RJME
Romanian Journal of
Morphology & Embryology
http://www.rjme.ro/

Original Paper

The potential value of diagnostic and predictive serum biomarkers for preeclampsia

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Abstract

Background: Preeclampsia (PE), one of the classes of hypertensive pregnancy disorders, is one of the three causes of maternal morbidity and mortality worldwide. The angiogenic and anti-angiogenic factors are useful markers in predicting and diagnosing PE. *Aim*: This study aims to detect and measure the serum level of some biomarkers [hypoxia-inducible factor-1 subunit alpha (HIF-1A), vascular endothelial growth factor (VEGF), interferon-gamma-inducible protein of 10 kDa (IP-10), matrix metalloproteinase-13 (MMP-13)] in patients with PE and their correlation with the severity of the disease, to find a good predictor for PE. *Patients, Materials and Methods*: This prospective study aims to monitor 48 pregnant women who address obstetric consultation and who present risk factors for PE, and a control group with characteristics similar to the study group. Patients were divided into three groups: Group I (*n*=15) including normal pregnant (NP) women with blood pressure <140/90 mmHg, without proteinuria, Group II (*n*=18) including patients with mild PE (MildPE), Group III (*n*=15) including patients with severe PE (SeverePE). The analysis of serum biomarkers was based on a quantitative sandwich enzyme-linked immunosorbent assay (ELISA), according to the manufacturer's instructions. *Results*: In our study, we found that all biomarkers investigated have higher concentrations in the serum of patients with SeverePE and MildPE than those in the control subjects (Group I, NP), the concentrations were increasing along with the disease activity. The means concentrations of HIF-1A, VEGF, IP-10, MMP-13, better correlated with indices in SeverePE group than in MildPE group. We found that VEGF was the biomarker that best correlates with indices that assess the severity of PE. The best separation of patients with SeverePE from those with MildPE can be done with the help of MMP-13 (82% accuracy), followed by VEGF (80.40% accuracy) and the least good detection being done by dosing IP-10. *Conclusions*: We can say that, due to high specificity diagnostic accuracy, determination of serum concentrations of MMP-13 and VEGF, could be useful in the diagnosis and distinguishing of patients with SeverePE and may prove useful in the monitoring of the disease course.

Keywords: preeclampsia, matrix metalloproteinase-13, vascular endothelial growth factor, hypoxia-inducible factor-1 subunit alpha.

Introduction

Preeclampsia (PE) is one of the classes of hypertensive pregnancy disorders (HPDs), according to the *American College of Obstetricians and Gynecologists* (ACOG) Guidelines in 2013 [1]. It seems that with the increase in the incidence of HPD in recent years, by its involvement in the long-term health of both mother and child, its importance will increase in the future, considering globally that PE is among the three causes that cause morbidity and mortality in mothers [2]. According to Wang's study, published in 2021, the HPD frequency increased to 18.08 million cases worldwide, so from 1990 to 2019, a total increase of 10.92% was observed [3].

The definition of PE is constantly changing. Even if new criteria were added, in an attempt not to use only proteinuria in the diagnostic formula, no more accurate detection was performed of women at risk of PE who had associated adverse events. Two of the most important medical societies, concerned about the study of PE, as are the *ACOG* and the *International Society for the Study of Hypertension in Pregnancy* have established new criteria and definitions, which have been applied in Europe, but through this approach, the number of women classified with mild PE (MildPE) has increased. In addition, angiogenic and antiangiogenic factors are accurate markers in predicting and diagnosing PE [4]. Thus, it was observed as a new onset hypertension, plus changes in angiogenic spectrum, as is either increasing the soluble fms-like tyrosine kinase-1 (sFlt-1)/placental growth factor (PlGF) ratio, as well as a drop only of PlGF, without other alternative diagnoses, is considered PE [5].

Diagnostic criteria for PE include high blood pressure after 20 weeks of gestation, as well as the presence of one or more changes during pregnancy: proteinuria, dysfunction of both the maternal organs and the uteroplacental territory [6].

The study of PE and the clarifications that have appeared over the years are extremely important because PE remains a disease of pregnancy, specific to it, representing a major cause of maternal and fetal morbidity and mortality globally [7]. Also, not to be overlooked are long-term maternal complications of PE, such as chronic hypertension, cardiovascular disease, and chronic kidney disease [8–10].

Aim

This study aims to detect and measure the serum level of some biomarkers [hypoxia-inducible factor-1 subunit alpha (HIF-1A), vascular endothelial growth factor (VEGF), interferon-gamma-inducible protein of 10 kDa (IP-10), matrix metalloproteinase-13 (MMP-13)] in patients with PE and their correlation with the severity of the disease, to find a good predictor for PE.

⌗ Patients, Materials and Methods

Subjects and clinical assessment

This prospective study aims to monitor 48 pregnant women who address obstetric consultation and who present risk factors for PE, and a control group with characteristics similar to the study group. The patients were diagnosed in the OpenMed Private Hospital and in the Department of Gynecology of the Filantropia Municipal Clinical Hospital, Craiova, Dolj County, Romania. Patients were divided into three groups: Group I (*n*=15) including normal pregnant (NP) women with blood pressure <140/90 mmHg, without proteinuria, Group II (*n*=18) including patients with MildPE, Group III (*n*=15) including patients with severe PE (SeverePE).

