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Possibilities for diagnosis and prediction of preterm labor at the present stage

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Abstract

Preterm birth is one of the main causes of perinatal morbidity and mortality, which does not tend to decrease in rate. The risk of death in premature babies is 25–35 times higher than that of full-term babies, and stillbirths are registered 8–13 times more often than in timely delivery. To date, there are no effective ways to prevent preterm birth. Therefore, the timeliness of therapy, which largely determines the outcome of pregnancy in general, depends on the effectiveness of assessing the likelihood of their development. At the International Federation of Gynecology and Obstetrics (FIGO) Congress (2018), preterm birth is identified as a problem that has not yet been solved at the current stage of science and technology development. The result of the unsolved problems is a situation wherein the modern world over the past 60 years there has been no decrease in the premature birth rate, which is 9.5% of births and annually ends with the birth of 15,000,000 premature babies. The study aimed to research modern methods of diagnosis and prediction of spontaneous preterm birth. An analytical method was used in the study: a detailed systematic analysis of modern domestic and foreign literature on the diagnosis and prognosis of preterm birth. We used eLibrary, Scopus, PubMed, MEDLINE, ScienceDirect, Cochrane Library bibliographic databases (until August 2020). The article deals with the diagnosis and prediction of preterm birth probability, which will optimize the management of patients from the risk group and, in the future, will reduce the rate of perinatal morbidity and mortality of premature babies. Despite a significant number of researches devoted to the study of possibilities for diagnosing and predicting spontaneous preterm birth, currently, there are no methods with absolute diagnostic value. Most existing studies indicate that when assessing the probability of preterm birth, a comprehensive approach should be preferred taking into account the results of several main and additional methods.

Keywords: preterm birth, uterine contractile activity, preterm termination of pregnancy, risk group, probability, predicting.

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Introduction. Preterm birth (PB) is a major cause of perinatal morbidity and mortality [1,2]. Every year, 13–15 million premature babies are born worldwide, which is about 5%–10% of the total number of births [1–3]. Neonatal mortality of premature newborns amounts to 28%, whereas total perinatal mortality associated exclusively with complications caused by prematurity accounts for about 50%–70% [3,4]. The incidence of premature newborns is many times higher than that of full-term newborns. Nowadays, almost every third child born prematurely suffers from cerebral palsy [4]. The severity and incidence of complications in premature newborns are proportional to the onset of PB [3,4]. The birth of children with extremely low body weight often results in disability, which can be expressed as a disorder of general psychomotor development and can be accompanied by blindness, deafness, cerebral disorders, and chronic lung diseases. In this regard, PB has become an urgent problem of modern health care [1,5].

Currently, PBs represent both a medical and social problem, which is associated with a high probability of a child's disability and is associated with high material and economic costs for society as a whole [3,5]. According to international studies, nursing one 22-week-old baby can cost several hundred thousand dollars. A contrast is seen between full-term and premature babies during further monitoring of the child's health and treatment in out-

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patient-polyclinic conditions, which is often depressing, since the quality of life of children born with extremely low body weight is far from perfect [5].

Health problems in premature infants can lead to socially associated conflicts in the family and subsequent pregnancy refusal amid fear of a similar unfavorable outcome [3]. According to Radzinsky, the main organizational disadvantage in the management of PB in obstetric institutions is the lack of their readiness to provide effective medical care [5]. In view of this, obstetricians currently face three main tasks, namely, the need to accurately determine the time of PB onset, timely preparation of the fetus for preterm birth through adequate and safe medications, and transportation of the fetus *in utero* to level III hospitals [3,6]. Predicting the time of PB onset is currently the most promising field of medical care, which enables the implementation of timely and successful organizational measures aimed at improving perinatal outcomes [6].

This study aimed to analyze current methods of diagnostics and prediction of spontaneous PB. An analytical method using a systematic analysis of modern Russian and international literature on the diagnostics and prediction of PB was performed. The study used information from eLibrary, Scopus, PubMed, MEDLINE, ScienceDirect, Cochrane Library, and the Federal Institute of Industrial Property until August 2020.

