Preterm premature rupture of membranes: prediction of risks in women of Zaporizhzhia region of Ukraine

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The etiology of preterm premature rupture of membranes (PPROM), which is responsible for approximately 30% cases of preterm birth (PTB) is not yet fully understood.
The aim *of the study was to create a mathematical model for prognostication of PPROM based on the anamnesis, clinical data, laboratory findings and genetics predictors.*
Material and methods. *The study involved 80 women with PPROM (between 26 and 34 weeks of gestation) and 50 women having term birth (>37 weeks of gestation) of Zaporizhzhia region of Ukraine. Anamnesis, clinical, laboratory data and single nucleotide polymorphism sequencing of interleukin1β (IL1β), tumor necrosis factor α (TNFα), interleukin4 (IL4), interleukin10 (IL10) and Relaxin 2 (RLN2) genes has been analyzed. Receiver operating characteristic analysis and multivariate logistic regression were used to PPROM predictors identification.*
Results. *We have identified prognostic anamnestic (history of preterm birth), clinical (cervical insuffiency, compromised uteroplacental and fetal circulation), microbiological (vaginal dysbiosis) and hematological criteria for intra-amniotic contamination and further development of PPROM and PTB: WBC>12.3×10⁹/L, GRAN>76%, LYM<19%, neutrophil lymphocyte ratio>3.87, Kalph-Kaliph leukocyte index of intoxication (LII) >3.4, Ostrovsky LII >2.8. Also we have found that GG genotype of IL10 gene polymorphism (rs1800872) leads to a 12.5-fold and CT genotype of RLN2 gene polymorphism (rs4742076) leads to a 17.0-fold increase in risk for PPROM.*
Conclusions. *The prognostic model that we have suggested is an adequate and convenient instrument for practical medical use, which allows for assessment of PPROM probability with a 85% sensitivity and a 72% specificity.*

Key words: *preterm premature rupture of membranes, risks prediction*

Pol Med J, 2020; XLVIII (288); 399–405

Przedwczesne, niedojrzałe pęknięcie błon: przewidywanie ryzyka u kobiet w regionie Zaporoża na Ukrainie

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Etiologia przedwczesnego pęknięcia błon (PPROM), która odpowiada za około 30% przypadków przedwczesnego porodu (PTB), nie jest jeszcze w pełni poznana.
Celem badania *było stworzenie modelu matematycznego do prognozowania PPROM na podstawie wywiadu, danych klinicznych, wyników badań laboratoryjnych i predyktorów genetyki.*
Materiał i metody. *W badaniu wzięło udział 80 kobiet z PPROM (między 26 a 34 tygodniem ciąży) i 50 kobiet urodzonych w terminie (> 37 tygodni ciąży) w regionie Zaporoża na Ukrainie. Analizowano anamnezę, dane kliniczne, laboratoryjne i sekwencjonowanie polimorfizmu pojedynczego nukleotydu genów interleukiny 1β (IL1β), czynnika martwicy nowotworów α (TNFα), interleukiny 4 (IL4), interleukiny 10 (IL10) i relaksyny 2 (RLN2). Do identyfikacji predyktorów PPROM zastosowano analizę charakterystyk pracy odbiornika i wielowymiarową regresję logistyczną.*
Wyniki. *Zidentyfikowaliśmy prognostyczne anamnestyczne (historia przedwczesnego porodu), kliniczne (niewydolność szyjki macicy, upośledzone krążenie macicy i płodu), mikrobiologiczne (dysbioza pochwy) i hematologiczne kryteria zanieczyszczenia wewnątrzamniotycznego i dalszego rozwoju PPROM i PTB: WBC > 12,3 × 10⁹/L, GRAN> 76%, LYM <19%, stosunek limfocytów granulocytów obojętnochłonnych > 3,87, wskaźnik zatruć leukocytów Kalph-Kaliph (LII) > 3,4, Ostrovsky LII > 2,8. Odkryliśmy również, że genotyp GG polimorfizmu genu IL10 (rs1800872) prowadzi do 12,5-krotnego, a genotyp CT polimorfizmu genu RLN2 (rs4742076) prowadzi do 17,0-krotnego wzrostu ryzyka PPROM.*
Wnioski. *Zaproponowany przez nas model prognostyczny jest odpowiednim i wygodnym instrumentem do praktycznego zastosowania medycznego, który pozwala na ocenę prawdopodobieństwa PPROM z czułością 85% i swoistością 72%.*

Słowa kluczowe: *przedwczesne pęknięcie błon, przewidywanie ryzyka*

Pol Merkur Lekarski, 2020; XLVIII (288); 399–405

According to the World Health Organization (WHO), preterm birth (PTB) is any birth occurring before 37 complete weeks of gestation or fewer than 259 days from the first day of the last menstrual period [38]. Complications of PTB were the leading cause of death in children younger than 5 years of age in 2016, accounting for approximately 16% of all deaths, and for 35% of all neonatal deaths [7]. PTB rates vary within 5 to 13% depending on the country, with 15 million preterm births each year on

a global scale [15,43]. In Poland, PTB incidence shows a downward trend with every year, but is still higher than in other European nations, at approximately 8% [2]. The incidence of PTB in Ukraine is estimated at 12% to 46% [45].

