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# Analysis of classical risk factors and additional candidate-predictors of preeclampsia in Bulgarian study group of pregnant women

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## I. Introduction

The American College of Obstetricians and Gynecologists (ACOG) and the National Institute for Health and Clinical Excellence (NICE) recommend screening for preeclampsia (PE) based on maternal risk factors. According to the NICE recommendation, any of the following high-risk factors (hypertension in previous pregnancy, chronic hypertension, chronic kidney disease, diabetes mellitus or autoimmune disease), or more than one moderate risk factor (nulliparity, age ≥40 years, BMI ≥35 kg/m$^2$, family history of preeclampsia or interpregnancy intervals of more than 10 years) is considered a high risk of preeclampsia. (www.nice.org.uk/guidance/ng133; https://www.guidelinecentral.com/summaries/organizations/national-collaborating-centre-for-womens-and-childrens-health) (Chaemsaithong P. et al. 2020). The predictive model recommended by ACOG and NICE essentially treats each risk factor as a separate screening test. Scientific data shows that PE screening based on the NICE and ACOG approaches has shortcomings, as the NICE recommendation achieves detection rate of only 41% and 34%, with a false positive percentage of 10% for premature and term preeclampsia, respectively ( Chaemsaithong P., et al (2020).

Of the various PE prediction models in the first trimester, special attention deserves the predictive model developed by the Fetal Medicine Foundation (FMF), namely the triple screen. It combines maternal factors and measurements of mean arterial pressure (MAP), uterine artery plasticity index and serum placental growth factor (PlGF). The FMF triple test has detection rate of 90% and 75% for predicting early and late PE, respectively, with a 10% false-positive rate. This model of screening is better than the traditional method, structured only by the risk factors of the mother. (Rolnik, D.L. et al. (2019)) So far, the best predictive ability for high risk of PE shows the mathematical model (Bayes' theorem - a formula for calculating the probability of occurrence of an event, after some information is already known about it), which combines prior information from maternal factors, obstetric and medical history, uterine artery PI, MAP and serum PAPP-A and PlGF of the mother, in 11 - 13 weeks of gestation (O'Gorman, N. et al. (2015); (Akolekar, R. et al. (2013). Wright D. et al. (2015).

The scientific community is focusing its efforts on the identification of biomarkers that can further increase the prognostic capacity of the diagnostic approaches for PE risk assessment in the first trimester of pregnancy. The data shows that combinations of biomarkers have better predictive efficiency than the single biomarkers. (1. Poon L.C. and Nicolaides K.H. (2014); (Al-Rubaie Z. et al. (2016)) (Chaemsaithong P. et al. (2018).

**Objective**: To assess and analyze the classical risk factors for preeclampsia (PE) and to propose inclusion of additional predictors (and ratios) in the risk constellation for assessment of high risk of the complication.

**Materials and methods**: In a two-stage retrospective study conducted from 30.01.2018 to 31.08.2020 at Outpatient Practice Fetal Medicine Ltd., Plovdiv, Bulgaria, 1511 pregnant women were examined through regular examinations. The primary data is obtained from their archived Medical Record. During the first stage (in 11 gestation week + 0 days – 13 gestation week + 6 days) was collected information about the maternal factors, medical and obstetric history and status. Second stage – through a telephone interview (conducted up to six months after the birth of the child): data on the mode of birth, weight of the newborn, PE occurrence, in

which gestation week /GW/ is PE onset, gestational hypertension (GH) and diabetes, intrauterine growth retardation (IUGR), whether they took Aspirin and in what dose, complications, etc. The patients are divided into groups: high risk (PE risk higher than 1: 150), and low PE risk, with or without onset of IUGR, GH, diabetes, etc.

**Inclusion criteria**: The study must be conducted between GW 11 + 0 days, up to GW 13 + 6 days of pregnancy or fetus size from 45 mm to 84 mm; viable fetus; single pregnancy; the woman must be 18 and over years of age; without serious mental and physical illnesses. Exclusion criteria: minors; multiple pregnancy; structural abnormalities of the fetus; abortion; ulcer and gastritis; coagulation disorders; Aspirin intolerance; termination of pregnancy on medical grounds; stillbirth.

The monitoring characteristics are mainly divided into 2 groups.

Factorial characteristics: age, education, concomitant diseases, smoking, parity, interval between two pregnancies, previous PE, BMI, IVF, etc. The arithmetic mean of the pulsatility indices of the uterine arteries (mean UtPI), mean arterial pressure (MAP), biochemical markers of the mother- angiogenic placental factors involved in trophoblast invasion and placental growth and development: Pregnancy associated plasma protein-A (PAPP-A); Placental growth factor (PLGF) etc.

Resultative characteristics: PE occurrence and in which week of gestation is the onset, the ability to predict early, midterm and late PE and the premature birth. Assessment of the predictive role of additional risk factors, forming new predictive ratios based on the classical predictors. Analysis of which predictors remain independent and what is their individual contribution both to the occurrence of PE (at each stage) and to IUGR and others.

## II. Research Methods:

- Documentary method: the medical files were obtained from the outpatient register, after obstetric and gynecological examinations, anamnestic data, biochemical and biophysical indicators, telephone interview, etc.
- Clinical method – anthropometric methods: height, weight, BMI, etc.
- Laboratory methods: the tests were performed on a specialized automated biochemical analyzer by immunofluorescence Perkin ELmer DELFIA Xpress. The following were studied: - Pregnancy associated plasma protein-A (PAPP-A); - Placental growth factor (PLGF).
- Ultrasound methods:
- Transabdominal ultrasound of UtPI - with a high-end device from the GE group (Voluson E6) by abdominal ultrasound with 4-6 MHz transducer. The uterine arteries are revealed by: sagittal image of the cervix; Doppler color flow mapping; Moving the transducer from side to side parallel to the cervix; The arteries are at the level of the inner axis of the cervical canal; Insonation window 2 mm wide to cover the entire container; Insonation angle: less than 30º; Maximum systolic velocity: more than 60 cm/ sec; Mean pulsatility index: mean PI (left + right / 2) - cut off 1.5.
- Transvaginal ultrasound of UtPI - transvaginal access with 5-7MHz probe in cases with technical impossibility to perform the transabdominal method (overweight, uterine fibroids, etc.). The same orientation and evaluation criteria apply as in the transabdominal examination, but with a higher threshold of the mean pulsatility index of the uterine arteries (mPI-UA).
- Mean arterial pressure (MAP) - according to the protocol of the Australian CVD Association - with automatic devices 3BTO-A2, Microlife.
- The risk calculation software used is FetView with calculator provided by Fetal Medicine Foundation.
- The diagnostic criteria for PE diagnosis are based on the ISSHP criteria for PE.