A database was set up based on the files prepared according to the protocol, with the consent of the patients, who will be dynamically monitored during pregnancy. All patients signed an informed consent being previously informed about the study.

The study participation procedure included several criteria. The inclusion criteria were patients older than 18 years, gestational age over 20 weeks of gestation, singleton pregnancy, pregnancies with risk factors for PE, absence of other pregnancy complications, presence of informed consent. The exclusion criteria were the presence of clinically manifest infections, severe liver and kidney disease, diabetes, pregnancies with fetal congenital anomalies, multifetal pregnancies, hematopoietic dysfunction, immunological disease, absence of informed consent.

The diagnosis of MildPE was established in pregnant women when the systolic blood pressure (SBP) values were less than or equal to 140–159 mmHg and the diastolic blood pressure (DBP) values less than or equal to 90–109 mmHg, as well as the presence or absence proteinuria less than or equal to 0.3 g/24 hours by dipstick test.

The diagnosis of SeverePE was established in pregnant women when the SBP values were greater than or equal to 160 mmHg and the DBP values were greater than or equal to 110 mmHg, as well as the presence or absence proteinuria greater than or equal to 5 g/24 hours by dipstick

test, or one of the following clinical manifestations: symptoms of severity manifested by headache, epigastric pain, the presence of intrauterine growth restriction (IUGR) by ultrasound, vision disorders, vomiting, to which can be added oliguria and thrombocytopenia.

Sample collection

Patients from both groups (study group and control group) provided the biological material, blood (approximately 5 mL of venous blood) which was collected in tubes without anticoagulant. After harvesting, the clot was separated by centrifugation (15 minutes at 3500 rpm) within four hours of harvesting, based on standard procedure. The serum tubes were sealed to avoid contamination and stored at +2°C to +8°C for three days if the samples were analyzed immediately or frozen at -20°C and even -80°C if the samples were worked over a longer period of time. The samples were coded with letters and numbers given in the order of collection.

Biomarkers investigations

The biomarkers investigations of the patients, included in the study, were determined with the support of the Department of Immunology, University of Medicine and Pharmacy of Craiova.

The method used in the laboratory was the enzyme-linked immunosorbent assay (ELISA) immuno-enzymatic method, quantitative, sandwich technique, going through all the steps specified by the manufacturer in the kits used. The ELISA method was performed with a standard optical analyzer, at 450 nm wavelength.

To determine the serum concentrations of the investigated biomarkers, commercial test sets designed specifically for each of the mediators were used: HIF-1A (Catalog # EHIF1A; Assay range: 81.92–20 000 pg/mL), VEGF (Catalog # KHG0111; Assay range: 23.8–1500 pg/mL), IP-10/CXCL10 (Catalog # KAC2361; Assay range: 7.8–500 pg/mL) and MMP-13 (Catalog # EHMMP13; Assay range: 8.23–6000 pg/mL) – Thermo Fisher Scientific (MA, USA).

Ethical issue

Being a study, in which investigated patients, we met all standards in terms of medical ethics, as recommended in the Helsinki Declaration of 1975, updated in 2008. Also, to comply with these standards, we obtained the approval for the study from the Commission of Ethics, Academic and Scientific Deontology, University of Medicine and Pharmacy of Craiova (Approval No. 134/17.09.2021).

Statistical analysis

Patients' data obtained from medical documents have been managed and then processed using Microsoft Excel (Data Analysis module). For statistical analysis of the parameters, we used GraphPad Prism 5 Trial Version (CA, USA). Furthermore, data normality was tested by using the Shapiro–Wilk test. Normal variables were presented as mean value with standard deviation (SD). The difference between the two groups was estimated by independent *t*-test and the Mann–Whitney *U*-test for normally distributed and skewed data, respectively. All tests were two-sided and *p*-values ≤0.05 were considered significant.

The existence of correlations between the investigated markers (HIF-1A, VEGF, IP-10, MMP-13), as well as the correlations between these biomarkers and indices that assess the severity of PE [SBP, DBP, platelets, creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT)], were interpreted by Pearson's linear correlation coefficient (r).

To determine diagnostic performance/accuracy of the analyzed serological markers, we used the analysis by receiver operating characteristic (ROC) curve to detect possible cut-off, which to use to differentiation of patients with MildPE from those with SeverePE. The analyzed cut-off values for each biomarker were accompanied by sensitivity (Sn), specificity (Sp), the Youden index useful in detecting the diagnostic performance of the investigated tests (Sn + Sp – 1), as well as the positive likelihood ratio [LR(+)] and the negative likelihood ratio [LR(-)]. The diagnostic accuracy was determined as the area under the ROC curve (AUC), accompanied by the 95% confidence interval (95% CI) and the statistical p.