Preterm rupture of membranes (PROM) occurs in approximately 2-5% of pregnancies before term and 8% of term gestations. If it happens before 37 weeks, researches describe it as preterm premature rupture of membranes (PPROM) [23,34]. This is an important factor for prenatal morbidity and mortality, and, frequently, prematurity [28], responsible for approximately 30% cases of PTB [32]. Lack of amniotic fluid in the uterus disturbs fetal lung development and promotes development of respiratory distress syndrome in neonates. On the other hand they have higher risk of severe inborn infection [34].

The etiology of PPROM is not yet fully understood due to it being a multifactorial syndrome involving complex interactions between genetically determined and environmental factors. The traditional factor contributing to PPROM is an infection, such as Chlamydia trachomatis, Trichomonas vaginalis, Neisseria gonorrhoea, candidiasis and group B Streptococcus, developing either immediately in the amniotic membrane or ascending from vagina or cervix [4,5,27]. Moreover, abnormalities of amniotic membrane, incompetent cervix, malposition of the fetus, age of gravida (less than 20 years or more than 35 years), blood type, multiple pregnancy, smoking, socio-economic status, obstetrical hemorrhage, spontaneous or artificial abortions and history of preterm delivery or premature rupture of membrane, nutrition deficits (including deficit of vitamin C); hyperactive uterus, narrow pelvis, habitual maternal physical stress and injuries, including coital injuries, internal check up and amniocentesis can be viewed as contributors [22].

Genetic predisposition to PTB has been confirmed by familial association, identification of genetic susceptibility and racial predisposition regarding the rates of PPROM and PTB. Polymorphisms in several genes have been assessed for their association with PPROM, including cytokines [32,44]. Human genome sequencing technology and the discovery of single nucleotide polymorphism (SNP) have been a breakthrough in the study of the impact of genetic code on quantitative changes in gene expression and further biological functioning of proteins [3,21]. The SNP-genes of the cytokines are mostly found in regulatory regions of the gene; they can directly influence transcriptional activity and levels of the cytokine in the blood. These genetic variations may influence the individual characteristics [9], which are involved in immunological responses to infection and act as triggers of PTB and/or PPROM [6,26,44]. However, the data about the polymorphism of signaling molecules in PPROM in a setting of preterm delivery in Ukrainian population are absent.

The aim of the study was to create a mathematical model for prognostication of preterm premature rapture of membranes in women of Zaporizhia region of Ukraine based on the anamnesis, clinical data, laboratory findings and genetics predictors.

MATERIAL AND METHODS

The research was performed at the Department of Obstetrics and Gynecology of Zaporizhia State Medical University, in Zaporizhia Regional Perinatal Center. The study group (SG) involved 80 women undergoing PTB induced PPROM with active contractions (between 26 and 34 weeks of gestation). The mean age of the study subjects was 29.60±6.30 years. The average time of PPROM occurrence was 31.03±2.48 weeks. PPROM was diagnosed on a visualization of amniotic fluid loss by sterile speculum examination and ultrasound examination. The control group (CG) consisted of 50 women having term birth without complications (>37 weeks of gestation). All enrolled patients in the study were Caucasian and of Ukrainian origin. The informed consent was obtained from all participants and the protocol was approved by the local ethics committee of Zaporizhzhia State Medical University.

Exclusion criteria were: multiple pregnancy, abnormal result of prenatal screening, anatomical or genetic defects of the fetus, gestational diabetes, severe arterial hypertension present before the pregnancy or pregnancy-induced (blood pressure ≥160/110 mm Hg), immunological disorders, maternal severe endocrine disorders, generalized infection.

Total counts of white blood cells (WBC), lymphocytes (LYM) and granulocytes (GRAN) in peripheral blood were assessed using a Swelab Alfa automatic hematological analyzer (Boule Medical AB, Sweden). Kalph-Kaliph leukocyte index of intoxication (Kalph-Kaliph LII) was calculated by the formula: (4×myelocytes + 3×metamyelocytes + 2×band neutrophils + 1×segmented neutrophils) × (plasmocytes +1) / [(monocytes + lymphocytes) × (eosinophils + 1)]. Ostrovsky leukocyte index of intoxication (Ostrovsky LII) was calculated by the formula: (metamyelocytes + myelocytes + band neutrophils + segmented neutrophils + plasmocytes) / (monocytes + lymphocytes + eosinophils).

We performed SNP sequencing of interleukin1β (IL1β (rs 1143627)), tumor necrosis factor α (TNFα (rs 1800629)), interleukin4 (IL4 (rs2243250)), interleukin10 (IL10 (rs1800872 and rs1800896)) and Relaxin 2 (RLN2 (rs4742076 and rs3758239)) genes in 50 randomly selected women of SG and each women of CG. Genotyping was performed with samples of total human DNA isolated from whole blood according to Manufacturer's instruction using a "DNA-express-blood-plus" reagent kit (Litech, Russia). Genotyping with TaqMan probes was performed on a CFX96™Real-Time PCR Detection Systems thermocycler (Bio-Rad Laboratories, Inc., USA). The polymerase chain reaction (PCR) for TaqMan genotyping was performed according to the instruction by Applied Biosystems (USA). The investigations were performed at the Molecular-Genetic Research Division of the Medical and Laboratory Center of Zaporizhzhia State Medical University.