Statistical methods: The data is processed with the SPSS statistical package. 21. Ver. and are significant at level of significance - $\alpha = 0.05$. The following statistical analyzes are used: descriptive analysis; $X^2$ - analysis (Chi-squaered test); Student's T-test; analysis of variance (One Way Anova), using last significant difference (LSD) or Dunnett's T3 for multiple intergroup comparisons; correlation analysis; graphical analysis.

## III. Results.

The mean age of the studied 1511 pregnant women is 29.91 ± 5.32 years (18 - 46 years).38 (2.9%) of them have developed PE, and GH is observed in 5.9%. In 85 (6.5%) participants is reported intrauterine growth retardation (IUGR). The cases in which there is bothIUGRand premature birth are a total of 30 (2.3%) of the total sample.The classification of the participants according to their risk of preeclampsia showed that 591 (39.1%) of the examined patients were reported as high-risk. All patients at risk higher than 1:150 were classified as high-risk, and it was recommended that they take Aspirin 150 mg every night from 12 to 36 week of gestation. 80.6% of the high-risk group took the medication regularly.

In order to refine and optimize the complexity of the risk constellation for PE, it was proposed to include additional predictors, as the strength and direction and their impact was compared with that of the

classical risk factors (Tables 1, 2 and 3). In the analysis made in Tables 1, 2 and 3, the classical biophysical and biochemical risk indicators were grouped and compared, as well as additional predictors for PE. The structuring is in two main groups - First main group (risk) - with subgroups of patients at high and low risk of PE, and Second main group (with or without PE) – with subgroups of women that did or did not develop PE.

Table 1 shows the comparisons of the average levels between the classical risk factors and the additional PE candidate-predictors. It was found that the mean serum level of PLGF in cases that were classified as PE high-risk is 0.60 ± 0.26 MoM, which is lower than the established mean level of the women that actually developed PE - 0.66 ± 0.26 MoM (i.e.we can say that this factor is underestimated as a predictor when assessing the PE risk).

The mean serum PAPP-A level in the cases of high risk of PE is 0.92 ± 1.52 MoM, which is significantly higher than in the women who subsequently develop PE - 0.83 ± 0.56 MoM (this factor is overestimated as a predictor when assessing the risk of PE). The data thus obtained indicates the need for more in-depth analysis and further studies on the specific contribution of PLGF and PAPP-A to determining the risk of PE.

The mean arterial pressure (MAP) in the women at high risk for PE is 93.73 ± 8.10 mmHg, and in those who had the complication it was 96.10 ± 7.25 mmHg (this risk factor was underestimated as a predictor of PE).

The PI of the right uterine artery in high-risk women averaged 2.06 ± 0.71, and in those who developed PE it was 1.93 ± 0.75 (this risk factor is overestimated as a predictor of PE). The PI of the left uterine artery in women at risk was 2.09 ± 0.66, and in those who developed PE it was 2.05 ± 76. The arithmetic mean of the pulsatility index of the two uterine arteries (mPI-UA) was 2.07 ± 0.53 in high-risk women and 1.99 ± 0.62 in pregnant women who developed PE. (i.e. this indicator is overestimated in risk assessment).

Table 2 presents the average differences between the classic and the proposed additional PE candidate-predictors.

This comparison uses the value of the statistical parameter - Student's T-test, which is an indirect indicator (absolute and comparable value) to determine the magnitude of the established intergroup differences.

In the group structured on the basis of PE risk (First main group), the highest statistically significant intergroup difference was obtained with PLGF, followed by: MAP; mPI-UA, PI of left and right uterine artery. The lowest value of the statistical parameter Student's t-criterion is in PAPP-A. When organizing the groups of women with PE and those without the disease, there is a rearrangement of the differences between the analyzed classical indicators. The highest value of the statistical parameter Student's t-criterion is the difference found in MAP - t = 5.026, followed by differences in: PLGF - t = 4.458; then PAPP-A, where - t = 2.275; PI of the left uterine artery - t = 1,685 and mPI-UA - t = 1,238.

An intergroup comparison was made between the additional candidate predictors for PE, and they were also compared as described above for the classic risk factors. Thecomparison of the cervix length shows that there is no significant difference between women who are in the high-risk group and those who are at low risk - p> 0.05. This is not the case, however, for the women who develop PE and those who do not, as here the difference is 0.812 mm - p> 0.05. In women who have PE, the average length of the cervix is shorter - 32.75 ± 4.47 mm, and in those who do not have PE, it is 33.56 ± 5.09 mm (Table 2).

The number of cigarettes smoked per day by the women in the two PE risk groups was established (Table 1). Women in the higher risk class smoke an average of three cigarettes per day, and women in the lower risk class smoke nearly 5 cigarettes per day (4.56 ± 4.39 cigarettes per day). None of the pregnant women who developed PE smoked, and those who did not develop PE reported smoking an average of 4.4 ± 6.17 cigarettes per day. The participants who are in the high-risk group for PE have an average number of births - 0.31 ± 0.51, and those defined as low-risk have 0.53 ± 0.60 births. When comparing the number of births when PE has already developed, the ratio is reversed and it is established that women who have PE have given birth 0.50 ± 0.60 times, and those who have not developed complications have given birth to fewer children - 0.44 ± 0.58 births.