Assessment of risk factors (RF) of spontaneous PB. For many years, risk groups have been identified in the Russian Federation, including those determining the probability of PB [4]. Risk groups generally include RFs related to as follows: (i) past pregnancies and/or previous surgeries (PB history, late miscarriages, two or more therapeutic abortions, more than four births, and uterine cervix [UC] conization or amputation), (ii) current pregnancy (low social and economic living standard, alcohol/nicotine/drug addiction, stressful work and/or family situation, age younger than 18 or older than 34 years, and periodontitis presence), and (iii) PB in this pregnancy (urinary tract infections, hyperthermia, uterine bleeding, placenta previa, polyhydramnios, premature UC "maturation," severe diabetes mellitus or other extragenital pathology, and trauma and surgery in the current pregnancy) [3,7]. However, the main RF is the presence of PB in the anamnesis [4]. Despite common knowledge of the described RFs, it is currently impossible to predict the onset of labor, which is associated with the multifactorial etiology of PB [2].

Given the lack of convincing data on the predictive value of scoring assessment of the risk of PB, diagnostic measures are currently of greater importance, as they could estimate the time and probability of the implementation of RF in the clinical presentation of PB [3,4]. A direct study of the tissues of the uterine-fetal-placental complex can provide accurate information about the state of pregnancy and calculate the probability of complications, including PB [2,8].

Meanwhile, considering the striving contemporary medicine for minimally invasive technologies, within the problem under consideration, more accessible, reliable, and less expensive technologies should be preferred. It should be noted that invasive procedures, such as amniocentesis or tissue biopsy to identify significant markers, can also cause PB [2,9–11]. For this reason, nowadays, along with instrumental and clinical research methods, preference is given to analyses of biological body fluids, such as whole blood/blood serum/plasma, urine, saliva, and cervicovaginal contents, which are the most accessible for diagnostics. However, they contain a large number of proteins and metabolites, in which the concentration can change in response to the initial stages of pathogenesis of threatening PB [2].

Evaluation of the efficiency of ultrasound predictors of spontaneous PB. According to the current clinical guidelines, nowadays, the assessment of UC length (cervicometry) is considered prognostically valuable in diagnosing the threat of PB [3,4]. With a UC length of 2.5 cm or less, the risk of PB increases by six times. Recently, much attention has been paid to ultrasound examination, but the sensitivity of gynecological examination and transabdominal echography is quite low and amounts to 25%–30% and 35%–40%, respectively [3]. At the same time, many researchers agree that the assessment of UC length by transvaginal echography, in contrast, is a rather valuable prognostic method [12–14]. However, with a UC length of more than 3 cm, the probability of the onset of labor within the next week is 1%; therefore, this technique is used as an indicator for predicting tocolysis efficiency and not PB.

With a UC length of less than 15 mm, the risk of childbirth earlier than week 32 of gestation is 50%, and with a UC length of more than 30 mm, the probability of labor onset within the next week is about 1% [3, 4]. Shortening of UC to less than 25 mm should be considered a signal for the entire complex of significant diagnostic measures [4]. In accordance with the current clinical guidelines, a patient with a UC length of 3.0 cm or more is not subject to hospitalization, and additional diagnostic measures are not required [3].

According to Radzinsky, the most reliable diagnostic method is a combination of cervicometry (main method) and biochemical methods (additio-

nal) [5]. To increase the diagnostic value of cervicometry, some researchers propose to evaluate a new criterion—the glandular index [15]. It is represented by a sonographically hypoechoic zone around the cervical canal of UC, which presumably histologically corresponds to the glandular zone around the cervical canal, which produces a mucous plug with a barrier function [15,16].

According to the study by Volkova et al., cervicometry in trimester I of pregnancy is useful for predicting the risk of spontaneous PB; however, it has low sensitivity (25.4%) because of the high number of false-positive (37.0%) and false-negative (97.6%) results. Meanwhile, the determination of the glandular index increases the sensitivity of cervicometry in diagnosing PB up to 26.8% and early PB up to 78.6%. A decrease in the glandular index reflects the premature maturation of the UC, and it can be considered a predictor of spontaneous PB [16].

