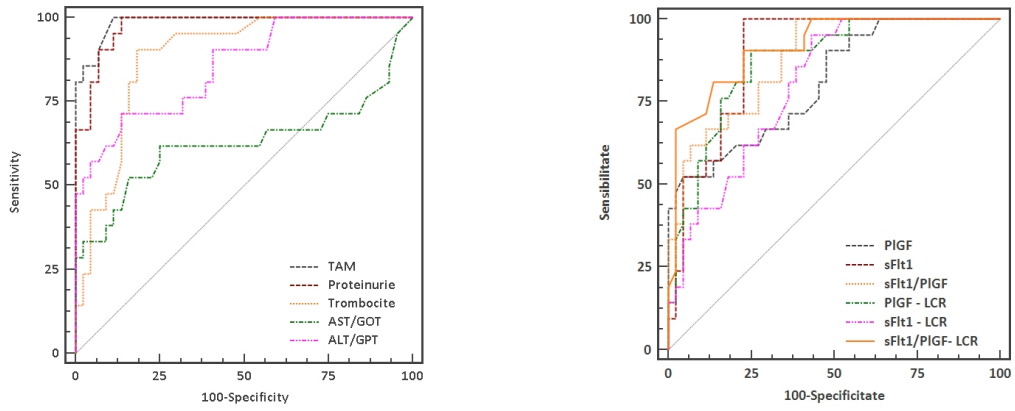

Fig. 4. Comparison of ROC curves and AUC for clinical parameters, laboratory and angiogenic factors in patients with preeclampsia

Significant prognostic value (p <0.001) was obtained for both serum angiogenic factors: PlGF (Sn 66.7%, Sp 70.5% and AUC = 0.808, cut-off ≤ 101 pg/ml), sFlt1 (Sn 100%, Sp 77.3% and AUC = 0.891, cut-off value of > 8497 pg/ml) and

sFlt1/PlGF ratio (Sn 80.9%, Sp 72.7%, AUC = 0.878, cut-off > 76.4). In contrast, PlGF-CSF proved to be the most powerful prognostic marker (Sn 90.5%, Sp 75.0%, cut-off > 9.3 pg/ml, AUC = 0.871, p <0.001), significantly better than sFlt1/PlGF-LCR ratio (AUC = 0.645, Sn 76.2%, Sp 54.6%, cut-off ≤ 2.1, p <0.05) and sFlt1-CSF (AUC = 0.531, Sn 61.9%, Sp 56.8%, cut-off ≤ 34.7 pg/ml, p = 0.687).

Summarizing, we can say that although serum sFlt1 has proved the most powerful indicator of preeclampsia severity, due to the poor results obtained for sFlt1-CSF it can not be safely used as a prognostic factor in preeclampsia. Therefore PlGF is a better overall marker for preeclampsia severity diagnosis.

Discussions

Recent studies claim that the etiology of preeclampsia is the imbalance between angiogenic (PlGF, VEGF) and anti-angiogenic factors (sFlt1, sEng)[6].

sFlt-1 is a soluble fms-like tyrosine-1 kinase protein (sVEGFR-1) that inactivates proteins involved blood vessels growth [7] and inhibits angiogenesis by binding to the free forms of VEGF and PlGF[8] and reducing the effects of angiogenic factors upon maternal endothelium and contributing to further development of hypertension and proteinuria. As a result, serum levels of sFlt-1, PlGF and VEGF in preeclampsia are altered and can be correlated with the severity of the disease[9] similar to the results obtained in our study.