Statistical analysis of the data was performed using Statistica 13.0 software package (StatSoft Software No. JPZ8041382130ARCN10-J). Receiver operating characteristic (ROC) analysis was used to determine the cut-offs for values of selected factors. The findings of the ROC analysis were given as mean areas under the ROC curve (AUC, area under the curve) built in terms of sensitivity (ST) and specificity (SP) values and the limits of the 95% confidence interval (95% CI). In order to assess the contribution of clinical, laboratory and molecular genetic factors to the probability of PPROM, relative risk and odds ratio (RR and OR) were calculated, with 95% confidence intervals (CI). If RR and OR values [CI 95%] are between 0 and 1, this corresponds to risk reduction; when RR and OR values [CI 95%] are equal to 1, this translates into the absence of effect and when RR and OR [CI 95%] are above 1, this translates into increased risk.

The decision whether to include a patient to the group of higher PPROM risk was informed by the sequential estimation method with LR+ and LR- threshold limits. The concept of the likelihood factor (LF) informs the interpretation of the test: this is the probability ratio of finding a certain test result in a subject with the disease in question compared to the probability of finding the test result in a subject without the disease. A positive likelihood factor (LR+) is the ratio of probability of obtaining a positive test result in a patient with the disease to the same probability in a patient without the disease (that is, the ratio of true positives to false positives). The negative likelihood factor (LR-) is the ratio of probability of obtaining a negative test result in a patient with the disease to the same probability in a patient without the disease (that is, the ratio of false negatives to true negatives).

$$LR+ = \frac{Se}{1-Sp} \qquad LR- = \frac{1-Se}{Sp}$$

The method of multivariate logistic regression was used to assess the risk factors for PROM in a setting of preterm delivery. By using the equations of logistic regression, we determined the specific predictors to influence the outcomes, and, using their values, we estimated the probabilities of individual outcomes.

Table 1. Operational characteristics of diagnostic efficacy of hematological indices as factors predicting PPROM and PTB
Tabela 1. Charakterystyka operacyjna skuteczności diagnostycznej wskaźników hematologicznych jako czynników prognozujących PPROM i PTB

Parameter	Cut-off point	Sensitivity, %	Specificity, %	The value of positive prognosis, %	The value of negative prognosis, %
WBC	> 12.3 ^{9}/L	63.75	88	61.54	38.46
LYM	≤ 19%	73.75	90	61.54	38.46
GRAN	> 76%	68.75	94	61.54	38.46
NLR	> 3.87	73.75	90	61.54	38.46
Kalph-Kaliph LII	> 3.42	36.71	92	60.77	39.23
Ostrovsky LII	> 2.76	67.09	70	61.24	38.76