In the group with high risk of PE, the average weight of the newborns from the previous birth is 3112.66 ± 565.407 g, and in the women with low risk of PE the average weight of previous newborn is 3241.153 ± 503.597 g. In women with developed PE, the average weight of children born from previous pregnancies is 2973.33 ± 769.85 g, compared to the weight of those born to women who have not developed PE - 3240.8 ± 497.561 g. The data shows that the indicator - low weight of the previous newborn (it is significantly lower in women with PE), should be included in the risk constellation, as well as shortening of the cervix.

Weight gain during pregnancy is an indicator that has similar mean values when determining whether patients are at high or low risk of PE. However, in case of developed PE, it was found that the weight gain in these women was 16.90 ± 8.72 kg, and in the cases without PE, it was - 15.45 ± 5.99 kg. This shows that the risk constellation must includerapid weight gain during pregnancy. The average difference in the comparison of the indicator "weight gain during pregnancy" in the First main group is 0.686 kg, and in the Second main group is

1.449 kg (here again we see more than twice higher difference in the cases of developed PE, compared to the risk groups).

The BMI ofthe high-risk pregnant women is 25.91 ± 5.16, and of the low-risk participants it is 23.42 ± 4.47. When grouping patients with and without PE, the BMI values were 27.589 ± 5.386 and 23.911 ± 4.800, respectively, MD = 3.678, p = 0.000. This is a key indicator in terms of importance and significance among the additional predictors according to the value of the Student's statistical t-criteria, and this applies to the data for both the first and the second main group.

From a practical medical point of view, it is necessary to pay more attention to the ranking of additional (candidate) predictors when considering whether or not PE has developed. In the Second main group, the first place is occupied by the difference found in the comparison of BMI. (Student's t = 4,571, p = 0,000). Second and third place in this group are taken by the differences between the weight of the newborns in this and previous births, respectively, the differences found are 454.200 g and 240.456 g. These differences are about twice as large as those found in the First main group - this and previous birth – 198.624 g and 128.590 g.The fourth place in the Second group, with the predictor-candidates thus ranked, is occupied by the interval between births, t = 0.964, p = 3.355, MD = 0.902 years. In fifth place in the same group is weight gain during pregnancy, t = 0.911, p = 0.365, MD = 1.449 kg. When this indicator is taken into account in determining the PE risk, the reported intergroup difference is 0.686 kg. This data shows that the shorter interval between births is more risky for PE, as in the first group the difference found for this indicator is 1,226 years, p = 0.001.In the ranking of the indicators in the Second main group in sixth and seventh place (according to the Student's criterion) is the difference in the cervical length *t* = 0.702 and the number of births *t* = 0.584, asaccording to the latter indicator it was confirmed that women who give birth for the first time are not at higher risk for PE. When comparing the differences found in the First and Second main groups, the average differences found in the length of the cervix are: 0.367 mm and 0.812 mm, respectively. The reported difference is twice as large in the second main group. We recommend that the data on the shortening of the cervix be taken into account and that this indicator be included in the risk constellation of PE.

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**Table 1 Comparison between classic and new candidates for PE predictability - First main group (PE risk assessment) and Second main group (with or without developed PE)**

| Final PE risk | | | | | With/ Without PE | | | |
|---|---|---|---|---|---|---|---|---|
| | | Count | Mean value | Standard deviation | | Count | Mean value | Standard deviation |
| Placental growth factor (PLGF) MoM | high PE risk | 560 | 0,6012 | 0,26067 | with PE | 35 | 0,6626 | 0,27764 |
| | low PE risk | 875 | 1,0487 | 0,49995 | without PE | 1122 | 0,8813 | 0,47877 |
| Pregnancy-associated plasma protein PAPP-A MoM | high PE risk | 582 | 0,9249 | 0,51568 | with PE | 38 | 0,8342 | 0,56066 |
| | low PE risk | 908 | 1,1071 | 0,63208 | without PE | 1167 | 1,0561 | 0,59257 |
| Mean arterial pressure (MAP) | high PE risk | 590 | 93,726 | 8,103701 | with PE | 38 | 96,0987 | 7,246760 |
| | low PE risk | 919 | 87,807 | 7,755571 | without PE | 1179 | 89,4309 | 8,072791 |
| Pulsatility index of right uterine artery (R Ut PI) | high PE risk | 591 | 2,059 | 0,70618 | with PE | 38 | 1,9284 | 0,75391 |
| | low PE risk | 920 | 1,7132 | 0,58798 | without PE | 1181 | 1,8482 | 0,63812 |
| Pulsatility index of left uterine artery (L Ut PI) | high PE risk | 591 | 2,0891 | 0,66116 | with PE | 38 | 2,0495 | 0,76434 |
| | low PE risk | 920 | 1,7571 | 0,58377 | without PE | 1181 | 1,8774 | 0,61448 |
| Arithmetic mean of the pulsation index of both uterine arteries (Mean Ut PI) | high PE risk | 591 | 2,0742 | 0,527498 | with PE | 38 | 1,9889 | 0,621968 |
| | low PE risk | 920 | 1,7351 | 0,462505 | without PE | 1181 | 1,8628 | 0,494931 |
| Additional indicators/ new probable candidate predictors for PE | | | | | | | | |
| Cervix length (мм) | high PE risk | 226 | 33,791 | 5,0773 | with PE | 20 | 32,750 | 4,4707 |
| | low PE risk | 325 | 33,424 | 5,1565 | without PE | 458 | 33,562 | 5,0903 |
| Smoked cigarettes per day | high PE risk | 3 | 3,00 | 1,000 | with PE | 0[a] | . | . |
| | low PE risk | 9 | 4,56 | 4,391 | without PE | 10 | 4,40 | 4,169 |
| Number of births | high PE risk | 590 | 0,31 | 0,551 | with PE | 38 | 0,50 | 0,604 |
| | low PE risk | 919 | 0,53 | 0,597 | without PE | 1180 | 0,44 | 0,581 |
| Weight of newborn in previous birth | high PE risk | 139 | 3112,66 | 565,407 | with PE | 18 | 2983,33 | 769,851 |
| | low PE risk | 389 | 3241,15 | 503,597 | without PE | 469 | 3224,08 | 497,561 |
| Interval between births | high PE risk | 137 | 7,16 | 4,517 | with PE | 18 | 5,28 | 3,392 |
| | low PE risk | 384 | 5,90 | 3,609 | without PE | 462 | 6,18 | 3,910 |
| Weight of newborn | high PE risk | 495 | 3088,94 | 536,014 | with PE | 38 | 2776,84 | 860,035 |
| | low PE risk | 804 | 3287,56 | 435,745 | without PE | 1174 | 3231,04 | 460,892 |
| Wight gain during pregnancy | high PE risk | 401 | 15,13 | 6,301 | with PE | 31 | 16,90 | 8,715 |
| | low PE risk | 653 | 15,81 | 6,050 | without PE | 955 | 15,45 | 5,987 |
| BMI | high PE risk | 575 | 25,9146 | 5,60255 | with PE | 37 | 27,5892 | 5,38574 |

