Review

Recent Advances and Challenges in the Early Diagnosis and Treatment of Preterm Labor

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Recent Advances and Challenges in the Early Diagnosis and Treatment of Preterm Labor

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Abstract: Births occurring before thirty-seven weeks of gestation are referred to as preterm births (PTBs), which is the primary cause of neonatal mortality and long-term disabilities. The unknown mechanism behind PTB makes diagnosis difficult, yet early detection is necessary for controlling and averting related consequences. The primary focus of this work is to provide an overview of the known risk factors associated with preterm labor and the conventional and advanced procedures for early detection of PTB, including multi-omics and artificial intelligence (AI)/machine learning (ML)-based approaches. It also discusses the principles of detecting various proteomic biomarkers based on lateral flow immunoassay and microfluidic chips, along with the commercially available point-of-care testing (POCT) devices and associated challenges. After briefing the therapeutic and preventive measures of PTB, the review summarizes with an outlook.

Keywords: preterm birth (PTB); premature rupture of membrane (PROM); biomarkers; lateral flow immunoassay (LFIA) device; microfluidics; multi-omics

1. Introduction

Live births occurring before 37 weeks of pregnancy because of spontaneous or deliberate induction of labor are referred to as PTB. Preeclampsia, placenta previa, fetal growth retardation, etc., directly endanger the health of the mother or the fetus and are classified as indicated PTB.[1] The four categories of PTB include late (34–37 weeks), intermediate (32–34 weeks), extremely preterm (28–32 weeks), and extreme (<28 weeks) preterm births, depending on gestational age.[2] Premature delivery is the primary cause of death for children under five worldwide.[3] Necrotizing enterocolitis, respiratory distress syndrome, periventricular leukomalacia, seizures, intraventricular hemorrhage, cerebral palsy, hypoxic-ischemic encephalopathy, visual and hearing impairments, infections, feeding issues, and many other short- and long-term morbidities are more common in preterm neonates.[4] The global PTB rate increased from 9.8% to 10.6% between 2000 and 2014, with an expected 13.4 million cases (1 in 10 newborns) in 2020. In 2020, India accounted for 3.02 million PTB, or almost 23% of all PTB globally, the most significant number of preterm births worldwide (**Figure 1a)** and the fourth in PTB rate after Bangladesh, Malawi, and Pakistan (**Figure 1b**).[5] Preterm labor (PTL) is characterized by mild abdominal pains, back pain, regular uterine contractions, watery or bloody vaginal discharge, increased volume of discharge, and rupture of membranes with water leakage.[6] Various factors are responsible for PTL, such as infection (endotoxin), which accounts for 25–40% of cases,[7] inflammatory mediators (IL1β, TNFα),[8] vaginal bleeding (hemorrhage)[9], uterine overdistension, [10,11], excessive amniotic fluid volume (polyhydramnios),[12] stress,[13] immunological complications, and preterm PROM (PPROM). Other significant determinants include the mother's demographics, gestational age, nutritional condition, history of pregnancy, psychological traits, smoking, alcohol intake, and biological and genetic factors, although the exact mechanism underlying PTB remains unclear.[14-17] PTL can be diagnosed using a variety of techniques, including cervical examination (including cervical dilation and length assessment),

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amniotic fluid examination using a sterile speculum, qualitative or quantitative biomarker identification, etc. [6]

The earlier reviews mainly concentrated on establishing a relationship between PTB and adult mortality, [18] the impact of high temperatures during pregnancy,[19] other risk variables,[16,20] and inflammation [21,22]. The others focused on diverse topics, including the estimation of PTB at the national, regional, and global levels, [4] the conventional prognostic methods for PTB or PROM, and comparative analysis,[6,23,24] the clinical practice guidelines (CPG) for PTB management,[25] the impact of progesterone on PTB rate in asymptomatic singleton and twin-pregnant women,[26] or placental alpha microglobulin-1 (PAMG-1) in threatened preterm delivery (TPD) [27] and so on. However, none has systematically reviewed the guiding principles and evolving technologies for early detection of PTB and associated POCT devices available in the market. Following an overview of PTB and its current global status, the present review addresses the early diagnosis of PTB using traditional and advanced tenchiques. It then provides an impression of the POCT devices available for PTB detection, such as microfluidic chips and lateral flow immunoassay test kits. After a brief mention of therapeutic and preventive alternatives for PTB, the review ends with a summary and outlook.