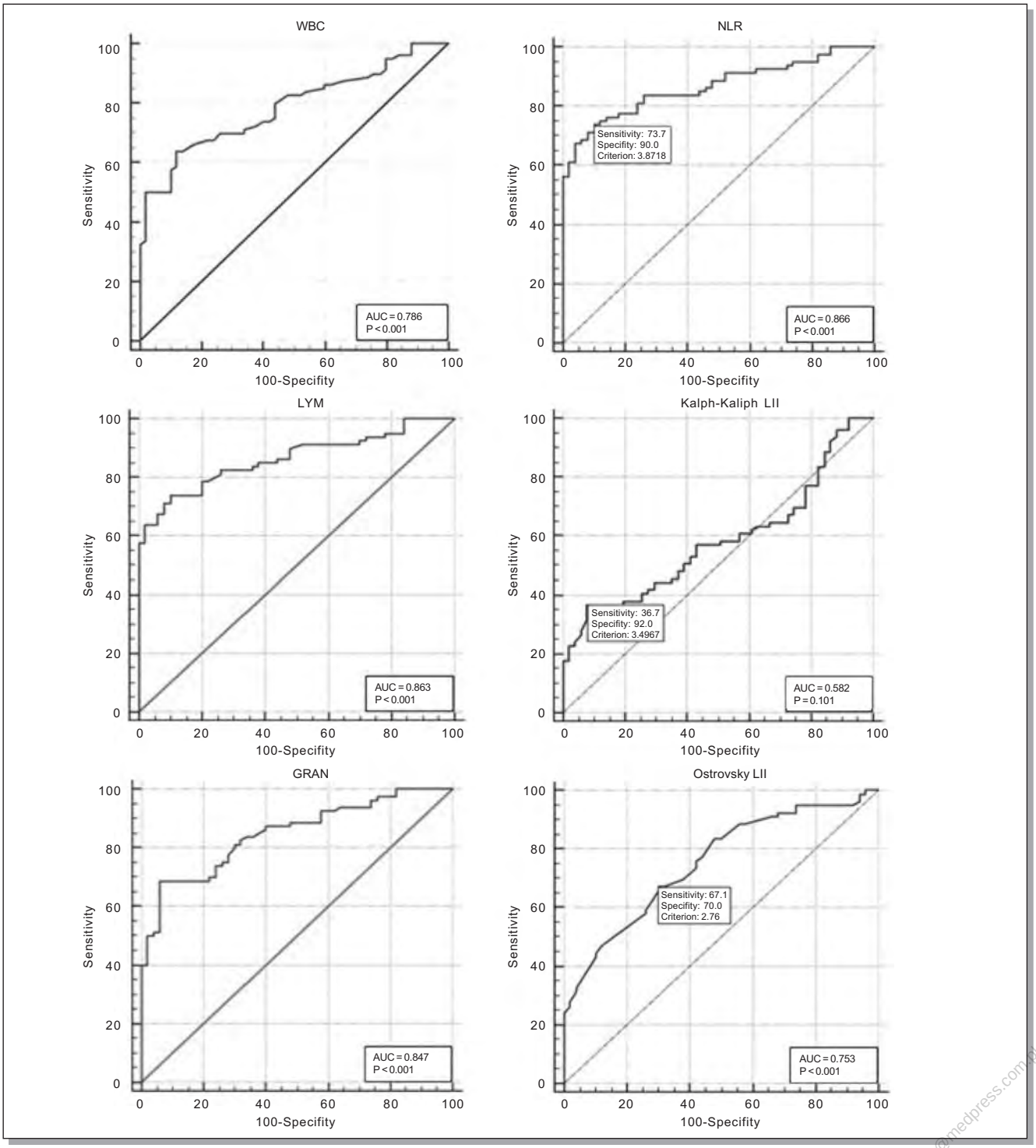

Figure 1. Receiver operating characteristic curves showed discriminatory power of white blood cells (WBC), lymphocytes (LYM), granulocytes (GRAN) count and leukocyte intoxication indices (Kalph-Kaliph LII, Ostrovsky LII and NLR) in peripheral blood on PPROM risk
Rycina 1. Charakterystyczne krzywe działania odbiornika wykazały moc dyskryminującą białych krwinek (WBC), limfocytów (LYM), liczbę granulocytów (GRAN) i wskaźniki zatrucia leukocytów (Kalph-Kaliph LII, Ostrovsky LII i NLR) we krwi obwodowej na ryzyko PPROM

RESULTS

In order to determine the diagnostic values of hematologic findings (WBC, GRAN and LYM) and LII as predictors of PPROM and initiation of preterm delivery at 26-34 weeks of gestation, we have performed a ROC analysis and established the average quality of these prognostic factors ($p<0.001$). The optimal cut-off values for prognostication of PPROM in a setting of preterm delivery (with respect to the criterion of maximally balanced sensitivity and specificity) were >12.3×10^9/L for WBC; <19.0% for LYM; >76.0% for GRAN; >3.87 for neutrophil lymphocyte ratio (NLR); >3.42 for Kalph-Kaliph LII and >2.76 for Ostrovsky LII (tab. 1).

The area under the curve (AUC) was 0.786 for WBC, 0.863 for LYM, 0.847 for GRAN, 0.866 for NLR and 0.753 for Ostrovsky LII, which suggests a good prognostic value of investigational predictors (fig. 1).

Using an integrative analysis of anamnesis, course of pregnancy and laboratory findings in patients of the study and control groups, we have identified 11 factors, which increased the risks for PPROM and initiation of preterm delivery (tab. 2).

The risk for PPROM at 26-34 weeks of gestation with subsequent preterm delivery was increasing in presence of well-known predictors, such as cervical insuffiency (OR=4.55, 95% CI 1.25-16.55), impaired vaginal microbiocenosis as reported by bacteriological assessment (OR=4.33, 95% CI 1.52-12.33) and compromised uteroplacental and fetal circulation (OR=4.64, 95% CI 1.48-14.53).

Detection of 95% sensitivity and 95% specificity was considered sufficient to inform the decision on assigning the patients to the group of higher PPROM risk using a sequential estimation method; that said, a positive likelihood factor (LR+) took on a value of 19, while a negative likelihood factor (LR-) took on a value of $^1/_{19}$ or 0.05. When predicting PPROM-associated preterm labor using RR values of the relevant predictor (tab. 2), it is possible to stratify gravidas into the strata of high risk (LR+=19 or >) or low risk (LR- = 0.05 or <) and to develop the algorithm for further pregnancy management and to optimize preventive measures.

In order to detect the most sensitive predictors of risk for development of preterm labor we used a method of multivariate linear stepwise regression analysis. WBC, LYM and GRAN have been identified as the most significant independent predictors in women of this category. The values of B coefficients are natural logarithms of odds ratios for the relevant variables. The presence of one or another independent variable increases the chances for obstetrical complications × EXP (B) times. The mathematical relationship between the dependent variable (the probability of preterm labor) and independent variables (those selected in the process of multivariate regression analysis) is described by the following multiple logistic regression equation:

$$Y = \frac{1}{1+e^{-x}}$$

where X =-8.85+0.19 × WBC – 0.09 × LYM + 0.12 × GRAN

Table 2. Comparative analysis of the factors predicting PPROM and risk for PTB
Tabela 2. Analiza porównawcza czynników prognozujących PPROM i ryzyko PTB

Parameter		Rates across the groups				RR [CI 95%]	OR [CI 95%]
		Abs., subjects		Relative, %			
		SG	*CG*	*SG*	*CG*		
GRAN, % > 76	Yes	55	3	68.75	6	11.46 [3.75-35.03]	34.47 [9.67-122.88]
	No	25	47	31.25	94		
History of preterm birth	Yes	13	1	16.25	2	8.13 [1.08-61.37]	9.51 [1.18-76.61]
	No	67	49	83.75	98		
LYM % ≤ 19	Yes	58	5	72.5	10	7.25 [3.10-16.97]	23.73 [8.25-68.22]
	No	22	45	27.5	90		
NLR >3.87	Yes	59	6	73.75	12	6.15 [2.85-13.26]	20.6 [7.6-55.84]
	No	21	44	26.25	88		
Kalph-Kaliph LII > 3.42	Yes	29	4	36.25	8	4.53 [1.68-12.23]	6.54 [2.11-20.23]
	No	51	46	63.75	92		
Cervical insuffiency	Yes	18	3	22.5	6	3.75 [1.15-12.22]	4.55 [1.25-16.55]
	No	62	47	77.5	94		
Compromised UPFC*	Yes	23	4	28.75	8	3.59 [1.31-9.87]	4.64 [1.48-14.53]
	No	57	46	71.25	92		
WBC ^{9}/L >12.3	Yes	51	9	63.75	18	3.54 [1.91-6.58]	8.01 [3.39-18.96]
	No	29	41	36.25	82		
Pathogenic bacteria in vaginal microbiocenosis	Yes	26	5	32.5	10	3.25 [1.32-7.98]	4.33 [1.52-12.33]
	No	54	45	67.5	90		
A resident of remote village/district	Yes	36	10	45	20	2.25 [1.22-4.14]	3.27 [1.43-7.50]
	No	44	40	55	80		
Ostrovsky LII > 2.76	Yes	53	16	66.25	32	2.37 [1.54-3.64]	6.63 [2.93-14.97]
	No	17	34	21.25	68		