At the same time, Martynenko et al. noted that the prognostically unfavorable ultrasound criterion for PB is a pathological change in the shape of the internal os (V- and U-shaped) along with shortening of the cervical canal to 25 mm or less [17]. According to Astafiev et al., the most significant prognostic sign is the uterocervical angle formed by the UC and lower uterine segment [18]. The uterocervical angle is measured by transvaginal echography based on the visualization of the angle created by two lines, namely, a line along the anterior wall of the uterus, including the isthmus and internal os, and a line parallel to the cervical canal through the internal and external os. An angle of more than 105° is mainly combined with a high risk of PB (sensitivity 81.3% and specificity 83.1%) [18].

The works by Kuznetsova et al., who determined the relationship between PB and retrochorial hematoma volume and between the human chorionic gonadotropin and pregnancy-associated plasma protein A (PAPP-A) levels in trimester I of pregnancy, are of interest. According to the data obtained, in pregnant women with retrochorial hematoma with a volume of more than 1 cm^3 and a PAPP-A level of less than 0.7 MoM, the risk of PB increases by 9.5 times, and with retrochorial hematoma of the same volume and a β-subunit level of human chorionic gonadotropin less than 0.4 MoM, the risk of PB increases by 5.5 times [19].

Some works by Kosyakova et al. defined relaxin as the most pathogenetically substantiated predictor of PB. High serum relaxin concentrations are associated with an 11-fold increase in the incidence of very early PB (weeks 22–26) and a 6-fold increase in early PB (weeks 27–32) [20].

Kurochka et al. used an integrated approach, including the determination of C-reactive protein and UC vascularization index. They believe that the probability of the onset of ultra-early PB is higher if the C-reactive protein level is 6 mg/L or more and UC vascularization index 20% or more. According to the study, the prognosis sensitivity and specificity are 89.6% and 85.7%, respectively. UC vascularization increases because of the onset of functioning of a special venous network in the uterus (sinusoidal venous lacunae) on the eve of labor, resulting in a "cavernous-like" transformation of the UC [21].

Makukhina et al. obtained interesting data and proposed an algorithm for calculating the probability of PB based on the analysis of ultrasound examination results. In a standard "cut through three vessels" transabdominal ultrasound section, the authors determined the ratio of the distance from the posterior surface of the sternum to the anterior wall of the aortic cross section (T_1) to the distance from the posterior surface of the sternum to the anterior surface of the thoracic vertebral body (T_2). In a standard transvaginal ultrasound sagittal section of UC, the UC length from the internal to the external os in millimeters (L) and the presence of congestion of echo-positive suspension over the internal os (S) were determined. The ratio is regarded as 0 points if the value of the ratio T_1/T_2 is not less than 0.40, 0.5 points with T_1/T_2 equal to 0.35–0.39, and 1.5 points with T_1/T_2 less than 0.35. If L is not less than 20 mm, 0 points are assigned, and 0.5 points with L less than 20 mm. In the absence of an echo-positive suspension over the internal os (S), 0 points are assigned, and 1 point in case of its presence. The risk of PB is calculated using the equation $p = T_1/T_2 + L + S$. If $p \geq 2$, the probability of the onset of PB within the next 14 days is high. The prognosis accuracy is 88.1%, sensitivity 84.6%, and specificity 89.5% [22]

Assessment of the probability of spontaneous PB by analyzing the most accessible environments of the body. Several authors prefer saliva as the medium, which is most accessible for analyzing the mother's body [2,23–25]. According to the authors, the steroid concentration in the saliva reflects the unbound, unconjugated, and, therefore, biologically active fraction of the hormonal profile of blood plasma. Since saliva samples are easy to collect and store, the authors consider the assessment of saliva hormones to be the optimal method for the instant diagnostics of threatening PB [23]. In particular, they examined the progesterone level in the saliva, and its concentration was significantly lower in women with developed PB compared with delivery at term ($p = 0.0009$) [23]. Given the

polyetiology of PB, the presence of such a significant marker seems to be a promising method for predicting PB. The authors also determined the absence of a correlation between fetal fibronectin and progesterone levels [2,23].