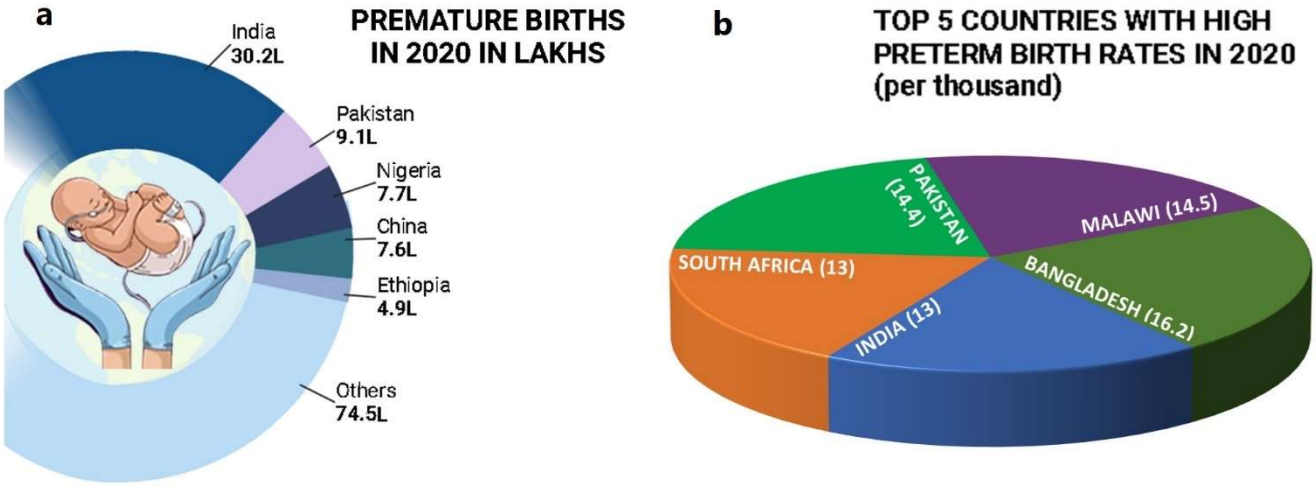

Figure 1. (a) in 2020, 13.4 million cases of preterm birth (PTB) around the globe, and India alone accounted for 3.02 million, the most significant number worldwide. (b) An estimate of PTB rate (per thousand births) in Bangladesh, Malawi, Pakistan, India, and South Africa. [5].

2. PTB/PTL risk prediction

PTL/PTB can be detected early using conventional and advanced procedures. The conventional techniques involve physical and chemical testing methods. In contrast, the advanced methods involve multi-omics studies for detecting biomarkers using a lateral flow or a microfluidic device and AI/ML-based procedures.

2.1. Physical testing methods

Physical testing includes the speculum examination, fern testing, and transvaginal ultrasound (TVUS) for cervical assessment. A speculum assessment ensures the rupture of amniotic membranes by observing the color and odor of pooled amniotic fluid.[28] The Ferning test indicates positive PROM when amniotic fluid produces a "ferning" or "fern-like" pattern on a slide due to crystallization.[23] TVUS, or endovaginal ultrasound, is used to examine parameters like cervical length (CL), cervical dilation, and uterocervical angle (UCA) in the female uterus, ovaries, cervix, and vagina. [29] For PTL prediction, the cut-off values used for UCA and CL are ≥110.97° and <3.4 cm, respectively, as listed in **Table 1**.[30]

2.2. Chemical testing method

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The chemical testing utilizes a nitrazine test for detecting the rupture of amniotic membranes by measuring changes in the pH of vaginal fluid from acidic (pH 3.8–4.5) to alkaline due to the mixing of amniotic fluid (pH ≥7.0), indicated by a change in nitrazine paper color from yellow to dark blue. However, there can be other reasons for an increase in vaginal fluid pH to alkaline, which may lead to false-positive results.[31]