⊡ Results

Characteristics of the study subjects

Analyzing the demographic parameter maternal age (mean ± SD), we found the absence of statistically significant differences between the groups investigated: SeverePE *vs* MildPE (29.90±6.43 *vs* 28.30±7.93 years, *p*=0.870), SeverePE *vs* NP (29.90±6.43 *vs* 28.90±6.84 years, *p*=0.738) and MildPE *vs* NP (28.30±7.93 *vs* 28.90±6.84 years, *p*=1.000).

Maternal weight was higher in the two groups of preeclamptic patient's *vs* NP women: SeverePE *vs* NP (84.90±7.97 *vs* 77.51±7.22 kg, *p*=0.018) and MildPE *vs* NP (83.45±7.97 *vs* 77.51±7.22 kg, *p*=0.020).

Regarding the body mass index (BMI), we found the existence of significant statistical differences between the group of patients diagnosed with PE *vs* NP women: SeverePE *vs* NP (31.34±4.42 *vs* 25.86±1.41 kg/m², *p*=0.004) and MildPE *vs* NP (33.50±7.17 *vs* 25.86±1.41 kg/m², *p*=0.014).

In our study, we observed that gestational age at birth, but also the birth weight of infants, were lower, the observations founded in both groups of preeclamptic patients *vs* control subjects.

Analyzing the indices that assess the severity of PE (SBP, DBP, platelets, creatinine, AST, ALT), we found higher statistically significant differences between the preeclamptic patients and NP women for the mean values of SBP, DBP: SeverePE *vs* NP (182.00±5.41 *vs* 119.00± 3.15 mmHg, *p*=0.004) and MildPE *vs* NP (149.00±7.60 *vs* 119.00±3.15 mmHg, *p*=0.014) for the SBP and SeverePE *vs* NP (110.00±3.46 *vs* 73.70±4.81 mmHg, *p*=0.004) and MildPE *vs* NP (92.90±7.53 *vs* 73.70±4.81 mmHg, *p*=0.014) for the DBP, respectively.

Except for the AST and ALT (means of serum concentrations were significantly higher in PE groups), the other indices analyzed (creatinine, urea, uric acid, platelets) showed normal serum values in both PE and NP groups.

Proteinuria, a parameter that assesses the severity of PE, was found in 10 (66.67%) patients in the SeverePE group and in six (33.33%) cases diagnosed with MildPE, respectively.

In Table 1, we detailed the most important demographic and clinical data for the three groups included in our study.

Table 1 – *Demographics and clinical characteristics of enrolled patients*

Parameter	NP (*n*=15)	MildPE (*n*=18)	SeverePE (*n*=15)
Maternal age [years] (mean ± SD)	28.90± 6.84	28.30± 7.93	29.90± 6.43
Maternal weight [kg] (mean ± SD)	77.51± 7.22	83.45± 7.97	84.90± 7.97
BMI [kg/m²] (mean ± SD)	25.86± 1.41	33.52± 7.17	31.34± 4.42
SBP [mmHg] (mean ± SD)	119.00± 3.15	149.00± 7.60	182.00± 5.41
DBP [mmHg] (mean ± SD)	73.70± 4.81	92.90± 7.53	110.00± 3.46
MAP [mmHg] (mean ± SD)	85.30± 8.13	113.00± 10.80	130.00± 7.85
PE debut [weeks]:			
▪ <34 (early onset PE)	–	4	7
▪ 34–37 (late onset PE)	–	14	8
Birth weight (percentiles)	16.00± 25.46	7.00± 14.34	75.00± 24.58
Parity:			
▪ nulliparous	5	11	6
▪ 1 childbirth	6	7	5
▪ >1 childbirth	4	–	4
Maternal comorbidities:			
▪ chronic arterial hypertension	–	3	3
▪ renal pathology	–	2	2
▪ diabetes	–	5	2
Diuresis:			
▪ normal	15	17	10
▪ oliguria	–	1	5
▪ proteinuria	–	6	10
Hemoglobin [g/dL] (mean ± SD)	11.50± 0.72	12.20± 1.39	11.70± 1.48
Platelets [No.×10³/µL] (mean ± SD)	170 267± 31 885	173 444± 56 943	157 067± 51 472
AST [U/L] (mean ± SD)	34.10± 23.30	45.90± 38.00	59.90± 23.90
ALT [U/L] (mean ± SD)	37.80± 28.70	64.50± 36.80	67.90± 44.30
Uric acid [mg/dL] (mean ± SD)	4.23± 1.18	5.74± 1.19	5.79± 1.19
Creatinine [mg/dL] (mean ± SD)	0.68± 0.12	0.82± 0.11	0.81± 0.14
Urea [mg/dL] (mean ± SD)	21.50± 1.25	27.10± 8.57	31.20± 8.59

ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; BMI: Body mass index; DBP: Diastolic blood pressure; MAP: Mean arterial blood pressure; NP: Normal pregnant; PE: Preeclampsia; SBP: Systolic blood pressure; SD: Standard deviation.

Serum biomarkers levels and clinical activity stage

We found that all biomarkers investigated have higher levels in the serum of patients with SeverePE and MildPE than those in the control subjects (Group I, NP), statistically significant differences.

