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Are neutrophil/lymphocyte ratio (NLR), platelet/lymphocyte ratio (PLR), and/or mean platelet volume (MPV) clinically useful as predictive parameters for pre-eclampsia?

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ABSTRACT

Objective: Pre-eclampsia (PE) is a severe pregnancy complication with significant maternal and neonatal morbimortality resulting in high health care costs. Prevention, mainly based on the administration of acetylsalicylic acid, is only possible if timely identification of high-risk patients can be realized in an easy, non-expensive and widely available way. This paper explores the clinical usability of neutrophil/lymphocyte

ratio (NLR), platelet/lymphocyte ratio (PLR), and/or mean platelet volume (MPV) in discriminating between women that will and those that will not develop PE.
Study design: Demographic data and laboratory results were retrospectively collected and compared in 2050 pregnant women (164 PE and 1886 controls) between 1 January 2014 and 31 January 2016.
Results: In the PE group, gravidity, parity, gestational age, and birth weight were significantly lower compared to the control group. Before the 20th pregnancy week, MPV was significantly elevated in the PE group compared to the controls ($p = 0.006$), hence analysis revealed an optimal cut-off point of 8.15 (sensitivity 66.7%, specificity 56.3%) for predicting PE. At the end of pregnancy, NLR and MPV appeared to be higher and PLR lower in the PE group compared to the controls, which strengthens the current knowledge on the pathogenesis of PE.
Conclusion: MPV is significantly elevated in the first half of pregnancy in women who later develop PE and might therefor be implemented in combination with other parameters in a PE prediction model.

Key words: pre-eclampsia, mean platelet volume, neutrophil, lymphocyte, prediction .

INTRODUCTION

Pre-eclampsia (PE) constitutes a major pregnancy complication, as it occurs in 2-8% of pregnancies and is associated with significant maternal and neonatal morbimortality resulting in high health-care costs [1-5]. It is a leading cause of maternal death in developing countries, where mortality is attributed to eclampsia, a result of untreated PE [1]. Regarding the mother, PE can lead to caesarean section, renal failure, liver failure, coagulopathy, stroke, adult respiratory distress syndrome, cardiac arrest, and eventually death [1, 6, 7]. There is widespread empirical evidence that PE is a major risk factor for cardiovascular disease later in life [8-10]. Neonatal risks are intra-uterine growth restriction and low birth weight (due to the placental dysfunction), perinatal death and iatrogenic prematurity [1, 3]. The main features of PE are hypertension and proteinuria [3]. PE is caused by placental dysfunction and placental hypoxia [3, 11, 12]. This leads to activation of immunological factors [13, 14], increased neutrophil counts [15, 16], thrombocyte activation [17-19], systemic inflammation, and endothelial dysfunction [20, 21]. To date, there is only symptomatic but no curative therapy for PE. Delivery is the only way to end the disease, but the timing of delivery should be weighed against the fetal risks of premature birth [1, 3, 7, 22]. In women with increased risk of PE, low dose acetylsalicylic acid is recommended as prevention of PE, but has a high number needed to treat [22-24]. According to the WHO, acetylsalicylic acid should be started at 75 mg per day before 20 weeks of pregnancy [25].

The significant morbidity and mortality call for a predictive test in early pregnancy concerning the future development of PE, in order to provide close follow-up and preventive measures.

Based on the pathogenesis of the disease, this study was designed to examine differences in serum inflammatory and thrombocyte factors, such as NLR (neutrophil / lymphocyte ratio), PLR (platelet / lymphocyte ratio), and MPV (mean platelet volume), between women who developed PE and healthy pregnant women. The purpose of this study is to implement these easy applicable parameters as low-cost predictive factors for the development of PE.

METHODS

Patient population

This retrospective cohort study was conducted at the Antwerp University Hospital (UZA). The study group consisted of all women who gave birth from 1 January 2014 until 31 January 2016. Patients were divided in two groups: a PE group and a healthy control group.

PE was diagnosed in accordance with the American Congress of Obstetricians and Gynecologists (ACOG) as hypertension (a systolic blood pressure of 140 mmHg or higher, or a diastolic blood pressure of 90 mmHg or higher, that occurs after 20 weeks pregnancy in a woman with previously normal blood pressure), and proteinuria (measured as 0.3 gram proteins or more in a 24-hour urine specimen) [7], or signs of other maternal organ dysfunction, such as renal insufficiency (elevated creatinine), liver involvement (elevated transaminases, right upper quadrant or epigastric abdominal pain), neurological complications (headache, hyperreflexia, visual scotoma) or hematological complications (thrombocytopenia, DIC, hemolysis) or signs of utero-placental dysfunction, such as fetal growth restriction [22]. No difference was made between severe or mild PE. Women with HELLP syndrome (hemolysis, elevated liver enzymes, and low platelet counts), were also considered to have PE, since HELLP syndrome is a more serious condition in the same spectrum of this disorder [22].