According to Maged et al., the sensitivity of the prognosis based on the analysis of progesterone concentration in saliva is 84%, and the specificity and accuracy are 90% and 89.8%, respectively [24]. Research by Priya et al. confirmed the predictive value of the progesterone level in saliva, and its threshold value is 2575 pg/mL. According to the authors, about 80% of PB up to week 34 occurs when the progesterone level is below the threshold [25]. Abuelghar et al. determined the presence of a statistically significant correlation between the UC length and progesterone concentrations in saliva; therefore, the authors considered a decrease in progesterone to be a more objective predictor of PB [26].

An increase in the estriol level in saliva over 2.3 ng/mL can also accompany the development of threatening PB (specificity 77% and sensitivity 71%) [27].

The study of urine to determine biomarkers of development of threatening PB also seems to be quite promising, since the urine test is noninvasive and quite simple [2]. In accordance with existing clinical guidelines, screening and treating asymptomatic bacteriuria are significant factors in preventing PB [3]. Meanwhile, the study by Hundley et al. did not reveal the clinical significance of urine culture as a predictor of PB ($p = 0.68$). The sensitivity and specificity of a positive relationship between the factors described are 0.7% and 98.6%, respectively. In view of this, Hundley and Chalermchockchareonkit believe that using routine urine culture tests to assess the probability of PB will be costly and uninformative [28,29].

Assessment of changes in the autonomic regulation as a predictor of spontaneous PB. According to several authors, autonomic regulation is of paramount importance in prolonging pregnancy under the influence of RF of PB [30,31]. The works by Rad'kov, who proposed to conduct a spectral analysis of heart rate variability for early diagnostics of threatening PB, are of practical interest. The authors recorded the electrocardiogram for 5 min in two positions, namely, in the supine position and on the right side. The registered biopotentials are converted by a computer program into a sequence of R–R intervals. The program conducts spectral analysis of heart rate variability using the parametric autoregressive Berg method. The result is a normalized power value (nHFs) in the high frequency range of the heart rate spectrum.

The results of the study, obtained in the supine position, reflect quantitatively the influence of the parasympathetic division of the autonomic nervous system on the contractile function of the uterus. With the nHFs index within the range of 24.0–32.4 normalized units and the nHF change coefficient when changing the supine position to the position on the right side (nHFr/s) in the range from −17.2% to 41.3% in trimester III of pregnancy, threatening PB is diagnosed [30]. However, the influence of the patient's vegetative status on the work of the cardiovascular system and the functional state of the myometrium may differ due to the peculiarities of adrenergic reactivity of the pregnant uterus, which does not allow using this method as a universal approach [31].

Assessment of the probability of spontaneous PB by blood tests. Considering the availability and minimal invasiveness of blood tests, it is logical to search for significant markers in the peripheral bloodstream. Meanwhile, the local remoteness of the vascular system periphery from gestational tissues cannot enable the determination of a universal predictor of PB [2]. Nevertheless, researchers are still trying to find the "gold standard" for early diagnostics of threatening PB [32].

For example, a biochemical study of the plasma of peripheral venous blood can determine the concentration of norepinephrine. According to Malyshkina et al., if its value is 167.6 pg/mL or less at a gestational age of 24–34 weeks, threatening PB should be diagnosed. The sensitivity, specificity, and accuracy of the pattern revealed are 80.3%, 78.4%, and 79.6%, respectively [31]. The decrease in the blood pool of norepinephrine in threatening PB is probably caused by its consumption when it binds to a large number of active α-adrenergic receptors that implement myometrial contraction. The mechanism for reducing β-adrenergic reactivity can be caused by increased synthesis of prostaglandins E_2 and $F_{2\alpha}$ due to the release of catecholamines of fetal origin. In addition, prostaglandin E_2 has an inhibitory effect on β-adrenergic receptors. However, the lack of a sufficient amount of norepinephrine can lead to the accumulation of cyclic adenosine monophosphate in the cell and direct activation of the expression of genes for cyclooxygenase-2, which is a key enzyme in the synthesis of prostaglandins that trigger myometrial contractions. Myometrial hypoxia, which develops in the presence of threatening PB, also reduces its β-adrenoreactivity but increases the sensitivity to α-adrenergic receptor agonists [31].