*uteroplacental and fetal circulation

In part, the chances for preterm delivery were significantly ($p<0.01$) higher in residents of remote village/district, a 2.3-fold difference (95% CI: 1.22-4.14), in women with GRAN >76%, a 11.5-fold difference (95% CI: 3.75-35.03); in women with WBC>12.3×10^9/L, a 3.5-fold difference (95% CI: 1.91-6.58); women with a history of preterm labor, a 8.1-fold difference (95% CI: 1.08-61.37); in women with LYM ≤19%, a 7.3-fold difference (95% CI: 3.10-16.97); in women with NLR increased over 3.9, a 6.2-fold difference (95% CI: 2.85-13.26); in women with Kalph-Kaliph LII increased over 3.4, a 4.5-fold difference (95% CI 1.68-12.23) and in women with Ostrovsky LII increased over 2.8, a 2.4-fold difference (95% CI: 1.54-3.64).

It should be emphasized that 16.3% patients of the SG (as opposed to 2% in the CG) had a history of preterm labor, suggesting a personal genetic predisposition to this complication. With the intent of integration of our previously published data [18], we were able to identify the genetic markers with statistically significant associations in the population of Zaporizhzhia region of Ukraine (tab. 3).

The protective effect is inherent to AG genotype of RLN2 gene polymorphism (rs3758239): RR=0.67, 95% CI: 0.05-0.54; CC genotype of RLN2 gene polymorphism (rs4742076): RR=0.59, 95% CI: 0.46-0.75 and TG genotype of IL10 gene polymorphism (rs1800872): RR=0.49, 95% CI: 0.32-0.73. In the population of Zaporizhia oblast of Ukraine, the CT genotype of RLN2 gene polymorphism (rs4742076) leads to a 17.0-fold increase in risk for PROM in preterm delivery (95% CI: 2.29-125.67); the GG genotype of IL10 gene polymorphism (rs1800872) leads to a 12.5-fold increase (95% CI: 3.07-50.85) and the AA genotype of RLN2 gene polymorphism (rs3758239) leads to a 1.7-fold increase (95% CI: 1.33-2.28), respectively.

in women with WBC>12.3×10^9/L; in women with an anamnesis of preterm labor; in women with LYM ≤19%; in women with NLR >3.9; in women with Kalph-Kaliph LII >3.4 and in women with Ostrovsky LII >2.8. The risk for PPROM at 26-34 weeks of gestation with subsequent preterm delivery was increased in presence of well-known predictors, such as cervical insuffiency, impaired vaginal microbiocenosis and compromised uteroplacental and fetal circulation.

A special place among signaling molecules is occupied by cytokines. IL1β and TNFα are important pro-inflammatory cytokines synthesized by active mast cells and macrophages. Conversely, IL4 and IL10 are ubiquitous anti-inflammatory cytokines chiefly produced by T cells [20,30]. Pregnant women are known to have reduced inflammatory responses and, respectively, increased anti-inflammatory responses [8,40].

The role of inflammation in the etiology of PTB is not clear, but elevated IL1β, TNFα, IL6 and IL8 levels in amniotic fluid have been reported in women with PTB [44]. IL1β regulates gene expression in the smooth muscle cells of the myometrium [1].

Table. 3. The genetic markers selected for prognostication of PPROM risk at 26-34 weeks of gestation and the risk for initiation of preterm delivery in the population of Zaporizhzhia region of Ukraine
Tabela 3. Markery genetyczne wybrane do prognozowania ryzyka PPROM w 26-34 tygodniu ciąży oraz ryzyko rozpoczęcia porodu przedwczesnego w populacji regionu moczowodu – Zaporoże

Parameter		Rates across the groups				RR [CI 95%]	OR [CI 95%]
		Abs., subjects		Relative, %			
		SG	*CG*	*SG*	*CG*		
CT genotype of polymorphism *RLN2 (rs4742076)*	Yes	17	1	34	2	17 [2.29-125.67]	25.24 [3.12-204.1]
	No	33	49	66	98		
GG genotype of polymorphism IL10 (rs1800872)	Yes	25	2	50	4	12.5 [3.07-50.85]	24 [5.16-111.73]
	No	25	48	50	96		
AA genotype of polymorphism *RLN2 (rs3758239)*	Yes	47	27	94	54	1.74 [1.33-2.28]	13.34 [3.61-49.41]
	No	3	23	6	46		
AG genotype of polymorphism *RLN2 (rs3758239)*	Yes	3	18	6	36	0.67 [0.05-0.54]	0.11 [0.03-0.42]
	No	47	32	94	64		
CC genotype of polymorphism *RLN2 (rs4742076)*	Yes	29	49	58	98	0.59 [0.46-0.75]	0.03 [0.00-0.23]
	No	21	1	42	2		
TG genotype of polymorphism IL10 *(rs1800872)*	Yes	18	37	36	74	0.49 [0.32-0.73]	0.19 [0.08-0.47]
	No	32	13	64	26		

DISCUSSION

Over the past 40 years in the world there is no tendency to reduce the number of PTB, and at least every tenth child on Earth (11.1%) is born before 37 complete weeks of gestation [13,14,17]. The levels of prenatal morbidity and mortality in PTB are determined by gestational age and weight of the fetus, as well as by the course of pregnancy and delivery. A distinctive position within the structure of prenatal morbidity and mortality in preterm neonates is held by the labor in a setting of PPROM [11,35,42].