Data collection

Data were obtained from pregnancy reports and laboratory results. Collected data were age, body mass index (BMI) at the beginning of pregnancy, abuses, and maternal chronic diseases such as kidney diseases, diabetes mellitus, and autoimmune diseases.

Obstetric data were GPA-status (gravidity, parity and abortion), gestational age, single or multiple gestation, personal and familial history of PE, neonatal birth weight, type of delivery (vaginal delivery or primary or secondary caesarean section) and the presence of gestational hypertension, gestational diabetes, gestational cholestasis, PE, HELLP, PPROM (preterm premature rupture of membranes), premature contractions without PPROM, placenta previa, vaginal blood loss, and fetal abnormalities. Proteinuria throughout pregnancy was evaluated using dipstick. Data on blood pressure and blood samples were taken at two occasions: before the 20th pregnancy week and right before primary caesarean section (PCS).

Since the UZA serves as a referral center, first trimester blood results of referred patients were collected through contacting general practitioners and referring gynecologists. Maternal venous blood samples in UZA were taken using vacuette tube. Ethylenediaminetetraacetic acid (EDTA) samples were analyzed using an ADVIA 120 Hematology System (Siemens healthcare®, Germany). Blood results involved complete blood count (CBC) with leukocyte differentiation and biochemical factors such as liver enzymes, creatinine, and glucose.

Exclusion criteria were multiple gestation, previous pregnancy with PE, kidney disease, diabetes mellitus, obesity (BMI ≥ 30kg/m²), gestational diabetes, cholestasis of pregnancy, chronic hypertension, inflammatory bowel diseases, thyroid disorders, auto-immune diseases, cardiovascular diseases, the use of acetylsalicylic acid during pregnancy, hepatitis B or C, human immunodeficiency virus, syphilis, signs of active infection, fetal death, and fetal chromosomal or morphological disorders.

Statistical analysis

Data analysis was executed using SPSS (Statistical Package for the Social Sciences) for Windows, version 24

(SPSS Inc., Chicago, IL).
Normal distribution of variables was tested with the Kolmogorov-Smirnov test. Independent T-test was used to compare mean NLR, PLR, and MPV between the PE and the control group for the first and second lab report for parametric data. Non-parametric data were analyzed using Mann-Whitney U-test. Outliers, defined as values > 2 standard deviations of the mean, were eliminated. Receiver operating characteristic (ROC) curves were used to determine the optimal cut-off level for NLR, PLR, and MPV in predicting PE. Binary multiple logistic regression analysis was performed to assess the independent predictors of PE. Statistical significance was defined as $p < 0.05$.

RESULTS

Patient characteristics

A total of 2050 patients were included, of which 164 pre-eclamptic patients. After applying the exclusion criteria and withholding cases with missing lab reports, 1495 patients remained in the control group and 118 patients in the PE group. (Table I) Regarding the second blood sample (right before labor), patients undergoing PCS were filtered, since being in labor (vaginal delivery or secondary caesarean section) may affect lab results. There remained 138 patients in the control group and 59 patients in the PE group with PCS. (Table II)

Blood pressure, serum inflammatory, and thrombocyte factors before 20th pregnancy week

Systolic and diastolic blood pressure were significantly elevated in the PE group. Leukocytes and thrombocytes were significantly higher in the PE group. No statistical significant difference was found between the PE and control group, in terms of NLR and PLR. MPV was significantly higher in the PE group, compared to the control group. (Table III) The optimal cut-off ratio for MPV is determined by ROC-analysis, as shown in figure 1. The area under the curve (AUC) is 0.652 (95% confidence interval 0.515-0.790). The optimal cut-off point is set at 8.15 with a sensitivity of 66.7% and a specificity of 56.3%. *"Logistic regression with MPV and maternal BMI before the 20th pregnancy week was statistically significant (Hosmer and Lemeshow test with significance 0.21, values > 0.05 are considered significant) in predicting PE. This model has an overall significance of p=0.02 and is able to divide 96.4% of the patients in the correct group (PE versus healthy pregnancy). The Nagelkerke R square however was 0.12, indicating a rather weak model quality."*