**Table 1 Comparison between classic and new candidates for PE predictability - First main group (PE risk assessment) and Second main group (with or without developed PE)**

| Final PE risk | | | | | With/ Without PE | | | |
|---|---|---|---|---|---|---|---|---|
| | | Count | Mean value | Standard deviation | | Count | Mean value | Standard deviation |
| Placental growth factor (PLGF) MoM | high PE risk | 560 | 0,6012 | 0,26067 | with PE | 35 | 0,6626 | 0,27764 |
| | low PE risk | 875 | 1,0487 | 0,49995 | without PE | 1122 | 0,8813 | 0,47877 |
| Pregnancy-associated plasma protein PAPP-A MoM | high PE risk | 582 | 0,9249 | 0,51568 | with PE | 38 | 0,8342 | 0,56066 |
| | low PE risk | 908 | 1,1071 | 0,63208 | without PE | 1167 | 1,0561 | 0,59257 |
| Mean arterial pressure (MAP) | high PE risk | 590 | 93,726 | 8,103701 | with PE | 38 | 96,0987 | 7,246760 |
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| Pulsatility index of right uterine artery (R Ut PI) | high PE risk | 591 | 2,059 | 0,70618 | with PE | 38 | 1,9284 | 0,75391 |
| | low PE risk | 920 | 1,7132 | 0,58798 | without PE | 1181 | 1,8482 | 0,63812 |
| Pulsatility index of left uterine artery (L Ut PI) | high PE risk | 591 | 2,0891 | 0,66116 | with PE | 38 | 2,0495 | 0,76434 |
| | low PE risk | 920 | 1,7571 | 0,58377 | without PE | 1181 | 1,8774 | 0,61448 |
| Arithmetic mean of the pulsation index of both uterine arteries (Mean Ut PI) | high PE risk | 591 | 2,0742 | 0,527498 | with PE | 38 | 1,9889 | 0,621968 |
| | low PE risk | 920 | 1,7351 | 0,462505 | without PE | 1181 | 1,8628 | 0,494931 |
| Additional indicators/ new probable candidate predictors for PE | | | | | | | | |
| Cervix length (мм) | high PE risk | 226 | 33,791 | 5,0773 | with PE | 20 | 32,750 | 4,4707 |
| | low PE risk | 325 | 33,424 | 5,1565 | without PE | 458 | 33,562 | 5,0903 |
| Smoked cigarettes per day | high PE risk | 3 | 3,00 | 1,000 | with PE | 0[a] | . | . |
| | low PE risk | 9 | 4,56 | 4,391 | without PE | 10 | 4,40 | 4,169 |
| Number of births | high PE risk | 590 | 0,31 | 0,551 | with PE | 38 | 0,50 | 0,604 |
| | low PE risk | 919 | 0,53 | 0,597 | without PE | 1180 | 0,44 | 0,581 |
| Weight of newborn in previous birth | high PE risk | 139 | 3112,66 | 565,407 | with PE | 18 | 2983,33 | 769,851 |
| | low PE risk | 389 | 3241,15 | 503,597 | without PE | 469 | 3224,08 | 497,561 |
| Interval between births | high PE risk | 137 | 7,16 | 4,517 | with PE | 18 | 5,28 | 3,392 |
| | low PE risk | 384 | 5,90 | 3,609 | without PE | 462 | 6,18 | 3,910 |
| Weight of newborn | high PE risk | 495 | 3088,94 | 536,014 | with PE | 38 | 2776,84 | 860,035 |
| | low PE risk | 804 | 3287,56 | 435,745 | without PE | 1174 | 3231,04 | 460,892 |
| Wight gain during | high PE risk | 401 | 15,13 | 6,301 | with PE | 31 | 16,90 | 8,715 |
| | low PE risk | 912 | 23,4217 | 4,47186 | without PE | 1170 | 23,9105 | 4,80098 |

**Table 2 Intergroup differences between classic and new candidates for PE predictability - First main group (PE risk assessment) and Second main group (with or without developed PE)**