The risk factors of preterm labor have traditionally included social factors (age, race, and lifestyle factors), anamnesis (different types of preterm delivery) and the special aspects of the particular gestation (bleeding, infectious and inflammatory complications, etc.). However, the system of risk assessment based on these factors is known for a very low level of efficacy and a large quantity of false positive results. False positives, in turn, lead to excessive diagnostic burden, unjustified hospitalization of gravidas and unwarranted treatment [35].

Using an integrative analysis of anamnesis, course of pregnancy and laboratory findings in patients of the study and control groups, we have identified 11 factors, which increased the risks for PPROM and initiation of preterm delivery. In part, the chances for preterm delivery were significantly (p<0.01) higher in residents of remote village/district; in women with GRAN >76%; It also significantly increases the expression and secretion of IL8, monocyte chemotactic protein 1, granulocyte macrophage colonystimulating factor, TNFα and IL6 in the epithelial cells in the female reproductive tract [25] and together with TNFα, stimulates the amnion, decidua and myometrium to express prostaglandins [6]. Moreover, IL1β promotes local progesterone metabolism, which is necessary for maintaining pregnancy [41].

TNFα is another important regulatory molecule during pregnancy, which mediates an inflammatory response and is also involved in labor activities such as membranes repture and uterine contractions [44]. High concentrations of TNFα are produced by pro-inflammatory Th17 cells, which are one of at least four sub-populations of CD4+ T-cells: T-helper of the first type (Th1), T-helper of the second type (Th2), T-helper17 (Th17), and regulatory T-cells – Treg cells [16,19]. *Wu L. et al.* analyzed the Treg cells bias against Th17 cells and whether it may play a role in the maintenance of pregnancy [39]. Therefore, up-regulation of the Treg population and down-regulation of the Th17 population might contribute to the outcome of pregnancy.

There are data that successful pregnancy is associated with aberrant expression of IL4 [29]. Furthermore, *El-Shazly et al.* indicated that placentas from women with PPROM and preterm delivery have higher levels of Th1-inducing cytokines and placentas from women after preterm delivery compared with

term delivery showed a bias towards the Th1 profile with significantly higher levels of IFN-γ and IL-2 as well as the Th1-inducing cytokine IL12 [10]. In the study performed by *A. Heinzmann* haplotypes IL13/IL4 in a German population were presented as associated with PTB [12].

In our study we did not found significant associations between SNPs of IL1β, IL4 and TNFα genes with increased risk for PPROM or protective effect in the population of Zaporizhzhia region of Ukraine.

IL10 is a well-known anti-inflammatory cytokine. Serum levels of IL10 are associated with PTB; however, the results are inconsistent with some authors reporting increased levels of IL10 in PTB, and others demonstrating increased IL10 levels to actually reduce the risk for PTB. Menon et al. demonstrated IL10 SNP to be significantly associated with PTB in Caucasian women [24]. At the same time, *Stonek F.* and *Metzenbauer M*. have not found any associations between IL10 and PTB [33].

We have found that in the population of Zaporizhzhia region of Ukraine the protective effect is inherent to TG genotype of IL10 gene polymorphism (rs1800872) and GG genotype of IL10 gene polymorphism (rs1800872) leads to a 12.5-fold increase in risk for PPROM.

Relaxin is a mammalian hormone with an important reproductive role. In humans, 3 relaxin genes have been described (RLN1, RLN 2, and RLN 3) [36] with RLN1 and RLN2 expressed in human decidual membrane and placenta. However, RLN2 is the predominant form also produced by the corpus luteum; in pregnancy it enters the systemic circulation. RLNs have been shown to produce dose-dependent increases in expression of specific genes and proteins and the activities of certain regulatory matrix metalloproteinases, which contribute to PPROM. They are also capable of direct modulation of production of pro-inflammatory cytokines [31].

We have found that in the population of Zaporizhzhia region of Ukraine the protective effect is inherent to AG genotype of RLN2 gene polymorphism (rs3758239) and CC genotype of RLN2 gene polymorphism (rs4742076). The CT genotype of RLN2 gene polymorphism (rs4742076) leads to a 17.0-fold increase in risk for PPROM and the AA genotype of RLN2 gene polymorphism (rs3758239) leads to a 1.7-fold increase, respectively.

A recent study within a homogeneous Danish population has demonstrated women who are homozygous for specific SNPs in the promoter region of RLN2 to be genetically susceptible to PTB [37] The study by *Rocha F.G. et al.* involved a population of strictly selected women with PTB or PPROM and term control subjects (>8000 samples from Filipino patients who gave birth at 34-36 weeks). The results obtained in these Filipino patients have found SNP rs4742076 in the RLN2 promoter to be associated with increased dRLN expression and PPROM, while SNP rs3758239 was associated with both PPROM and PTB [31].