Blood pressure, serum inflammatory, and thrombocyte factors right before PCS

Right before PCS, blood pressure was significantly elevated in the PE group, with hypertensive ranges both in systolic as in diastolic blood pressure, which is a known symptom of PE. Leukocytes were not significantly different between the two groups. Neutrophils were significantly higher, and thrombocytes were significantly lower in the PE group, compared to the control group. There was a significantly higher NLR, a lower PLR and a higher MPV, in the PE group compared to the control group. (Table IV) Corresponding ROC curves are shown in figures 2, 3, and 4. AUC with their optimal cut-off points for NLR, PLR, and MPV are listed in Table V. Logistic regression analysis with NLR, PLR, and MPV was borderline significant (Hosmer and Lemeshow test with 0.05 significance, values > 0.05 are considered significant). Logistic regression with NLR and PLR as variables is promising, with a significance of 0.791. This model gives a correct prediction of PE in 80% of the cases. (Formula: Logit (p) = -3.911 + 3.520*NLRcutoffpoint + 2.789*PLRcutoffpoint) Transforming the model in ROC curve analysis had an AUC of 0.870 (95% confidence interval 0.790-0.949), as shown in figure 5. The optimal cut-off for the probability of PE was 0.32, with a sensitivity of 83.9% and a specificity of 70.5%. The positive likelihood ratio for this cut-off point was 2.84 and the negative 0.23.

DISCUSSION

The pathogenesis of PE exists of two consecutive stages. The first stage occurs at the maternofetal junction where a deficient invasion of cytotrophoblasts in the uterine wall and the spiral arteries leads to reduced utero-placental arterial flow and inadequate perfusion of the placenta [3, 11, 12]. This leads to hypoxia and the release of reactive oxygen species, which further contributes to placental oxidative stress and placental dysfunction [3]. This hypoxic state also induces inflammation through the release of chemokines, pro-inflammatory cytokines, anti-angiogenic factors and the activation of monocytes and neutrophils [13]. The neutrophil plays an important role in the pathogenesis of PE. Activation of neutrophils occurs by exposure to oxidized lipids secreted by the placenta, when they pass the intervillous space [26-28].
The second stage of PE starts when these activated neutrophils infiltrate maternal vascular tissue and is associated with maternal systemic vascular inflammation [13, 26, 29]. This leads to thrombocyte activation, vasoconstriction, hypertension, endothelial dysfunction, and end-organ ischemia [3, 5, 12]. For this reason, the clinical stadium of PE is characterized by hypertension, proteinuria, edema, headache, scotoma, coagulopathy, and renal and hepatic dysfunction [3, 9, 14]. Systemic inflammation occurs in normal pregnancies. There is a shift towards Th2 (suppressor T-helper) lymphocytes in normal pregnancies, which leads to suppression of Th1 cytokines, which in turn enables maternal immune tolerance to the fetus, whereas in PE there is a shift towards the Th1 response, an immune maladaptation, and a hyper-inflammatory state [12, 14, 30]. Based on this pathogenesis cascade, there have been many studies about predictive factors for PE, but consensus about a significant and useful predictive parameter has not yet been achieved [31]. Some studies described blood

pressure [32] or uterine artery Doppler velocimetry (an increased pulsatility index alone or combined with bilateral notching) [31, 33, 34] in the screening for PE. Biochemical parameters that have been tested as predictive factors are mostly based on the hyper-inflammatory state, anti-angiogenesis or platelet activation specific for PE. Recently under research in this area is the increase in soluble FMS-like tyrosine kinase 1 (sFlt-1) and soluble endoglin (sEng) and decrease in placental growth factor (PlGF), caused by an imbalance in the VEGF signaling pathway [6].

Endothelial dysfunction in PE leads to uncontrolled intravascular thrombocyte activation with increased thrombocyte consumption in the maternal peripheral circulation [17-19]. Different studies showed that thrombocytes were significantly decreased [35, 36] and mean platelet volume (MPV) was significantly increased [5, 37-42] in women who developed PE, compared to women who did not develop PE [17, 19, 43-45]. This difference was even demonstrated in the first trimester of pregnancy [17, 39]. MPV is an indicator of thrombocyte size, synthesis, and function [19, 41]. MPV is increased in PE, more than in normal pregnancy, as a result of thrombocyte activation and aggregation [19, 37]. Thrombocyte activation in PE is related to the change in the coagulation process between thrombocytes and the damaged endothelial cells [35, 36, 46].