Thus, when we compared the mean levels between the SeverePE group and NP group, we obtained the following levels, exemplified in Table 2: HIF-1A (4720±1030 pg/mL *vs* 3030±654 pg/mL *p*=0.0001), VEGF (1700±598 pg/mL

vs 762±296 pg/mL, *p*<0.0001), IP-10 (311±84.1 pg/mL *vs* 183±36.9 pg/mL, *p*=0.0003), MMP-13 (1180±184 pg/mL *vs* 412±57.9 pg/mL, *p*<0.0001).

When we compared the mean levels between the MildPE and NP, we obtained: HIF-1A (3790±1070 pg/mL *vs* 3030±654 pg/mL, *p*=0.048), VEGF (1120±547 pg/mL *vs* 762±296 pg/mL, *p*=0.038), IP-10 (247±80.9 pg/mL *vs* 183±36.9 pg/mL, *p*=0.029), MMP-13 (925±199 pg/mL *vs* 412±57.9 pg/mL, *p*<0.0001).

Serum levels of all biomarkers investigated showed

differences between subgroups of patients with PE disease who had various stages of clinical activity, with the aggravation of the disease activity, the levels also increased (Figures 1 and 2). The levels in the SeverePE group were statistically higher than in the MildPE group: HIF-1A (4720±1030 pg/mL *vs* 3790±1070 pg/mL, *p*=0.036), VEGF (1700±598 pg/mL *vs* 1120±547 pg/mL, *p*=0.003), IP-10 (311±84.1 pg/mL *vs* 247±80.9 pg/mL, *p*=0.041), MMP-13 (1180±184 pg/mL *vs* 925±199 pg/mL, *p*=0.0019).

Table 2 – *Serum biomarkers levels for patients with MildPE, SeverePE and NP*

Parameter (mean±SD)	Levels in Group III (*n*=15)			Levels in Group II (*n*=18)			Levels in Groups		
	SeverePE	*NP*	*p*	*MildPE*	*NP*	*p*	*SeverePE*	*MildPE*	*p*
HIF-1A [pg/mL]	4720± 1030	3030± 654	*0.0001**	3790± 1070	3030± 654	*0.048**	4720± 1030	3790± 1070	*0.036**
VEGF [pg/mL]	1700± 598	762± 296	*<0.0001**	1120± 547	762± 296	*0.038**	1700± 598	1120± 547	*0.003**
IP-10 [pg/mL]	311± 84.1	183± 36.9	*0.0003**	247± 80.9	183± 36.9	*0.029**	311± 84.1	247± 80.9	*0.041**
MMP-13 [pg/mL]	1180± 184	412± 57.9	*<0.0001**	925± 199	412± 57.9	*<0.0001**	1180± 184	925± 199	*0.0019**

HIF-1A: Hypoxia-inducible factor-1 subunit alpha; IP-10: Interferon-gamma-inducible protein of 10 kDa; MMP-13: Matrix metalloproteinase-13; NP: Normal pregnant; PE: Preeclampsia; SD: Standard deviation; VEGF: Vascular endothelial growth factor; SD: Standard deviation. *Statistically significant correlations.
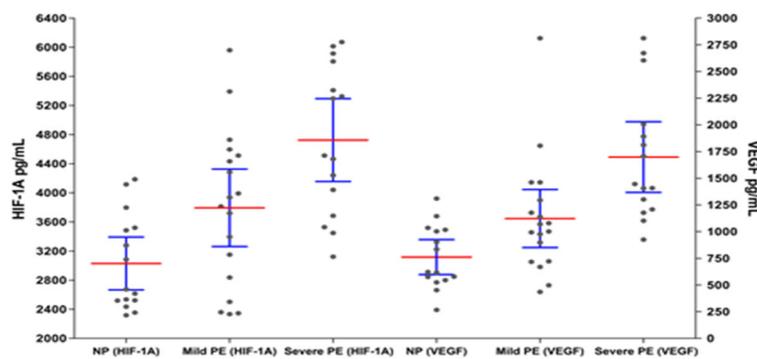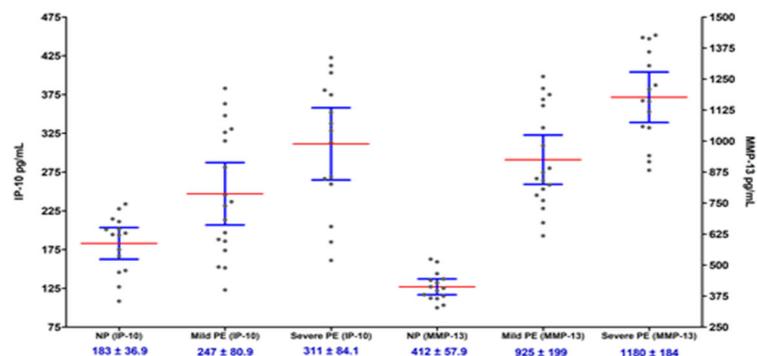

Figure 1 – *HIF-1A and VEGF levels in serum of patients with SeverePE, MildPE and NP; black circles represent level of HIF-1A and VEGF (pg/mL) in individual serum samples; red lines represent mean values accompanied by a SD, represented as blue horizontal bars. SD: Standard deviation; HIF-1A: Hypoxia-inducible factor-1 subunit alpha; NP: Normal pregnant; PE: Preeclampsia.*