The application of proteomic analysis, which initiates significant progress in elucidating previously unknown mechanisms of disruption of

molecular and cellular processes that ensure the development of pregnancy and enables to substantiate scientific ideas about the importance of specific protein substances as biomarkers of both the physiological and pathological course of pregnancy, is currently acquiring special diagnostic significance in PB [33].

Pogorelova et al. referred regulatory molecules, namely, E-cadherin, Janus kinase 2, endoplasmin, heat shock protein 8, gelsolin, transgelin-2, antioxidant enzymes (peroxiredoxins 2 and 3 and superoxide dismutase), and vascular endothelial growth factor A to the proteins, in which expression is reduced with the risk of PB. The authors revealed that in case of PB risks, there was an increase in the expression for the differentiation factor of the pigment epithelium, transcription factor S-II, ribosomal protein S6-kinase α_3, interleukin-6 (IL-6), insulin-like growth factor-binding protein 1, and bikunin [33].

Various bacterial pathogens are also triggers of spontaneous PB in 40%–60% of cases. Notably, infectious and inflammatory causes, as a rule, become inducers of preterm labor in the period from weeks 22 to 34 of gestation [34].

One of the earliest predicting methods is the method of Radkov et al., which assesses the probability of PB by analyzing the levels of IL-1 in the serum of peripheral blood and IL-6, an inflammatory marker, which is a tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), at a gestational age of 8–10 weeks. The risk of PB should be calculated using the following equation:

$$p = \frac{1}{1 + e^{z}},$$

where p is the value of the risk of PB; e is the base of the natural logarithm ($e \sim 2.72$).

The z value is calculated using the equation as follows:

$$z = 1.207 \times \text{IL-1} + 0.723 \times \text{IL-6} – 1.823 \times \text{TRAIL} + 5.361,$$

where 1.207, 0.723, and −1.823 are nonstandardized coefficients b; 5.361 is constant (regression coefficient b0); IL-1, IL-6, and TRAIL are values of their level in blood serum.

If $p > 0.47$, the risk of PB is considered high. High sensitivity (80.8%) and specificity (69.8%) of the method are because chronic inflammatory diseases in patients often lead to recurrent miscarriage and are associated with a high probability of PB [35].

The data of Levkovich et al. indicate that other ILs can also be considered predictors of PB. The authors propose to determine the level of IL-33 in blood serum using enzyme immunoassay in case of threatened miscarriage at a term of 18–22 weeks of gestation [36]. IL-33 expression in decidual stromal cells is necessary at the stage of pro-inflammatory implantation; however, its prolonged expression can lead to loss of pregnancy, since IL-33 has a pro-inflammatory effect on mast cells, basophils, eosinophils, monocytes, macrophages, natural killer cells, and activated neutrophils, causing the activation of NF-κB and MAP kinases and synthesis of various cytokines and chemokines. Premature activation of this pro-inflammatory pathway can lead to impaired pregnancy tolerance and PB [37]. An IL-33 level of 0.98 pg/mL and higher is associated with a high risk of PB (sensitivity 96% and specificity 97%) [37].

It should be borne in mind that the most common infections complicating pregnancy are etiologically associated with gram-negative bacteria, the cell wall of which includes lipopolysaccharides (endotoxins) and heteropolymers that can have a toxic effect on organs and systems. In their works, Bondarenko and Dobrokhotova determined that the concentration of bacterial polysaccharides exceeding 8.36 EU/mL of blood plasma at weeks 24–34 of gestation is associated with the onset of PB [38].