PE is also associated with an increase in leukocyte count, more than in normal pregnancy, and this increase is mainly due to the increase in neutrophil count [15, 16]. Lymphocytes are decreased in PE [15]. An increase in neutrophils and a decrease in lymphocytes results in an increased NLR in PE, compared to normal pregnancies. Few studies focused on this increased NLR, but there were no homogeneous conclusions for the use of NLR as a predictive factor for PE [4, 5, 26, 30, 47-51]. Regarding PLR, there were also inhomogeneous results [4, 5, 49]. The minority of studies examined blood results in the first trimester of pregnancy [4, 49]. The majority of previous papers about NLR/PLR and MPV however, were limited to late pregnancy [5, 26, 30, 47, 48, 50, 51]. To the best of our knowledge, this is the first large retrospective cohort study comparing early (<20weeks) and late (3rd trimester) MPV, NLR, and PLR values between normal pregnancies and pre-eclamptic pregnancies. Consistent with most of the previous studies and the current knowledge on the pathogenesis of PE, our study demonstrates that NLR and MPV are significantly higher and PLR significantly lower in established PE. We thus hypothesize that PE is associated with an enhanced inflammatory response and platelet activation due to systemic inflammation and endothelial dysfunction. The observation that MPV is already augmented in early pregnancy, leads to the conclusion that this systemic inflammation and thrombocyte activation is already present in early pregnancy and MPV can serve as an additional predictor of PE. The weakness of our logistic prediction model with NLR and BMI before 20 weeks, suggests that these factors alone are not capable of correctly predicting PE. However, in combination with other parameters (pulsatility index uterine artery, familial history, anti-angiogenic factors) they might be of additional value in the prediction of PE.

Recent literature proposes NLR as an interesting prognostic factor of cardiovascular disorders since this ratio is significantly increased and since an elevated NLR gives a higher risk for cardiovascular morbidity and mortality [52-54]. For this reason, NLR can be suggested as a prognostic factor in PE for future cardiovascular disease. Future research is required to study whether a higher NLR in PE is associated with a higher prevalence of long-term cardiovascular disease.

LIMITATIONS

Limitations to our study are due to the retrospective character of the study, resulting in missing data in the obstetric files. Patients were mostly followed in private practiced hospitals and only transferred to the UZA after the diagnosis of PE. Because of this, their first blood results, before the 20th pregnancy week, were collected from other laboratories. As a result, leukocyte differentiation was often not executed in other laboratories, which caused missing data for NLR and PLR. The blood results before the 20th pregnancy week were thus derived from different laboratories, and were taken at different gestational weeks before the 20th week, which makes it difficult to compare these blood results between patients. Other limitations to our study were the retrospective model, in which confounding remains possible, and the lack of stratification in the PE group between severe and mild PE.

CONCLUSION

This study examined NLR, PLR, and MPV as predictive markers for PE. Before the 20th pregnancy week, only MPV was significantly elevated in future PE compared to healthy pregnancy. However, the discriminative power of MPV is, in our opinion, not strong enough to recommend use as a single parameter in clinical practice. There might be a place to implement MPV determination in combination with other parameters in a PE prediction model. At the end of pregnancy (right before PCS), there was a significantly higher NLR, a lower PLR, and a higher MPV, in the PE group compared to the control, which supports the current knowledge of the pathogenesis of PE. Further research is needed on predictive factors for PE, with the purpose of starting preventive treatment in these women, since PE is a pregnancy complication with serious implications for mother and child.

DISCLOSURE OF INTEREST

The authors report no conflicts of interest.

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TABLES

Table I: Patients characteristics

	Pre-eclampsia (n=118)	Controls (n=1495)	p-value*
Maternal age (years)	28.91 ± 4.91	30.20 ± 5.19	***0.006***
Nulliparous, n (%)	96 (81.4%)	697 (46.6%)	***0.00***
Smoking, n (%)	6 (0.08%)	87 (0.08%)	0.95
BMI at beginning pregnancy (kg/m²)	23.60 ± 3.66	22.69 ± 3.12	0.13
Gestational age at delivery (weeks)	32.94 ± 4.02	38.11 ± 3.29	***0.00***
Neonatal birth weight (g)	2216 ± 3802	3199 ± 1147	***0.006***

Data expressed as mean ± standard deviation, median (minimum-maximum) or number (%).
* p-value <0.05 = statistically significant difference between pre-eclampsia group and control group.

Table II: Patients characteristics (PCS)

	Pre-eclampsia (n=59)	Controls (n=138)	p-value*
Maternal age (years)	28.03 ± 5.06	31.96 ± 4.50	***0.00***
Nulliparous, n (%)	48 (81.4%)	51 (37.0%)	***0.00***
Smoking, n (%)	3 (0.09%)	7 (0.07%)	0.74
BMI at beginning pregnancy (kg/m²)	22.79 ± 3.11	23.13 ± 2.63	0.72
Gestational age at delivery (weeks)	30.98 ± 2.74	38.58 ± 0.80	***0.00***
Neonatal birth weight (g)	1475 ± 522	3298 ± 438	***0.00***

Data expressed as mean ± standard deviation, median (minimum-maximum) or number (%).
* p-value <0.05 = statistically significant difference between pre-eclampsia group and control group.