| | | Rank | t | P | Mean difference | PE | Rank | t | p | Mean difference |
|---|---|---|---|---|---|---|---|---|---|---|
| Placental growth factor MoM | final PE risk high PE risk | **1** | -22,182 | 0,000 | -0,4474 | with PE without PE | **2** | -4,458 | 0,000 | -0,2186 |
| Pregnancy-associated plasma protein A MoM | low PE risk high PE risk | **6** | -6,083 | 0000 | -0,1821 | with PE without PE | **3** | -2,275 | 0,023 | -0,2218 |
| Mean arterial pressure | low PE risk high PE risk | **2** | 14,214 | 0,000 | 5,91881 | with PE without PE | **1** | 5,026 | ,000 | 6,6678 |
| Pulsatility index of right uterine artery (R Ut PI) | low PE risk high PE risk | **5** | 9,915 | 0,000 | 0,3462 | with PE without PE | **6** | 0,758 | 0,449 | 0,0802 |
| Pulsatility index of left uterine artery (L Ut PI) | low PE risk high PE risk | **4** | 9,963 | 0,000 | 0,3319 | with PE without PE | **4** | 1,685 | 0,092 | 0,1720 |
| Arithmetic mean of the pulsation index of both uterine arteries (Mean Ut PI) | low PE risk high PE risk | **3** | 12,786 | 0,000 | 0,3390 | with PE without PE | **5** | 1,238 | 0,223 | 0,12615 |
| Additional indicators/ new probable candidate predictors for PE | | | | | | | | | | |
| Cervix length (мм) | low PE risk high PE risk | **7** | 0,826 | 0,409 | 0,3667 | with PE without PE | **6** | -0,702 | 0,483 | -0,8122 |
| Smoked cigarettes per day | low PE risk high PE risk | **8** | -0,590 | 0,568 | -1,556 | with PE without PE | … | **...** | … | …. |
| Number of births | low PE risk high PE risk | **2** | -7,311 | 0,000 | -0,220 | with PE without PE | **7** | 0,584 | 0,559 | 0,056 |
| Weight of newborn in previous birth | low PE risk high PE risk | **5** | -2,498 | 0,013 | -128,490 | with PE without PE | **3** | -1,967 | 0,050 | -240,746 |
| Interval between births | low PE risk high PE risk | **4** | 3,279 | 0,001 | 1,262 | with PE without PE | **4** | -0,964 | 0,335 | -0,902 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Weight of newborn | low PE risk<br>high PE risk | **3** | -6,951 | 0,000 | -198,624 | with PE<br>without PE | **2** | -3,241 | 0,002 | -454,200 |
| Wight gain during pregnancy | low PE risk<br>high PE risk | **6** | -1,759 | 0,079 | -0,686 | with PE<br>without PE | **5** | 0,919 | 0,365 | 1,449 |
| BMI | low PE risk<br>high PE risk | **1** | 9,012 | 0,000 | 2,4929 | with PE<br>without PE | **1** | 4,571 | 0,000 | 3,678 |

It is important to emphasize that the comparison of the PE predictors between the high-risk group and the group with developed PE, showed significant differences in the comparison of almost all indicators (Table 3).

**Analysis of classical predictors**

In classical risk factors the difference between the MAP of the women in the high-risk group and those with PE shows the highest Student's coefficient. The difference here is 2.372 mmHg, p = 0.000. The second biggest difference, according to the calculated t-criterion, is in PLGF (difference of MD = 0.059 MoM (Table 3), t = 5.342, p = 0.000), followed by the difference in PI of right uterine artery - MD = 0.131, t = 4,527, p = 0,000. The next largest intergroup difference - fourth in rank (according to Student's t-test), is between PAPP-A, and the difference found here is 0.091 mIU / L, t = 4.254 ml, p = 0.000. The intergroup difference in mPI-UA - fifth in rank, is also significant - 0.085, p = 0.000.

**Analysis of additional risk factors**

Among the high-risk cases and those who developed PE, the biggest reported difference (first in rank) is in the weight of the newborns, with the difference here of 312.097 grams, p = 0.000. The next largest intergroup difference is in the number of births - 0.186 births, p = 0.000. The third largest difference is in BMI, as the difference here is 1,674, p = 0.000. Fourth in rank is weight gain during pregnancy, with an intergroup difference of 1,773, p = 0.000. In the fifth place is the birth interval of 1.881 years, p = 0.000. The difference in the length of the cervix is 1.041 mm, p = 0.002. The mean weight of the children from the previoius birth showed a significant intergroup difference of 129.332 g, p = 0.008. The last two differences rank sixth and seventh in the analyzed additional predictors for PE.

**Table 3 Comparison between the classical and the additional predictors between women at high risk for PE and women with developed PE**

| | t | rank | p | Mean difference | Confidence interval for the mean | |
|---|---|---|---|---|---|---|
| | | | | | lower | upper |
| **Classical predictors** | | | | | | |
| Placental growth factor MoM | -5,342 | 2 | 0,000 | -0,0588 | -0,0805 | -0,0372 |
| Pregnancy-associated plasma protein AMoM | 4,254 | 4 | 0,000 | 0,0909 | 0,0489 | 0,1329 |
| Mean arterial pressure(MAP) | -7,112 | 1 | 0,000 | -2,3725 | -3,02782 | -1,71735 |
| Pulsatility index of right uterine artery (RUtPI) | 4,527 | 3 | 0,000 | 0,13149 | 0,0744 | 0,1885 |
| Pulsatility index of left uterine artery (LUtPI) | -0,219 | 6 | 0,827 | -0,0059 | -0,0594 | 0,0475 |
| Arithmetic mean of the pulsation index of uterine arteries (Mean Ut PI) | 3,930 | 5 | 0,000 | 0,08527 | 0,04266 | 0,12789 |
| **Additional predictors** | | | | | | |
| Cervix length (mm) | 3,081 | 6 | 0,002 | 1,0407 | 0,375 | 1,706 |
| Number of births | -8,215 | 2 | 0,000 | -0,186 | -0,23 | -0,14 |
| Weight of newborn in previous birth | 2,697 | 7 | 0,008 | 129,332 | 34,51 | 224,16 |
| Interval between births | 4,873 | 5 | 0,000 | 1,881 | 1,12 | 2,64 |
| Weight of newborn | 12,954 | 1 | 0,000 | 312,097 | 264,76 | 359,43 |
| Wight gain during pregnancy | -5,634 | 4 | 0,000 | -1,773 | -2,39 | -1,15 |
| BMI | -7,166 | 3 | 0,000 | -1,67437 | -2,1333 | -1,2155 |

**Relationships between biochemical and physical indicators used to predict preeclampsia**

In order to optimize and refine the predictive power of mathematical models for PE risk, we have structured four relationships between the biochemical and biophysical indicators used to predict PE, namely: mPI-UA / PLGF; mPI-UA / PAPP-A; MAP / PAPP-A, and MAP / PLGF (Table 4).

In the case without preeclampsia and gestational hypertension (GH), the mPI-UA / PLGF ratio is 2,898 ± 2,720. In pregnant women with GH this ratio is 2.636 ± 1.648. In the caseswith PE, the value of the ratio increases significantly and becomes - 4.002 ± 3.746. It can be seen that in women with GH it is even slightly lower than in pregnant women without complications.

The mPI-UA / PAPP-A ratio, in pregnant women without hypertensive complications,is 2,376 ± 1,653. In the caseswith PE this ratio increases significantly and it is 3.839 ± 2.999, and in the participants with GH it is 3.325 ± 3.491.