Figure 2 – *IP-10 and MMP-13 levels in serum of patients with SeverePE, MildPE and NP; black circles represent a level of IP-10 and MMP-13 (pg/mL) in individual serum samples; red lines represent mean values accompanied by a SD, represented as blue horizontal bars. SD: Standard deviation; IP-10: Interferon-gamma-inducible protein of 10 kDa; MMP-13: Matrix metalloproteinase-13; NP: Normal pregnant; PE: Preeclampsia.*

Correlations between HIF-1A, VEGF, IP-10, MMP-13 and the severity of PE

Another objective of our study was to identify the correlations between the mean levels of the investigated biomarkers (HIF-1A, VEGF, IP-10, MMP-13), as well as the correlations between these biomarkers and indices that assess the severity of PE (SBP, DBP, platelets, creatinine, AST, ALT).

We observed that HIF-1A, VEGF, IP-10, MMP-13 better correlated with indices in SeverePE group than in MildPE group (Tables 3 and 4).

In SeverePE group, we noticed that VEGF, HIF-1A and MMP-13 correlated negatively but statistically significantly with SBP: *r*=-0.134, *p*=0.027, weak negative correlation for VEGF; *r*=-0.376, *p*=0.045 fairly well correlation for HIF-1A; *r*=-0.234, *p*=0.041 fairly well correlation for MMP-13, respectively (Table 3).

Analyzing the data obtained, we found that VEGF was the biomarker that best correlates with indices that assess the severity of PE: platelets (strong positive correlation, *r*=0.709, *p*=0.003) and creatinine (fairly well correlation, *r*=0.424, *p*=0.015).

An important observation in the SeverePE group is

the significant negative correlation between VEGF and IP-10 (r=-0.408, p=0.031). We also found that HIF-1A was the only biomarker correlated with AST (fairly well significant negative correlation, r=-0.485, p=0.047).

Table 3 – *Correlations between HIF-1A, VEGF, IP-10, MMP-13 and SBP, DBP, in the SeverePE group*

	DBP	Platelets	Creatinine	AST	ALT	HIF-1A	VEGF	IP-10	MMP-13
SBP	r=0.030 p=0.914	r=-0.204 p=0.465	r=0.177 p=0.528	r=0.073 p=0.796	r=0.381 p=0.161	***r=-0.376 p=0.045***	***r=-0.134 p=0.027***	r=0.280 p=0.312	***r=-0.234 p=0.041***
DBP		r=-0.129 p=0.647	r=-0.239 p=0.392	r=-0.209 p=0.455	r=0.137 p=0.625	r=0.049 p=0.863	r=-0.071 p=0.802	r=0.282 p=0.308	r=-0.088 p=0.754
Platelets			r=-0.088 p=0.754	r=-0.151 p=0.590	r=-0.365 p=0.181	r=0.023 p=0.936	***r=0.710 p=0.003***	r=-0.441 p=0.099	r=-0.021 p=0.940
Creatinine				r=0.352 p=0.198	r=0.390 p=0.151	r=-0.421 p=0.118	***r=0.424 p=0.015***	r=-0.331 p=0.227	r=0.258 p=0.353
AST					r=-0.014 p=0.962	***r=-0.485 p=0.047***	r=-0.053 p=0.851	r=0.066 p=0.817	r=-0.259 p=0.352
ALT						r=-0.109 p=0.700	r=-0.024 p=0.932	r=0.069 p=0.805	r=-0.130 p=0.644
HIF-1A							r=-0.159 p=0.572	r=0.393 p=0.147	r=-0.382 p=0.159
VEGF								***r=-0.408 p=0.031***	r=-0.200 p=0.475
IP-10									r=-0.390 p=0.151

ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; DBP: Diastolic blood pressure; HIF-1A: Hypoxia-inducible factor-1 subunit alpha; IP-10: Interferon-gamma-inducible protein of 10 kDa; MMP-13: Matrix metalloproteinase-13; PE: Preeclampsia; r: Pearson's correlation coefficient; SBP: Systolic blood pressure; VEGF: Vascular endothelial growth factor. *Statistically significant correlations.

Analyzing the data obtained in the MildPE group, we found that the biomarkers investigated in our study were less correlated with indices that assess the severity of PE. VEGF was the only biomarker that correlated best, meeting positive correlation with IP-10 (strong correlation, r=0.519, p=0.027) and SBP (weak correlation r=0.268, p=0.028) (Table 4).