CONCLUSIONS

According to our study, reliable prognostic criteria for intraamniotic contamination and further development of PPROM and preterm labor at antenatal stage include hematological indices (WBC>12.3×10^9/L, GRAN>76%, LYM<19%, NLR>3.87), integrative index of anamnesis, course of pregnancy and the presence of CT genotype of RLN2 gene polymorphism and GG genotype of IL10 gene polymorphism. The prognostic model that we have suggested is an adequate and convenient instrument for practical medical use, which allows for assessment of PPROM probability with a 85% sensitivity and a 72% specificity.

REFERENCES

1. Bartlett SR, Sawdy R, Mann GE.: Induction of cyclooxygenase-2 expression in human myometrial smooth muscle cells by interleukin-1beta: involvement of p38 mitogen-activated protein kinase. J Physiol, 1999; 520: 399-406.
2. Bidzan M, Bieleninik Ł.: Conditions of the Quality of Life and Life Satisfaction of Mothers of Preterm Babies in Poland. J Socialomics, 2013; 2: e117.
3. Bilous II, Korda MM, Krynytska IY, et al.: Nerve impulse transmission pathway-focused genes expression analysis in patients with primary hypothyroidism and autoimmune thyroiditis. Endocrine Regulations, 2020; 54(2): 109-118.
4. Boiko I, Golparian D, Krynytska I, et al.: Antimicrobial susceptibility of Neisseria gonorrhoeae isolates and treatment of gonorrhoea patients in Ternopil and Dnipropetrovsk regions of Ukraine, 2013-2018. APMIS, 2019; 127(7): 503-509.
5. Boiko I, Golparian D, Krynytska I, et al.: High prevalence of Chlamydia trachomatis, Neisseria gonorrhoeae and particularly Trichomonas vaginalis diagnosed using US FDA-approved Aptima molecular tests and evaluation of conventional routine diagnostic tests in Ternopil, Ukraine. APMIS, 2019; 127(9): 627-634.
6. Bukowski R, Sadovsky Y, Goodarzi H, et al.: Onset of human preterm and term birth is related to unique inflammatory transcriptome profiles at the maternal fetal interface. Peer J, 2017; 5: e3685.
7. Chawanpaiboon S, Vogel JP, Moller AB, et al.: Global, regional, and national estimates of levels of preterm birth in 2014: a systematic review and modelling analysis. Lancet Glob Health, 2019; 7: e37-46.
8. Christian LM, Porter K, Karlsson E, et al.: Serum proinflammatory cytokine responses to influenza virus vaccine among women during pregnancy versus nonpregnancy. Am J Reprod Immunol, 2013; 70: 45-53.
9. Demikhov O, Dehtyarova I, Demikhova N.: Actual aspects of public health policy formation on the example of Ukraine. Bangladesh Journal of Medical Science, 2020; 19(3): 358-364.
10. El-Shazly S, Makhseed M, Azizieh F, et al.: Increased expression of pro-inflammatory cytokines in placentas of women undergoing spontaneous preterm delivery or premature rupture of membranes. Am J Reprod Immunol, 2004; 52: 45-52.
11. Gomez-Lopez N, Romero R, Plazyo O, et al.: Preterm labor in the absence of acute histologic chorioamnionitis is characterized by cellular senescence of the chorioamniotic membranes. Am J Obstet Gynecol, 2017; 217: 592e1-592e17.
12. Heinzmann A, Mailaparambil B, Mingirulli N, et al.: Association of Interleukin 13/4 and toll-like receptor 10 with preterm births. Neonatology, 2009; 96(3): 175-181.
13. Khmil M, Khmil S, Marushchak M, et al.: Reproductive hormone metabolism in women with infertility due to polycystic ovary syndrome depending on the constitutional body types. Pol Merkur Lekarski, 2020; 285: 152-156.
14. Khmil M, Khmil S, Marushchak M.: Hormone Imbalance in Women with Infertility Caused by Polycystic Ovary Syndrome: is There a Connection with Body Mass Index? OAMJMS, 2020; https://www.id-press.eu/mjms
15. Koullali B, Oudijk MA, Nijman TAJ, et al.: Risk assessment and management to prevent preterm birth. Semin Fetal Neonatal Med, 2016; 21: 80-88.
16. Koval HD, Chopyak VV, Kamyshnyi OM, et al.: Transcription regulatory factor expression in T-helper cell differentiation pathway in eutopic endometrial tissue samples of women with endometriosis associated with infertility. Cent Eur J Immunol, 2018; 43(1): 90-96.
17. Lamont RF.: Spontaneous preterm labour that leads to preterm birth: An update and personal reflection. Placenta, 2019; 79: 21-29.
18. Lyubomirskaya ES, Kamyshnyi AM, Krut YY, et al.: SNPs and transcriptional activity of genes of innate and adaptive immunity at the maternal-fetal interface in woman with preterm labour, associated with preterm premature rupture of membranes. Wiadomosci Lekarskie, 2020; 73(1): 25-30.
19. Marushchak M, Krynytska I, Milevska L, et al.: The changes of activity of effector caspase cascade components in case of alimentary obesity in rats. Bangladesh J Med Sci, 2017; 16(2): 252-258.
20. Marushchak M, Maksiv K, Krynytska I.: The specific features of free radical oxidation in patients with chronic obstructive pulmonary disease and arterial hypertension. Pol Merkur Lekarski, 2019; 279: 95-98.
21. Marushchak M, Maksiv K, Krynytska I.: ACE gene I/D polymorphism and arterial hypertension in patients with COPD. Pneumologia, 2019; 68(3): 114-119.
22. Maryuni M, Kurniasih D.: Risk factors of premature rupture of membrane. Kesmas: National Public Health J. 2017; 11(3): 133-137.
23. Meller CH, Carducci ME, Ceriani Cernadas JM, et al.: Preterm premature rupture of membranes. Arch Argent Pediatr, 2018; 116(4): e575-e581.
24. Menon R, Fortunato SJ, Edwards DR, et al.: Association of genetic variants, ethnicity and preterm birth with amniotic fluid cytokine concentrations. Ann Hum Genet, 2010; 74: 165-183.
25. Mosher AA, Rainey KJ, Giembycz MA, et al.: Prostaglandin E2 represses interleukin 1 beta-induced inflammatory mediator output from pregnant human myometrial cells through the EP2 and EP4 receptors. Biol Reprod, 2012; 87(7): 1-10.
26. Musilova I, Kutová R, Pliskova L, et al.: Intraamniotic inflammation in women with preterm prelabour rupture of membranes. Plos One, 2015; 10(7): e0133929.
27. Nakubulwa S, Kaye DK, Bwanga F, et al.: Genital infections and risk of premature rupture of membranes in Mulago Hospital, Uganda: a case control study. BMC Res Notes, 2015; 8: 573.
28. Petit C, Deruelle P, Behal H, et al.: Preterm premature rupture of membranes: Which criteria contraindicate home care management? Acta Obstet Gynecol Scand, 2018; 97: 1499-1507.
29. Piccinni MP. : T cell tolerance towards the fetal allograft. J Reprod Immunol, 2010; 85: 71-75.