2.3. Multi-omic biomarker studies

'Omics' refers to genomics, transcriptomics, proteomics, and metabolomics, frequently used to investigate disease biomarkers.[32] Multiple omic studies often predict PTB by finding molecules linked with various pathways (**Figure 2a**), as listed in **Table 2**. [33]

2.3.1. Genomic biomarkers:

Genomics study uses sequencing or microarray technology to analyze the gene expression level between gestational periods and sample types. Wnt signaling molecules, genes associated with inflammation and infection, such as EBF1, TIMP2, [34,35] COL4A3, [35,36] TNF, [37-40] and the candidate gene for schizophrenia, i.e., ABCA13, have been identified as PTB biomarkers through the multiple target studies, though their mechanisms remain unclear. [41-44]. Recently, microRNAs (miRNA) and their mature forms (miR) have been associated with PTB [45-47]. miRNAs are non-coding RNAs crucial in regulating gene expression [48]. For instance, TNF receptor genes (TNFR1 and TNFR2), TNFRSF6 gene, and gene variants of Toll-like receptors may be associated with an increased risk of PPROM and PTB [36,39,40,49-51]. According to Zhang et al., the genes like WNT4, AGTR2, RAP2C, EEFSEC, and AGTR2 are directly linked to gestational duration, while the genes EEFSEC, AGTR2, and EBF1 are associated with preterm delivery. [41]

2.3.2. Transcriptomic biomarkers:

Transcriptomics provides information on the abundance of multiple mRNA transcripts in a biological sample.[52] Five studies reported different miRNA levels in PTB [46,47,53-55]; for instance, miR-142 and five other miRNAs were identified for inducing shorter gestational duration.[47,55] miRNA transcripts related to EBF1 and MIR4266, MIR3612, MIR1251, and MIR601 are associated with spontaneous preterm birth (sPTB),[56] whereas Toll-like receptor (4TLR4) mRNA and interleukin-6 receptor (IL-6R) genes are associated with PTB. [57] [58]

2.3.3. Proteomic biomarkers:

Numerous protein biomarkers for PTB have been identified by extensive proteomics research. [59] Elevated lipocalin-type prostaglandin D2 synthase levels in cervicovaginal fluid (CVF) [58] or inflammatory interleukins (ILs) can cause PTB or PPROM by raising prostaglandin levels, which stimulate smooth muscle contraction in the uterus.[60-62] Numerous specific and nonspecific protein biomarkers for PTB have been identified in biological fluids, including amniotic fluid, vaginal secretions, urine, cervical mucus, plasma, and saliva.[63] Fetal fibronectin (fFN), placental alpha macroglobulin-1 (PAMG-1), and phosphorylated insulin-like growth factor binding protein-1 (PhIGFBP1) are considered to be the specific biomarkers for early prediction of PTB. There are also a few nonspecific biomarkers like ferritin, pregnancy-associated plasma protein-A (PAPP-A), urocortin-1, prolactin, matrix metalloproteases (MMPs), C-reactive protein (CRP), corticotrophin-releasing hormone (CRH), ILs, thrombin-antithrombin (TAT) complex, tumor necrosis factor-α (TNF-α), etc., which are used along with the specific biomarkers for the risk prediction of PTB.

fFN, the extracellular matrix (ECM) protein, is highly concentrated between the trophoblast and decidua, acting as a glue for holding the amniotic sac to the endometrium. PAMG-1 is a placental glycoprotein with a glue-like property similar to fFN. [64] fFN and PAMG-1 can be detected in CVF before 22 weeks of pregnancy and beyond 34 weeks till delivery, and its concentration is either undetectable or falls below 50 ng/mL during the intervening period. Therefore, the fFN level in the CVF is monitored to identify the risk of sPTB. If there are no indications of bleeding, cervicovaginal

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lesions, cervix dilatation <3 cm, or tears of the amniotic membrane, swabs should only be taken for a fFN test before any digital vaginal inspection.[65] The presence of fFN and PAMG-1 during 24 to 34 weeks indicates disruption of amniotic–endometrium interface, indicating a high risk of PROM. Chen et al. identified phIGFBP1 as a highly sensitive biomarker in the CVF for diagnosing PROM (choriodecidual disruption); a positive test indicates a 6.9-fold greater risk of PTB. [66] Usually, IGFBP1, or the non-phosphorylated form of PhIGFBP1, appears in amniotic fluid after 13 weeks of pregnancy, and the degree of phosphorylation persistently increases until the end of pregnancy.