Table 4 – *Correlations between HIF-1A, VEGF, IP-10, MMP-13 and SBP, DBP, in the MildPE group*

	DBP	Platelets	Creatinine	AST	ALT	HIF-1A	VEGF	IP-10	MMP-13
SBP	r=-0.056 p=0.824	r=-0.271 p=0.277	r=0.033 p=0.897	r=0.043 p=0.865	r=-0.115 p=0.650	r=-0.032 p=0.901	***r=0.268 p=0.028***	r=0.274 p=0.270	r=0.306 p=0.217
DBP		r=0.154 p=0.543	r=0.380 p=0.120	r=-0.437 p=0.070	r=0.448 p=0.062	r=-0.062 p=0.808	r=0.202 p=0.422	r=0.070 p=0.783	r=-0.161 p=0.523
Platelets			r=-0.043 p=0.865	r=-0.613 p=0.007	r=-0.244 p=0.330	r=-0.090 p=0.723	r=-0.047 p=0.852	r=0.193 p=0.442	r=-0.381 p=0.119
Creatinine				r=0.161 p=0.523	r=0.167 p=0.509	r=-0.066 p=0.794	r=0.231 p=0.357	r=0.282 p=0.257	r=-0.186 p=0.460
AST					r=0.829 p=0.001	r=0.362 p=0.140	r=-0.280 p=0.260	r=-0.181 p=0.472	r=0.257 p=0.304
ALT						r=0.288 p=0.246	r=-0.301 p=0.225	r=-0.108 p=0.670	r=-0.064 p=0.802
HIF-1A							r=-0.282 p=0.257	r=0.037 p=0.884	r=-0.067 p=0.793
VEGF								***r=0.519 p=0.027***	r=0.334 p=0.175
IP-10									r=-0.162 p=0.520

ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; DBP: Diastolic blood pressure; HIF-1A: Hypoxia-inducible factor-1 subunit alpha; IP-10: Interferon-gamma-inducible protein of 10 kDa; MMP-13: Matrix metalloproteinase-13; PE: Preeclampsia; r: Pearson's correlation coefficient; SBP: Systolic blood pressure; VEGF: Vascular endothelial growth factor. *Statistically significant correlations.

Diagnostic performance of the investigated parameters

As in the case of other biomarkers investigated in our studies, in this study we aimed to determine the diagnostic accuracy for the analyzed serological markers, using the analysis of the ROC curve. We compared the ROC curves for the four parameters studied in the groups of patients with PE and obtained a diagnostic performance in identifying PE, increased for MMP-13 and VEGF. The diagnostic performances are presented in Table 5.

As can be seen in Table 5, the best separation of patients with SeverePE from those with MildPE can be done with the help of MMP-13 (82% accuracy), followed by VEGF (80.40% accuracy) and the least good detection being done by dosing IP-10 (Figure 3).

The serum MMP-13 level indicated presence of SeverePE with 82% accuracy, using the cut-off value of 1056 pg/mL for better separation between patients with SeverePE and MildPE (95% CI: 0.679–0.961, p=0.002). Also, we got for MMP-13: the Youden index for assessing the performance of a diagnostic test was 0.522, with Sn and Sp equal to 72.22% and 80.00%, respectively, and LR(+)=8.33 and LR(-)=1.07.

The serum VEGF level indicated presence of SeverePE with 80.40% performance, using the cut-off value of 1193 pg/mL for better separation between patients with SeverePE and MildPE (95% CI: 0.650–0.957, p=0.003). The other statistical information that characterizes the performance of use, for VEGF were: the Youden index was 0.589, with Sn and Sp equal to 72.22% and 86.67%, respectively, and LR(+)=5.00 and LR(-)=1.01.

The data obtained at the ROC curves indicate that the other two biomarkers also have a fairly good diagnostic accuracy, but lower than those of MMP-13 and VEGF: HIF-1A (accuracy of 71.70%, with an optimal cut-off value of 4449 pg/mL, 95% CI: 0.541–0.892, *p*=0.034) and IP-10 (71.10% accuracy, with an optimal cut-off value of 297.20 pg/mL, 95% CI: 0.532–0.890, *p*=0.039).

Table 5 – *Diagnostic accuracy of the analyzed serological markers, using ROC curve analysis*

Parameter	AUC performance	Cut-off value	*p*-value	Sensitivity [%]	Specificity [%]	Youden index
MMP-13	0.820	1056.00	*0.002*	72.22	80.00	0.522
VEGF	0.804	1193.00	*0.003*	72.22	86.67	0.589
HIF-1A	0.717	4449.00	*0.034*	72.22	60.00	0.322
IP-10	0.711	297.20	*0.039*	66.67	60.00	0.267

AUC: Area under curve; HIF-1A: Hypoxia-inducible factor-1 subunit alpha; IP-10: Interferon-gamma-inducible protein of 10 kDa; MMP-13: Matrix metalloproteinase-13; ROC: Receiver operating characteristic; VEGF: Vascular endothelial growth factor.
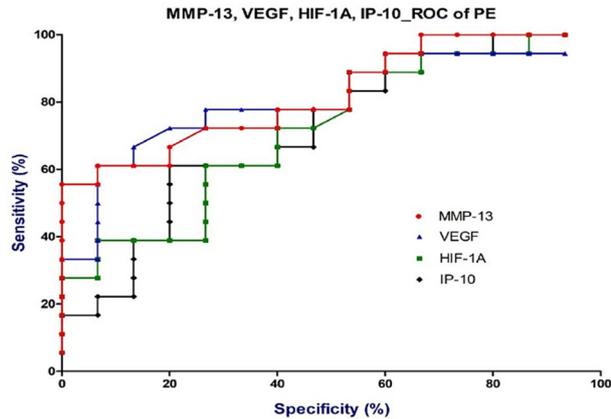