Malyshkina et al., in the presence of clinical signs of threatening PB at a gestational age of 24–34 weeks, recommended to determine the indicator of resistance of active coagulation factor V to activate protein C in blood plasma, and if its value is 0.94 or lower, the onset of PB is expected. According to the authors, the recommended method can predict PB with high accuracy (75.6%). The specificity and sensitivity of the method are 85.7% and 66.7%, respectively [39].

Sotnikova et al. proposed to determine the level of transforming growth factor β in blood serum, considering that its concentration could predict the onset of PB after week 34 of gestation, so PB is predicted at 2000 pg/mL or less. The method could predict PB with high accuracy (95.3%), sensitivity (96.5%), and specificity (92.8%) [40]. Meanwhile, PB prediction after week 34 of gestation is of low value for clinical practice, since in most cases, the management of patients at this term does not require tocolysis or fetal respiratory distress syndrome prevention [3,6].

The method of Posiseeva et al. is deemed to be more promising than that of Sotnikova, as it involves determining the probability of PB after week 28 of gestation. In the serum of peripheral blood before and after the course of preserving therapy, the quantitative indicator of nitrate ions should be determined, and if it increases by 10% or more relative to the initial value, the onset of PB is predicted [41]. However, the method has several significant disadvantages, such as the need for

repeated blood tests, the possibility of prognosis only after preserving therapy with a gestational age of 28 weeks or more, and the low accuracy (89.5%), sensitivity (92%), and specificity (84.6%) [40, 41]. The authors pointed out that a nitrite ion concentration of 1.15 μM/L or higher should be considered a criterion for threatening PB at week 33 of gestation or more with an accuracy of 96.7%, specificity of 100%, and sensitivity of 97.7% [42].

Another interesting method for predicting PB was presented by Borzova et al., according to which the ratio of indicators of the relative level of $CD25^+$ to $IL\text{-}2^+$ lymphocytes, equal to 1.0 or more at a gestational age of 6–12 weeks, has a rather high accuracy (82.6%), sensitivity (90.0%), and specificity (76.9%) of PB prognosis [43]. The relative level of $CD46^+$ monocytes of 51.7% or less can also be attributed to predictors of PB at weeks 22–33 of gestation. The accuracy of this relationship is 93%, sensitivity 84%, and specificity 100% [44]. Panova et al. suggested considering the relative level of $CD62L^+$ neutrophils of 65% or more as a predictor. The accuracy, sensitivity, and specificity of the relationship amount to 90% [45].

The ratio of the activity of interferon-α to interferon-γ over 5 is also accompanied by the development of PB [46]. After week 20 of gestation, threatening PB is often accompanied by a decrease of up to 20% or less in the relative level of T-lymphocytes carrying structures on their surface that react with fertility α2-microglobulin. The prediction accuracy of this relationship is 94% [46].

The main disadvantage of the methods described includes the lack of the ability to determine the time of PB. The time of PB onset can be predicted using the method of Tyutyunnik et al. It consists of determining the catalase activity in the peripheral blood plasma and calculating the probability of PB using the equation as follows:

$$p = \frac{1}{1 + e^{2,86679 - 0,06653 \times Katalaz}},$$

where p is the probability of PB; *Katalaz* is the level of catalase activity (U/mL); e is the base of the natural logarithm (e ~ 2.72). At $p \geq 0.5643984$, the onset of PB is predicted within the next 7 days [47].

In addition, to predict PB, Tyutyunnik, based on a logistic regression model using the simultaneous determination of the total amount of extracellular deoxyribonucleic acid (DNA) by real-time polymerase chain reaction (PCR) and IL-8 by enzyme immunoassay in blood plasma in pregnant women at weeks 22–36 of gestation, proposed the equation as follows:

$$p = \frac{1}{1 + e^{4,1 - 4 \times ИЛ\text{-}8 - 0,0001 \times овДНК}},$$

where p is the probability of developing PB; IL-8 is its level (pg/mL); ovDNA is the level of the total amount of extracellular DNA (GE/mL); e is the base of the natural logarithm (e ~ 2.72).