The MAP / PAPP-A ratio in the patients without PE and GHis 112.881 ± 68.792. In the cases with PE it is 181.474 ± 131.507. In the caseswith GH it is 163.723 ± 130.614. It is established that this ratio is much higher in the cases with PE, followed by those with GH. The MAP / PLGF ratio in the subjects without complications was 134,984 ± 96,599. It mainly increases in pregnant women with PE and becomes 181.238 ± 133.457, and in those studied with GC it is 142.438 ± 82.124.

Three of the calculated ratios can be used as predictors for GH: mPI-UA / PAPP-A, with a ratio of 3.325 ± 3.491. As well as the ratios MAP / PAPP-A and MAP / PLGF – 163.723 ± 130.614 and 142.438 ± 82.124.

**Table 4.Mean ratios between biochemical and physical indicators used to predict preeclampsia**

| Ratio | | Count | Minimum | Maximum | Mean | Standard deviation |
|---|---|---|---|---|---|---|
| Without PE and GH | mPI-UA /PLGF | 1122 | ,36 | 48,92 | 2,8984 | 2,7205 |
| | mPI-UA /PAPP-A | 1167 | ,24 | 13,44 | 2,3761 | 1,6528 |
| | MAP/PAPP-A | 1165 | 15,02 | 547,39 | 112,8813 | 68,7915 |
| | MA /PLGF | 1120 | 22,41 | 1441,67 | 134,9839 | 96,5986 |
| PE | mPI-UA /PLGF | 35 | ,65 | 23,15 | 4,0025 | 3,7456 |
| | mPI-UA /PAPP-A | 38 | ,45 | 11,96 | 3,8399 | 2,9897 |
| | MAP/PAPP-A | 38 | 38,35 | 549,17 | 181,4737 | 131,5073 |
| | MAP/PLGF | 35 | 66,61 | 865,00 | 181,2376 | 133,4565 |
| Gestational Hypertension | mPI-UA /PLGF | 71 | ,57 | 10,70 | 2,6364 | 1,6476 |
| | mPI-UA /PAPP-A | 74 | ,23 | 23,75 | 3,3248 | 3,49090 |
| | MAP/PAPP-A | 74 | 38,68 | 786,11 | 163,7225 | 130,6137 |
| | MAP/PLGF | 71 | 37,36 | 501,67 | 142,4378 | 82,1235 |

In Table 5, the four ratios are compared using analysis of variance: mPI-UA/ PLGF; mPI-UA/ PAPP-A MAR/ PAPP-A, and MAR/ PLGF. It was established that the four ratios show significant differences in their mean values in the compared groups. For the first ratio the value of the statistical criterion *F* is 3.226, p = 0.040; For the second ratio is F = 19.642, p = 0.000; for the third - F = 29.146, p = 0.000; for the fourth - F = 3.985, p = 0.019.

Table 6 compares the ratios between each group of women through multiple comparisons, as the participants were grouped into pregnant women without hypertensive complications, women with preeclampsia, and cases with GH. The mPI-UA/ PLGF ratio showed significant differences between cases without hypertensive complications and those with PE. The difference found for this ratio is MD = 1.104, p = 0.018. The other statistically significant difference in this ratio is between the cases with developed PE and those with GH - MD = 1.366, p = 0.015.

The intergroup comparison for the mPI-UA / PAPP-A ratio showed the following significant differences: between pregnant women without hypertensive complications and those with preeclampsia - MD = 1.464, p = 0.000, and between pregnant women without complications and those with gestational hypertension - MD = 0.949, p = 0.000.

In the MAP / PAPP-A ratio, a significant difference was found between the participants without complications and those who develop preeclampsia MD = 68,592, p = 0,000. There is a significant difference also between the women without complications and with GH, as the difference found is smaller – MD = 50.841, p = 0.000. There is no significant difference between subjects who developed HG and those with preeclampsia for the reported ratio.

The MAP / PLGF ratio showed a significant difference between the subjects without hypertensive complications and those with preeclampsia - MD = 46.253, p = 0.006. For this ratio there is a significant difference between the cases with gestational hypertension and those with preeclampsia, as the difference found is 38,799, p = 0.053. The difference here is significant with 90% reliability of the results.

**Table 5 Comparison of the relationships between biochemical and biophysical indicators used to predict preeclampsia**

| | | Count. | Mean | Standard deviation | F | p |
|---|---|---|---|---|---|---|
| mPI-UA /PLGF | No complication | 1122 | 2,8984 | 2,72054 | | |
| | Preeclampsia | 35 | 4,0025 | 3,74563 | | |
| | Gestational hypertension | 71 | 2,6364 | 1,64766 | 3,226 | 0,040 |
| | Total | 1228 | 2,9147 | 2,71000 | | |
| mPI-UA /PAPP-A | No complication | 1167 | 2,3761 | 1,65287 | | |
| | Preeclampsia | 38 | 3,8399 | 2,98973 | | |
| | Gestational hypertension | 74 | 3,3248 | 3,49090 | 19,642 | 0,000 |
| | Total | 1279 | 2,4745 | 1,88509 | | |
| MAP/PAPP-A | No complication | 1165 | 112,8813 | 68,79154 | | |
| | Preeclampsia | 38 | 181,4737 | 131,50731 | | |
| | Gestational hypertension | 74 | 163,7225 | 130,61376 | 29,146 | 0,000 |
| | Total | 1277 | 117,8686 | 77,84283 | | |
| MAP/PLGF | No complication | 1120 | 134,9839 | 96,59860 | | |
| | Preeclampsia | 35 | 181,2376 | 133,45656 | | |
| | Gestational hypertension | 71 | 142,4378 | 82,12350 | 3,985 | 0,019 |
| | Total | 1226 | 136,7360 | 97,28743 | | |