In normal pregnancy, proangiogenic factor PlGF is increased from the first two trimesters of pregnancy. In contrast, for patients with preeclampsia this factor is decreased and the anti-angiogenic factor sFlt -1 is greatly increased and remain so throughout pregnancy[10]. Also, the mean blood pressure is negatively correlated with plasma levels of PlGF and positively correlated with sFlt-1/PlGF ratio[9]. In preeclampsia, blood pressure is negatively correlated with blood levels of PlGF and positively with the ratio of the two factors sFlt-1/PlGF. sFlt -1 values in patients with preeclampsia are better correlated with increased disease severity. It was found that the sFlt-1/PlGF ratio is more accurate in predicting preeclampsia than independent measurement of the factors[11]. Our study confirms significant changes of angiogenic markers in preeclampsia. We have shown that PlGF is significantly decreased in preeclampsia and it correlates negatively with MAP (R = - 0.58) and

proteinuria (R = - 0.54) while sFlt1 and sFlt1/PlGF ratio are significantly increased and positively correlated with preeclampsia severity (R = 0.48 - 0.71).

Many studies have shown that the early and accurate diagnosis of preeclampsia requires the presence of the main symptoms (hypertension and proteinuria), as well as evaluating the balance between proangiogenic and anti-angiogenic factors[12].

It was suggested that these angiogenic factors also have significant alterations in the cerebrospinal fluid in patients with preeclampsia. These changes in the cerebrospinal fluid may lead to endothelial dysfunction and increased vascular permeability of the blood-brain barrier leading to hypertensive encephalopathy recently called posterior reversible encephalopathy syndrome (PRES)[13]. The mechanisms by which women with preeclampsia develop seizures are not yet known. There are two hypotheses concerning the onset of seizures targeting cerebral vascular function and cerebral blood flow autoregulation during increases in blood pressure and angiogenic factors are directly involved in both cases[14]. Pathophysiological mechanisms producing the PRES syndrome are not fully known. The most supported theory is that severe hypertension causes an imbalance of cerebral circulation autoregulation leading to hypoperfusion, endothelial cell injury, blood-brain barrier disruption and vasogenic edema[15].

By analyzing the relationship between serum and CSF levels of angiogenic factors we have shown a 4 fold increase of the blood-brain barrier permeability for PlGF and 2 fold for sFlt1 in patients with preeclampsia. For PlGF, we proved a strong negative correlation between serum and CSF (R = - 0.75) while for sFlt1 no such correlation was found (R = - 0.04). These results suggest that altered levels of CSF PlGF and sFlt1 in preeclampsia are not due to their interaction but to the changes in blood-brain barrier permeability that favors PlGF with a molecular weight (16 kD) more than 10 fold lower than sFlt1 (180 kD).

Recent studies have associated PRES syndrome triggering with lumbar puncture in pregnant women. Torillo et al. described this syndrome in 2007 in patients with severe preeclampsia subjected to combined spinal - epidural anesthesia prior to caesarean surgery[16].

Based on the observations of these studies and other literature data on the posterior reversible encephalopathy syndrome (PRES), we can state that patients with severe preeclampsia are at increased risk of developing this syndrome

secondary to spinal anesthesia. To be able to avoid the triggering of this syndrome by spinal contraindicating spinal anesthesia, it is therefore necessary to determine which patients with preeclampsia are at the higher risk. Some researchers have suggested routine magnetic resonance imaging in patients with increased risk of of PRES syndrome, but it is a costly and time-consuming investigation[17].

We therefore recommend that serum placental growth factor (PlGF) should be routinely tested to detect patients at high risk of developing severe preeclampsia and therefore increased risk to develop posterior reversible encephalopathy syndrome postpartum.

Conclusions

Our results confirm the decrease of serum PlGF in patients with preeclampsia, in contrast to its increase in cerebrospinal fluid as well as elevated serum sFlt1.

Significant negative correlation between serum and cerebrospinal fluid levels was obtained for PlGF, but not for sFlt1.

It was also confirmed the prognostic value of serum and CSF PlGF as well as serum sFlt1 as they all significantly correlate with the severity of preeclampsia.

These results indicate the potential use of serum PlGF in medical practice for the selection of pregnant women with preeclampsia and high-risk of posterior reversible encephalopathy syndrome and establishing an absolute contraindication to locoregional anesthesia in patients with indication of delivery by cesarean operation.

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