The level of intracellular iron-storage protein ferritin ≥ 37.5 ng/mL during 24-37 weeks of gestation can be correlated with infection, inflammation, and limited expansion of maternal plasma volume, which are associated with an elevated risk of PTL. [67,68]. PAPP-A is a growth-promoting enzyme that catalyzes the insulin growth factor (IGF) released by insulin-like growth factor binding protein, facilitating endometrial invasion. It appears in circulation after blastocyst implantation. A low serum concentration of PAPP-A after 24 weeks of pregnancy is significantly correlated with placental dysfunction, stillbirth, PTB, intrauterine or fetal growth restriction, and fetal death. [69] The neuropeptide urocortin, produced by amnion and chorion, encourages prostaglandin-mediated delivery through uterine contraction. A high level of urocortin in the blood and amniotic fluid is associated with PTL, indicating that the peptide has a crucial role in the onset of the condition.[70] Prolactin, a lactation-related polypeptide hormone, is released by chorion, decidua, and amnion, reaching the maximum level (7 μg/mL) in amniotic fluid during the second trimester and stays elevated throughout pregnancy. Detection of prolactin in CVF during 24-36 weeks of pregnancy may indicate decidual membrane rupture, which can be used as a PTB biomarker.[71,72] MMPs are zinc-dependent proteolytic enzymes produced by the placenta and fetal membranes that break down collagen I and IX, an essential component of the fetal membrane, causing cervical dilatation and fetal membrane rupture.[73] CRP, a nonspecific marker of infection and inflammation secreted by hepatocytes rises in peripheral blood during amnionitis and intrauterine infection. The CRP level also rises in maternal serum during PTL; therefore, it can be a good predictor of PTB. [74,75] CRH, a placenta-derived hypothalamic peptide, is highly expressed in maternal and fetal plasma. It stimulates parturition through interaction with estrogen, adrenal hormones, prostaglandins, and oxytocin. Study shows that an elevated CRH level (>23.7pg/ml) can be associated with a high risk of PTB.[76] The precise role of inflammatory markers in PTB is unknown. The pro-inflammatory cytokines, especially IL-6, IL-1β, and TNF-α levels in the blood, can directly link with PTB. TNF-α is produced in maternal and fetal tissues, which regulate immune cells and induce apoptotic cell death[77,78]. TNF-α may serve as a biomarker for early prediction of PTL and PROM.[79-81] TAT complex is produced after thrombin deactivation as result of coagulation in response to uterine bleeding.[82] Elevated level of TAT is associated with high risk of PTB and can predict PTB with a sensitivity, specificity, PPV, NPV of 50, 91, 80, and 71%, respectively with a cut-off value of > 8ng/ml.[83] However, insufficient studies and inconsistent findings limit their clinical diagnostic application. Their diagnostic utility is also less due to their association with multiple diseases.

Table 1. Performance metrics of various PTL/PTB detection methods.

Sr. no	Markers	Sample	Period (weeks)	Detection limit	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Ref
I. Physical Method									
1.	Cervical length	NA	22–24	< 25 mm	47	89	37	93	[84]
2.	UCA	NA	18-36	≥111°	65.1	43.6	29.8	77.3	[30]
3.	Ferning test	NA	34-37	NA	84.5	78.2	79.5	83.5	[85]