**Table 6 Multiple comparisons of the relationships between biochemical and biophysical indicators used to predict preeclampsia**

| Dependent Variable | Preeclampsia / Gestational hypertension | (J) Preeclampsia / Gestational hypertension | Mean difference | Standard error | p | 95% Confidence Interval | |
|---|---|---|---|---|---|---|---|
| | | | | | | Lower Bound | Upper Bound |
| mPI-UA /PLGF | No complication | Preeclampsia | -1,1041 | ,4643 | ,018 | -2,015 | -,1932 |
| | | Gestational hypertension | 0,26197 | ,3310 | ,429 | -,3875 | ,9114 |
| | Preeclampsia | No complication | 1,104 | ,4643 | ,018 | ,1932 | 2,0151 |
| | | Gestational hypertension | 1,366 | ,5586 | ,015 | ,2700 | 2,4622 |
| | Gestational hypertension | No complication | -,2619 | ,3310 | ,429 | -,9114 | ,3875 |
| | | Preeclampsia | -1,366 | ,5586 | ,015 | -2,4622 | -,2700 |
| mPI-UA /PAPP-A | No complication | Preeclampsia | -1,463 | ,3063 | ,000 | -2,064 | -,8629 |
| | | Gestational hypertension | -,9486* | ,2227 | ,000 | -1,385 | -,5116 |
| | Preeclampsia | No complication | 1,4638 | ,3063 | ,000 | ,8629 | 2,0647 |
| | | Gestational hypertension | ,5151 | ,3708 | ,165 | -,2124 | 1,2427 |
| | Gestational hypertension | No complication | ,9486* | ,2225 | ,000 | ,5116 | 1,3857 |
| | | Preeclampsia | -,5151 | ,3707 | ,165 | -1,2427 | ,2124 |
| MAP/PAPP-A | No complication | Preeclampsia | -68,592* | 12,558 | ,000 | -93,229 | -43,955 |
| | | Gestational hypertension | -50,841* | 9,1327 | ,000 | -68,758 | -32,924 |
| | Preeclampsia | No complication | 68,592* | 12,558 | ,000 | 43,955 | 93,2291 |
| | | Gestational hypertension | 17,751 | 15,203 | ,243 | -12,075 | 47,5780 |
| | Gestational hypertension | No complication | 50,841* | 9,132 | ,000 | 32,924 | 68,758 |
| | | Preeclampsia | -17,751 | 15,203 | ,243 | -47,578 | 12,075 |
| MAP/PLGF | No complication | Preeclampsia | -46,257* | 16,659 | ,006 | -78,937 | -13,570 |
| | | Gestational hypertension | -7,458 | 11,877 | ,530 | -30,756 | 15,848 |
| | Preeclampsia | No complication | 46,2537* | 16,659 | ,006 | 13,570 | 78,937 |
| | | Gestational hypertension | 38,799 | 20,044 | ,053 | -,5252 | 78,124 |
| | Gestational hypertension | No complication | 7,453 | 11,877 | ,530 | -15,848 | 30,756 |
| | | Preeclampsia | -38,799 | 20,044 | ,053 | -78,124 | ,5252 |

*. The mean difference is significant at the 0.05 level.

## IV. Discussion

In recent years, a number of studies have found (mainly as a consequence of the moving of the Down syndrome screening from the second to the first trimester) that four potentially useful indicators for PE screening can be added: arterial pressure measurements, mPI-UA and quantification of the levels of two placental proteins (PAPP-A and PlGF) in the mother's blood (O'Gorman, N. et al. (2015)). This study makes a re-evaluation of the classical predictors included in a mathematical model (Bayes theorem), which summarizes information from maternal factors, obstetric and medical history, PI of the uterine artery, mean arterial pressure (MAP) and serum PAPP-A and PlGF, when studied in pregnancy at 11 - 13 week of gestation.This model

actually identifies a significant number of women who are at high risk for early PE (O'Gorman, N. et al. (2015); Akolekar, R. et al. (2013)). (Wright D. et al. (2015)

Many authors believe that the most reliable screening test (indicator) is the pulsatility index of the uterine artery. The data collected in this study does not confirm this conclusively, but the high mPI-UA was identified as a highly influential and independent risk factor for intrauterine fetal retardation (data available with the author). The main mechanism for the development of PE has been found to be impaired trophoblast invasion (Khong, TY. Et al. (1986); Granger, JP. Et al. (2001)).

Doppler ultrasound is a non-invasive method for assessing blood flow to the placenta. The finding that poor placental perfusion,caused by increased PI of the uterine artery is associated with the development of PE, supports the theory that PE is a consequence of impaired placentation. The results of previous Doppler studies done in the first and second trimesters, as well as histological examinations of the maternal spiral arteries in the uterine wall, also confirm this hypothesis (Papageorghiou, AT et al. (2002); Plasencia, W. et al., 2007). (Olofsson, P. et al. (1993)).

mPI-UA in women with PE is affected by: gestational age, maternal age, weight, race and history of PE in previous pregnancies (Tayyar, A. et al. (2015). The mPI-UA value is higher in 11 - 13 week of gestation in those who subsequently develop PE and there is a significant negative linear correlation between mPI-UA and the gestational age at birth (O'Gorman, N. et al. (2015)).

Some authors have found that there are reduced levels of PlGF and PAPP-A, although it is assumed that PAPP-A does not contribute to the prediction model for PE (Copel J., et al. (2020).

Women who are predisposed to developing PE have high blood pressure during the first and second trimesters of pregnancy (Cnossen, JS. Et al. (2008), which was confirmed in our study bythe screening done between 11 to 13 week of gestation (Tables 1 and 2).

It is established that in both the first and second trimesters of pregnancy, decreased serum concentrations of PlGF and PAPP-A precede the clinical manifestation of PE (Tidwell, SC. Et al. (2001); Krauss, T. et al. (2004); (Crispi, F. et al. Llurba (2008); Erez, O. et al. (2008); (Akolekar, R. et al. (2008)), which was also confirmed by our results.

There is a significant positive linear correlation between serum PlGF and PAPP-A levels and the gestational age at which childbirth occurs (Akolekar, R. et al. (2013)). This observation further confirms that PE is a pathophysiological condition that is caused by a broad etiological spectrum (O'Gorman N. et al. (2016), including an immune component (Gencheva D. et al. (2021).