Table III: Blood pressure and hematological parameters before the 20th pregnancy week

	Pre-eclampsia group	Control group	p-value*
Systolic BP (mmHg)	125.53 ± 15.40	118.67 ± 12.78	***0.025***
Diastolic BP (mmHg)	74.05 ± 8.46	68.12 ± 9.32	***0.007***
Hemoglobin (g/dL)	12.53 ± 1.19	12.65 ± 1.95	0.693
Hematocrit (%)	37.19 ± 3.21	36.62 ± 3.32	0.171
MCV (fL)	87.15 ± 5.51	85.72 ± 9.94	0.237
MCHC (g/dL)	33.87 ± 1.26	34.27 ± 2.58	0.216
Leukocytes (*10^9/L)	19.67 ± 29.16	8.70 ± 2.31	***0.003***
Neutrophils (*10^9/L)	7.89 ± 10.04	5.63 ± 2.32	0.214
Lymphocytes (*10^9/L)	2.96 ± 4.91	1.83 ± 0.71	0.205
NLR	2.81 ± 0.95	3.08 ± 1.07	0.173
Thrombocytes (*10^9/L)	265.75 ± 67.62	249.07 ± 57.53	***0.022***
MPV (fL)	8.64 ± 1.17	8.06 ± 0.87	***0.006***
PLR	128.86 ± 49.95	132.29 ± 39.74	0.662

Data expressed as mean ± standard deviation.
BP = blood pressure, MCV = mean corpuscular volume, MCHC = mean corpuscular hemoglobin concentration, NLR = neutrophil/lymphocyte ratio, MPV = mean platelet volume, PLR = platelet/lymphocyte ratio.
* p-value <0.05 = statistically significant difference between pre-eclampsia group and control group.

Table IV: Blood pressure and hematological parameters right before PCS

	Pre-eclampsia group	Control group	p-value*
Systolic blood pressure (mmHg)	161.22 ± 21.56	122.31 ± 12.29	***0.00***
Diastolic blood pressure (mmHg)	101.14 ± 10.91	72.62 ± 8.21	***0.00***
Hemoglobin (g/dL)	12.09 ± 3.48	11.46 ± 1.11	0.186
Hematocrit (%)	33.85 ± 4.73	33.34 ± 2.79	0.468
MCV (fL)	86.10 ± 5.20	82.36 ± 6.13	***0.00***
MCHC (g/dL)	34.52 ± 1.60	34.37 ± 1.15	0.537
Leukocytes (*10^9/L)	13.27 ± 4.54	12.34 ± 15.34	0.656
Neutrophils (*10^9/L)	11.33 ± 4.22	6.49 ± 2.05	***0.00***
Lymphocytes (*10^9/L)	1.70 ± 0.62	1.77 ± 0.49	0.533
NLR	6.79 ± 2.84	3.60 ± 1.17	***0.00***
Thrombocytes (*10^9/L)	151 ± 73.89	232.96 ± 63.40	***0.00***

MPV (fL)	9.51 ± 1.21	8.90 ± 1.17	***0.005***
PLR	91.47 ± 47.48	129.05 ± 40.89	***0.0003***

Data expressed as mean ± standard deviation.
BP = blood pressure, MCV = mean corpuscular volume, MCHC = mean corpuscular hemoglobin concentration, NLR = neutrophil/lymphocyte ratio, MPV = mean platelet volume, PLR = platelet/lymphocyte ratio.
* p-value <0.05 = statistically significant difference between pre-eclampsia group and control group

Table V: AUC with optimal cut-off point for NLR, PLR, and MPV right before PCS

	NLR	PLR	MPV
AUC	0.863	0.732	0.642
95% confidence interval	0.783-0.944	0.616-0.848	0.544-0.741
Optimal cut-off point	3.92	109	8.85
Sensitivity	84.4%	69.7%	69.8%
Specificity	69.4%	66%	50%

FIGURE LEGENDS

Figure 1: Receiver operating characteristic (ROC) curve for MPV before the 20th pregnancy week.

Figure 2: ROC curve for NLR right before PCS.

Figure 3: ROC curve for PLR right before PCS.

Figure 4: ROC curve for MPV right before PCS.

Figure 5: ROC curve for logistic model with NLR and PLR right before PCS.