Figure 3 – *ROC curves for MMP-13, VEGF, HIF-1A, IP-10, in groups of patients with PE. HIF-1A: Hypoxia-inducible factor-1 subunit alpha; IP-10: Interferon-gamma-inducible protein of 10 kDa; MMP-13: Matrix metalloproteinase-13; PE: Preeclampsia; ROC: Receiver operating characteristic; VEGF: Vascular endothelial growth factor.*

Discussions

Analyzing the clinical characteristics of the investigated patients, we noticed a statistically significant difference between PE, with the two classes: MildPE and SeverePE, and the control group, in terms of BMI and preterm delivery. In this case, the results obtained by us are consistent with the study of Spradley *et al.* [11], which shows that there are common mechanisms between obesity and the risk of PE, and the study by Davies *et al.* [12], which shows that PE significantly influences preterm delivery.

In our study, we found that all investigated biomarkers (HIF-1A, VEGF, IP-10, MMP-13) have higher serum levels of patients with SeverePE and MildPE than those in control subjects, statistically significant differences, which was to be expected if we refer to the pathogenesis of PE. The levels in the SeverePE group were statistically higher than in MildPE group.

The main mediator of hypoxia is HIF-1, which participates in the coding of proteins associated with angiogenesis [13].

Correlating the mean levels of the HIF-1A in the SeverePE group and indices that can give us data on the severe evolution of PE (SBP, DBP, platelets, creatinine, AST, ALT), we found that HIF-1A was the only biomarker correlated with AST (fairly well significant negative correlation, *r*=-0.485, *p*=0.047) and negatively but statistically significantly with SBP (fairly well correlation, *r*=-0.376, *p*=0.045). In the MildPE group, we found that HIF-1A biomarker was less correlated with indices that assess the severity of PE. The levels in the SeverePE group were statistically higher than in the MildPE (4720±1030 pg/mL *vs* 3790±1070 pg/mL, *p*=0.036). The data obtained at the ROC curves indicate that the HIF-1A has a fairly good diagnostic accuracy, but lower than those of MMP-13 and VEGF (accuracy of 71.70%, with an optimal cut-off value of 4449.00 pg/mL, 95% CI: 0.541–0.892, *p*=0.034).

According to the studies of Rath *et al.* [14] and Depoix *et al.* [15], the increase in serum HIF-1A level may be responsible for the installation of endothelial dysfunction and the change in the status of oxidative stress, by decreasing it. All these phenomena are closely related to placental hypoxia considered as a preliminary factor for hypoxia in PE, causing an imbalance in the action of angiogenic factors, affected by the transcription of the *VEGF* gene that acts by counteracting angiogenesis. Some studies provide evidence that inflammatory cytokines may induce HIF-1A in nonhypoxic conditions [16]. But, despite all these studies, how HIF-1A remains elevated in PE as well as its role in producing PE is still unclear.

Many angiogenic factors play an important role in the occurrence of PE, but so far, the pathogenesis of PE has not been fully clarified. According to some researchers, poor remodeling of the spiral arteries and reduced maternal blood supply leads to hypoxia and ischemia in the placenta. Under these conditions, by producing anti-angiogenic factors by the trophoblast, hypoxia and endothelial dysfunction are accentuated [17–21]. The VEGF is an extremely important factor for placental vascular initiation and development, being involved in both physiological and pathological angiogenesis [22]. Under normal pregnancy, serum VEGF levels can be very low, below the lower limit of detection in most commercially available kits.

The serum VEGF levels were higher in the SeverePE group (1700±598 pg/mL *vs* 1120±547 pg/mL, *p*=0.003) than in the MildPE group. This is congruent with studies by Awad & El-Hamedi [23] who found that the serum VEGF level was significantly higher in SeverePE than in MildPE. In contrast, in Atakul's study [24] no significant difference was found between mild PE and SeverePE. Also, we found that the VEGF was the biomarker that best correlates with indices that assess the severity of PE: the platelets (strong positive correlation, *r*=0.709, *p*=0.003), the creatinine (fairly well correlation, *r*=0.424, *p*=0.015), and with the SBP (weak positive correlation, *r*=0.268, *p*=0.028). The ROC curve showed that the serum VEGF level

indicated presence of SeverePE with 80.40% performance, using the cut-off value of 1193.00 pg/mL for better separation between patients with SeverePE and MildPE (95% CI: 0.650–0.957, p=0.003).

IP-10, known as a C-X-C motif chemokine ligand 10 (CXCL10, CXC family chemokine), has proinflammatory properties and is known to modulate angiogenesis [25].