At $p > 0.5$, PB occurs in most cases. The sensitivity of the equation is 75.6%, and the specificity is 82.9% [48].

Assessment of the probability of spontaneous PB by analyzing cervicovaginal contents. An increase in the hydrogen ion concentration (pH value) of the vaginal environment over 4.4 (alkalization) is also considered a significant prognostic sign of PB [4,49]. For verification, any express methods (test strips, vaginal speculum with indicators, or special gloves) or laboratory data that complement bacterioscopic and bacteriological study of the contents of the cervical canal and the posterior vaginal fornix can be used [4]. The latter are used not so much to make a prognosis but to quantify possible pathogens of the inflammatory process and determine their sensitivity to antibiotics, which, if performed timely, can prevent the development of PB [4].

The study of cervicovaginal contents should especially be noted, which can be of important diagnostic value for monitoring the health of both pregnant women and the fetus, since it represents a complex mixture of secretions obtained from the vagina, endocervix, decidual endometrium, and amniochorion [2]. In addition, analysis of vaginal scrapings and UC is minimally invasive and safe. In terms of topical diagnostics, such studies seem to be the most promising since the UC and vagina are structural and functional elements of the birth canal. Currently, many studies have been conducted on the search for predictors of PB in cervicovaginal secretion [2,50–53].

A similar technology was proposed by Tyutyunnik et al. Their method involves scraping the epithelium of the cervical canal from a pregnant woman and performing PCR to determine the ratio of the expression levels of messenger ribonucleic acid (mRNA) of the *TLR4/CD68* and *TNF/GATA3* genes. To avoid degradation, the material (scrapings from the cervical canal) was placed in a 1.5 mL plastic tube with 500 μL of transport medium for RNA stabilization (visualizing solution from the reagent kit for nucleic acid isolation) immediately after sampling. The method can store the material at a temperature of −20°C for 6 months.

Next, the value of the canonical linear discriminant function is calculated using the following equation:

$$z = -\ln(TLR4/CD68) \times 1,152 + \ln(TNF/GATA3) \times 1,608 - 1,526,$$

where z is the canonical linear discriminant function; TLR4/CD68 is the ratio of expression levels

of mRNA of genes *TLR4* and *CD68*; TNF/GATA3 is the ratio of the expression levels of mRNA of the *TNF* and *GATA3* genes.

The probability of PB is calculated using binary logistic regression, which significantly increases the accuracy, as follows:

$$p = \frac{1}{1 + e + z},$$

where p is the probability of PB; z is the canonical linear discriminant function; e is the base of the natural logarithm ($e \sim 2.72$).

With a probability of 67% or more, the development of PB is predicted within the next 7 days [54].

The presence of urogenital infection triggers a cascade of reactions that contribute to the development of PB. For this reason, the study of epithelial cells of the cervical canal mucous membrane helped determine several predictors of PB. Signaling receptors of innate immunity include toll-like receptors (TLR), which represent a family of signaling molecules that recognize conservative molecular patterns of various pathogens, including viruses, bacteria, and fungi.

Gankovskaya et al. used real-time PCR to determine the level of expression of the *TLR2* gene in epithelial cells, during which the regularity that PB occurs with an increase in the level of TLR2 more than five times than the reference values was determined. The authors achieved high sensitivity (98.6%) and specificity (76.7%) because of using the TLR gene expression as a quantitative indicator, which determines the cascade of biochemical reactions mediated by increased synthesis of cytokines and, accordingly, prostaglandins, which cause myometrial hypertension [55].

From week 28 of gestation, the study of the endocervical epithelium helped determine the level of expression of the gene for the antimicrobial peptide β-defensin 1 using reverse transcription and real-time PCR. When its value is below 25.0×10^3 copies of complementary DNA relative to 10 copies of complementary DNA of β-actin, PB is predicted (sensitivity 86.7% and specificity 90.0%) [56].