According to Zumaeta M. et al. (2020) the longer interval between pregnancies (but we did not confirm this dependency), the higher body mass index and the higher share of women of African racial origin, the higher incidence of chronic hypertension, type 1diabetes mellitus, systemic lupus erythematosus or antiphospholipid syndrome, family history of PE, assisted reproduction, and a lower incidence of smoking are risks for this complication. (Zumaeta M. et al. (2020). In this study the comparison of the above indicators in women with PE did not prove the negative role of the longer pregnancy interval, however, higher body mass index and lower smoking frequency were confirmed as risk factors (Tables 1 and 2).

The connection between maternal smoking and low birthweight is well known (Wills R.A. and Coory M.D. (2008)).Smoking in the first trimester doubles the incidence of SGA and infants with low birthweight (Raisanen S. et al. (2014)), while long-term smoking during pregnancy increases the probability of overweight in 3-year-old boys (Suzuki K. etal. (2014). The interventions that promote smoking cessation during pregnancy reduce the cases of infants with low birthweight, increase the average birth weight and reduce obesity in boys (Suzuki K. et al. (2014); Lumley J. et al. al. (2009). Smoking cessation at any time during pregnancy improves fetal biometrics in later trimesters (Abraham M. et al. (2017)). Contrary to the above findings, it is important to emphasize that none of the smokers developed PE (Table 1).

Table 3 compares the average levels of the variables in the high-risk group compared to those in the pregnant women with PE. The purpose of this comparison is to compare the extent to which the average levels of the variables in the high-risk group correspond to or differ from those in the women who actually develop PEsubsequently. In this way, a certain reassessment and analysis of the predictive role and value of all these indicators is made.

In the Second main group (subgroups with or without PE) the ranking of the analyzed variables done using the Student's t-criterion, determines the first place for the intergroup difference in MAP. In second and third place are the differences found in PLGF and PAPP-A, and in fourth place is the registered difference in PI of the left uterine artery, in fifth place is in mPI-UA, and in sixth place is in PI of the right uterine artery.

The intergroup differences found for the levels of the indicators in the cases falling into the high-risk group for PE, and those that actually developed it later, show that it is necessary to further analyze and include other candidate-predictors. In view of the current data, it would be appropriate and in favor of the clinical practice to pay more attention to the relative contribution of the classical indicators - MAP and PLGF.

The most significant difference between women with and without PE in the analyzed biophysical and biochemical parameters was found in MAP, followed by the difference in PLGF, PAPP-A and mPI-UA. Of the additional indicators/ predictors analyzed, the difference in BMI was the first, followed by the weight of the newborn, the weight of newborn in previous birth, weight gain during pregnancy, cervical length and number of births.

This study also showed that none of the smokers developed PE.

The largest difference reported between high-risk pregnant women and those who actually develop PE is in MAP, followed by the difference in PLGF, then PAPP-A and mPI-UA. Of the additional predictors, the most significant difference between high-risk women and those who developed PE was reported in newborn weight, number of births, BMI, weight gain during pregnancy, birth interval, and cervical length.

Tables 4, 5 and 6 examine the relationships between biochemical and biophysical factors for PE, comparing the mean values in the cases without PE, compared with those with developed PE and GH. In pregnant women who have developed PE, these ratios increase statistically significantly compared to women without PE and may be a better indicator of the prognosis of a high risk of PE.

The analysis of the newly introduced relationshipsshows that both the mentioned significant differences, and their absence, can indicate which ratio is appropriate to be a good indicator for establishing a high risk of preeclampsia, or to register at high risk for GH. Our suggestion here is to include gestational hypertension in the system for assessment of high risk and, accordingly, to propose recommendations to reduce this risk. The ratios where there are no significant differences between uncomplicated cases and those with GH (they respectively cannot be used as predictors of GH) are: between mPI-UA / PLGF and between MAP / PLGF.The data show that low levels of PAPP-A are likely to have a stronger negative effect on PE but not on GH. These significant differences indicate that the two ratios cannot be predictors of a high risk of gestational hypertension, and cannot be recommended for inclusion in the clinical practice, using the software program for analysis of standard measured predictors of preeclampsia. The pregnant women with gestational hypertension also have high levels of these ratios, as they are more evident when the ratio includes PAPP-A. This indicator clearly plays a more significant role in the occurrence of GH than PLGF.

According to our data, predictors of gestational hypertension can be the following ratios (taking into account their increase): mPI-UA / PAPP-A and MAP / PAPP-A.

According to the current data for the prediction of PE, it is appropriate to include in the diagnosis not so much the absolute values of biophysical and biochemical parameters, but their ratios should be taken into account. This due to the fact that when comparing their average values in high-risk and in women with actually developed PE instead of coincidence, there are significant differences.

The proposed ratios can be used in standard programs calculating the risk of PE with the established in classical indicators.

## V. Conclusion

In conclusion, a further analysis of the contribution of classical biophysical and biochemical parameters is needed, as in women who have developed PE, their role seems to be modified according to their previously expected contribution in determining the risk of PE (embedded in the mathematical model for calculating the risk of PE). The risk constellation for the prediction of PE is duly and perfectly acceptable to be expanded with other new indicators or candidate predictors, namely: cervical length, the weight of the child at previous birth, data on IUGR, BMI (our data show that BMI over 24 is high risk for the development of PE), the number of births, weight gain during pregnancy and more.

This study alsoconfirmed that smoking has some protective role against the risk of PE. BMI takes first place (I rank) among the additional risk factors, according to the ranking made for the purposes of this study. The following routinely studied indicators are included as reliable additional predictors in the risk constellation for PE (we offer opportunity to supplement and optimize the model of Bayes' theorem): IUGR (is the most significant and independent predictor), followed by low birthweight in previous birth (according to available data), BMI and shortening of the cervix.

The proposed new ratios between biophysical and biochemical predictors: mPI-UA / PLGF; mPI-UA / PAPP-A MAR / PAPP-A and MAR / PLGF are more reliable indicators for determining the high risk of PE, and can be used directly with popular software programs for calculating the risk of PE.

Effective predictors of gestational hypertension may be the ratios: mPI-UA / PAPP-A and MAP / PAPP-A, taking into account their increase.

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