In our study, the serum IP-10 levels in the SeverePE group were statistically higher than in the MildPE group, IP-10 (311±84.1 pg/mL vs 247±80.9 pg/mL, p=0.041), meeting a strong positive correlation with the VEGF. These results are similar to those reported by Gotsch et $al.$ [26] who found that in the women with normal pregnancies the mean values of serum level of the IP-10 are higher than in non-pregnant women, and in pregnancies with PE, the mean values of serum level of IP-10 were higher than in normal pregnancy. In another study, in patients with pregnancy associated with PE, plasma levels of CXCL10 were found to be elevated compared to the control group, while in pregnancies associated with early-onset PE plasma levels of CXCL11 were elevated compared to the control group [27]. ROC curve analysis of this parameter in our study showed that the IP-10 has a fairly good diagnostic accuracy, but lower than those of the MMP-13 and VEGF (71.10% accuracy, with an optimal cut-off value of 297.20 pg/mL, 95% CI: 0.532–0.890, p=0.039).

In a recent study by Stefańska et $al.$, in 2021 [28], using a set of immune markers that would be potential to diagnose PE, it was shown that the IP-10 levels were different in cases with PE vs cases with gestational hypertension, higher values being in cases with PE. But unfortunately, of the 53 immunomarkers used, only four markers showed their effectiveness and among them, the IP-10 was not found.

One of the extremely important functions of matrix metalloproteinases (MMPs), zinc-dependent enzymes, is the invasion of proliferating cells in neighboring tissues, by degrading the extracellular matrix and activating endothelial vascular cells, thus intervening in the regulation of placental angiogenesis. It is currently known the role that the MMP-2 and MMP-9 have in endometrial remodeling in pregnancy by facilitating cell migration and angiogenesis [29]. Types I and III collagen play a central role in maintaining structural stability and regulating cell differentiation. It has been shown that collagen turnover in the uterus is altered in PE, by the involvement of collagens, MMP-1 and MMP-13, resulting in abnormal uterine remodeling [30].

In our study, we found a significant difference regarding the mean levels between the MildPE and NP (925±199 pg/mL vs 412±57.9 pg/mL, p<0.0001), as between SeverePE and NP (1180±184 pg/mL vs 412±57.9 pg/mL, p<0.0001). Also, the levels in the SeverePE group were statistically higher than in the MildPE group. We observed that the MMP-13 better correlated with indices in SeverePE group than in MildPE group. In SeverePE group, we noted fairly well correlation for the MMP-13 (r=-0.234, p=0.041).

According to the studies of Whitley & Cartwright [31] and Reister et $al.$ [32], the MMPs expression in preeclamptic patients appears to be significantly reduced. Extravillous trophoblast cells (EVT), in which the MMPs expression occurs, strongly express the receptor for leukemia inhibitory factor (LIF), which is known as an inhibitor of the MMPs expression. But Laskowska et $al.$, in their study [33], found

that the evolution of the MMP in preeclamptic women is different: the activity of some MMPs is increased, with high values of the MMP-2 and MMP-13 in SeverePE. The MMP-3 had higher levels only in early-onset SeverePE, while the activity of other MMPs was lower, as in the case of the MMP-9. Regarding the MMP-13, our study presents similar conclusions. Montagnana et $al.$ [34] found the same data in their study.

When evaluating diagnostic accuracy (diagnostic performances and Youden index for assessing the performance of a diagnostic test) of the HIF-1A, VEGF, IP-10, MMP-13, obtained that MMP-13 and VEGF have higher Sn and Sp as markers of the PE [the MMP-13 had LR(+)=8.33, LR(-)= 1.07 with 72.22% Sn, Sp equal to 80.00% and the Youden index was 0.522; the VEGF had LR(+)=5.00, LR(-)=1.01 with 72.22% Sn, Sp equal to 86.67% and an Youden index of 0.589].

Nikolov et $al.$ in their brief review have shown that the MMP-1, MMP-13, and their tissue inhibitors of MMP-1 and MMP-2 (TIMP-1 and TIMP-2, respectively), may be promising serological markers for PE. But the findings are still controversial, it is not known for sure whether the changes are the cause or the consequence of PE [35].

We also found that the MMP-13 and VEGF, although have the same Sn as the HIF-1A (72.22% Sn, Sp equal to 60.00% and an Youden index of 0.322), have a higher Sp. In the case of the IP-10, we obtained both lower Sn and Sp, although it had a diagnostic accuracy of 71.10%.

After performing this analysis of the study, we can hypothesize that due to the high diagnostic Sp of the MMP-13 and VEGF, these parameters can separate patients with SeverePE from those with MildPE. Also, these biomarkers can be used together with other indices (SBP, DBP, platelets, creatinine, AST, ALT) that assess the severity of PE, in diagnosing SeverePE forms.

⮞ Conclusions

As a result of complex statistical analysis and according to the obtained results, we can say that HIF-1A, VEGF, IP-10, MMP-13, better correlated with indices in the SeverePE group than in the MildPE group. Also, by comparing the ROC curve for all the four parameters studied in PE, we noticed that MMP-13 and VEGF have a higher diagnostic utility as markers of PE, so they may have a high potential to be a predictive marker for PE in combination with indices that assess the severity of PE (SBP, DBP, platelets, creatinine, AST, ALT), and being able to even monitor the evolution of the disease over time. And the other two markers, HIF-1A and IP-10 have fairly good diagnostic accuracy but are lower than those of MMP-13 and VEGF.

Authors' contribution
Mirela Radu and Anda Lorena Dijmărescu equally contributed to the manuscript.

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