30. Putilin DA, Kamyshnyi AM.: Changes of glut1, mTOR and AMPK1á GENE expression in pancreatic lymph node lymphocytes of rats with experimental diabetes mellitus. Med Immunol (Russia), 2016; 18(4): 339-346.
31. Rocha FG, Slavin TP, Li D, et al.: Genetic associations of relaxin: preterm birth and premature rupture of fetal membranes. Am J Obstet Gynecol, 2013; 209(3): 258.e1-8.
32. Romero R, Friel LA, Velez Edwards DR, et al.: A genetic association study of maternal and fetal candidate genes that predispose to preterm prelabor rupture of membranes (PROM). Am J Obstet Gynecol, 2010; 203(4): 361.e1-361.
33. Stonek F, Metzenbauer M, Hafner E, et al.: Interleukin-10-1082 G/A promoter polymorphism and pregnancy complications: results of prospective cohort study in 1616 pregnant women. Acta Obstet Gynecol Scand, 2008; 87(4): 430-433.
34. Swiatkowska-Freund M, Traczyk-Los A, Partyka A, et al.: Perinatal outcome in preterm premature rupture of membranes before 37 weeks of gestation. Ginekologia Polska, 2019; 90 (11): 645-650.
35. Tchirikov M, Schlabritz-Loutsevitch N, Maher J, et al.: Mid-trimester preterm premature rupture of membranes (PPROM): etiology, diagnosis, classification, international recommendations of treatment options and outcome. J. Perinat, 2017; 46(5): 465-488.
36. Tevz G, McGrath S, Demeter R, et al.: Identification of a novel fusion transcript between human relaxin-1(RLN1) and human relaxin-2 (RLN2) in prostate cancer. Mol Cell Endocrinol, 2016; 420: 159-168.
37. Vogel I, Hollegaard MV, Hougaard DM.: Polymorphism in the promoter region of relaxin-2 and preterm birth: involvement of relaxin in the etiology of preterm birth. In Vivo, 2009; 23(6): 1005-1010.
38. Vogel JP, Chawanpaiboon S, Moller AB, et al.: The global epidemiology of preterm birth. Best Pract Res Clin Obstet Gynaecol, 2018; 52:3-12.
39. Wu L, Luo L, Zhang Y, et al.: Alteration of Th17 and Treg cells in patients with unexplained recurrent spontaneous abortion before and after lymphocyte immunization therapy. Reprod Biol Endocrinol, 2014; 12: 74.
40. Yýlmaz Y, Verdi H, Taneri A, et al.: Maternal-fetal proinflammatory cytokine gene polymorphism and preterm birth. DNA Cell Biol, 2012; 31: 92-97.
41. Zachariades E, Mparmpakas D, Pang Y, et al.: Changes in placental progesterone receptors in term and preterm labour. Placenta, 2012;33:367-372.
42. Zare-Bidaki M, Sadrinia S, Erfani S, et al.: Antimicrobial properties of amniotic and chorionic membranes: a comparative study of two human fetal sacs. J Reprod Infertil, 2017; 18(2): 218-224.
43. Zeitlin J, Szamotulska K, Drewniak N, et al.: Preterm birth time trends in Europe: a study of 19 countries. BJOG, 2013; 120(11): 1356-1365.
44. Zhu Q, Sun J, Chen Y.: Preterm birth and single nucleotide polymorphisms in cytokine genes. Transl Pediatr, 2014; 3(2): 120-134.
45. Zhywieckaja-Denisova AA, Vorobiev II, Tkachenko VB.: Preterm birth: modern approaches to their prevention. Neonat Surg Perinat Med, 2012; 1(3): 46-49.

Disclosure: The authors declare no conflict of interest.

Received: 10.07.2020
Revised: 08.09.2020
Accepted: 26.10.2020

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