The definition of fetal fibronectin is considered the most pathogenetically justified, since this protein of the extracellular matrix is involved in the process of attachment of the membranes to the decidual membrane of the uterus. In the case of the development of threatening PB, fibronectin penetrates into the cervical canal and vagina. The absence of fetal fibronectin in the vaginal contents at the gestational age of 22–35 weeks guarantees the absence of PB within the next 14 days with 99.2% probability [4].

Meanwhile, the results of a meta-analysis by Leitich et al. focused on the assessment of the role of fibronectin as a predictor of PB, indicating that the test sensitivity in the development of PB is 71% within the next 7–14 days and 59% within the next 21 days [57]. The results of the study by Leitich et al. are probably determined by the fact that fibronectin is found in 3%–4% of women at a gestational age of 24–26 weeks [3].

A fibronectin test can be false-positive in almost 50% of women due to vaginal examination, unprotected intercourse, vaginal bleeding, or amniotic fluid leaks on the eve of the study [2, 53]. Meanwhile, many researchers have concluded that this test cannot be used as a method for preclinical diagnostics of threatening PB, which significantly reduces the possibility of timely start of therapy aimed at prolonging pregnancy [2, 53, 55].

A study by Boots et al. reported a sufficient predictive value of the fibronectin test, with a sensitivity of 75%–76% and specificity of 79%–82% [58]. The latest research by Dawes et al. pointed out that a fibronectin level of 200 ng/mL or more should be an indication for hospitalization of a pregnant woman [53]. According to the authors, the hospitalization of patients with lower fibronectin levels does not improve perinatal outcomes in the long term [53].

An alternative method for determining the risk of PB is the test to detect highly phosphorylated insulin-like growth factor-binding protein-1 (IGFBP-1), which is a decidual membrane protein. When the chorion and membrane are separated, the decidual protein enters the cervical canal [4]. A negative result indicates a low risk of PB within 7 days after the test. The predictive value of a negative result is 94% [3]. At the same time, Ting et al. believe that the tests for IGFBP-1 and fibronectin are comparable with each other [59]. The main advantage of the IGFBP-1 test is the independence of the result from the presence of semen or traces of blood in the secretion [53]. The results of the comparison of IGFBP-1, fibronectin, and other biochemical markers of cervicovaginal secretions indicate that currently, there are no other significant methods for predicting PB than the test for IGFBP-1 and fibronectin [60].

Assessment of the composition of amniotic fluid as a predictor of PB. Assessment of the amniotic fluid composition, evidently, serves as a significant research method to effectively determine the probability of PB. This is because the amniotic fluid represents the environment of the fetus, contains products of its secretions, and refers directly to the uterine-placental complex. Thus, studies by Khodjaeva et al., who proposed the collection of amniotic fluid by transabdominal amniocentesis with subsequent use of hematological and bioche-

mical automatic analyzers, indicate that the number of lamellar bodies ($p = 0.048$) and neutrophils ($p = 0.048$) and the level of total protein ($p = 0.049$) differ statistically significantly between term delivery and PB [61]. Meanwhile, the amniocentesis procedure is a highly invasive research method capable of inducing the development of PB; therefore, the rationality of the widespread use of this method in patients at risk of spontaneous PB is debatable [2,9–11].

Thus, despite the established RFs and presence of various research methods, none of them has an absolute diagnostic or predictive value [4]. Some authors believe that fetal fibronectin, α-fetoprotein, C-reactive protein, and IL-6 have the highest predictive accuracy [2]. However, given the polyetiology of PB, this complex of studies cannot be considered universal; therefore, any single prediction algorithm cannot be preferred without considering the initial cause [53].

Conclusion. Despite numerous studies focused on investigating the possibilities of diagnostics and prediction of PB, there are currently no methods with an absolute diagnostic or predictive value. Most of the existing studies indicate the fact that in assessing the probability of PB, an integrated approach should be preferred, considering the results of several sensitive and specific methods.

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