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II. Chemical Method									
1.	Nitrazine Test	Amniotic fluid	28-36	NA	87.3	80.9	82.1	86.4	[85]
III. Biomarker-based method									
Specific Biomarkers									
1.	fFN	CVF	23-34	≥ 50 µg/mL	66.7	87.9	36.4	96.2	[86]
2.	PAMG-1	CVF	24-34	≥ 4 pg/ml	90.0	93.8	78.3	97.4	[87]
					66.7	98.6	75	97.9	[88]
3.	IGFBP-1	CVF	20-35	≥ 30 µg/ml	89.5	94.1	94.4	88.9	[89]
					83.3	84.4	41.7	97.4	[90]
					70	74	48	88	[91]
Nonspecific biomarkers									
1.	Ferritin	Serum		≥37.5 ng/ml	78.7	68.7	71.5	76.3	[68]
2.	CRP	Serum	≤20	≥5.27 mg/l	75	86.1	37.5	96.87	[74]
3.	Prolactin	CVF	24-36	>7 ng/mL	78	80	88.64	64.52	[72]
			20-40	9.5 ng/L	87.03	75	75.80	86.53	[71]
			28-36	30 ng/L	95	78	93	84	[92]
4.	Urocortin-1	Amniotic fluid	13-28	≥57.88 pg/mL	81.8	40.0	40	82	[93]
5.	CRH	Serum	24-36	10.45 pg/ml	80	100	100	55.56	[76]
6.	ACTH	Serum	24-36	14.65 pg/ml	80	100	100	55.56	[76]
7.	MMP-8	Amniotic fluid	20 to 36	>30 ng/mL	82.4	78.0	36.0	97.7	[94]

UCA: uterocervical angle; PAMG-1: Placental Alpha Macroglobulin-1; fFN: Fetal fibronectin; CVF: cervicovaginal fluid, CRH: corticotrophin-releasing hormone, CRP: C-reactive protein; MMP: Matrix Metalloprotease, ACTH: Adrenocorticotrophic hormone;

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2.3.4. Metabolomic biomarkers:

Metabolomics investigates the cellular metabolites associated with specific biological conditions.[95] The metabolite profile of blood, urine, amniotic fluid, CVF, etc., can be obtained by mass spectrometry analysis followed by nuclear magnetic resonance imaging. Study shows that elevated levels of metabolites such as glutamate, prostaglandins, dulcitol, urocanic acid, N-acetyl glutamine, 1-methyladenine, salicylamide, oleic acid, diglyceride, etc. [36,96-99] On the other hand, low levels of glutamine, pyruvate, inositol, alanine, pyroglutamic acid, glutamine, galactose, hexose cluster 3 and 5, inositol, urea, glycerophospholipids (phosphatidylcholines, phosphatidylinositol) and sphingolipids (ceramides), etc., were associated with PTB risks.

2.3.5. Multi-omic biomarkers:

Stelzer et al. conducted a longitudinal multi-omics study involving the metabolome, proteome, and immunome to find putative PTB biomarkers (**Table 2**). [100] Using a combination of genomics and proteomics, IL-6 polymorphisms and MMP-9 levels were successfully correlated with PTB. [101] Similarly, gene polymorphisms of TLR4 and TNF-α were linked with TLR4 mRNA level using a combination of transcriptomic and genomic studies, which established elevated TLR4 mRNA expression may serve as a possible biomarker for PTB. [57]

Table 2. Genomic, transcriptomic, proteomic and metabolomic biomarkers.

Sr. No.	Identified biomarkers	Phenotype	Ref.
Genomic biomarkers			
1	ABCA13	PTB	[44]
2	microRNAs (miRNA) and miR	PTB	[45-47]
3	TIMP2	Inflammation and infection	[34,35]
4	COL4A3	Inflammation and infection	[35,36]
5	TNF	Inflammation and infection	[37-40]
6	TNF1 and TNF2	PTB	[40], [49], [50]
7	TNFRSF6	PPROM	[39], [36]
8	Toll-like receptor	PPROM	[41]
Transcriptomic biomarkers			
9	miR-21, miR-142, miR-30e, miR-148b, miR-29b and miR-223	↓ Gestational period	[47]
10	MIR4266, MIR1251, MIR601 and MIR3612	↑ sPTB risk	[55]
11	LINC00870 and LINC00094	↑ PTB risk	[55]
12	TLR4	↑ PTB risk	[56], [57]
13	IL-6R		[58]
Proteomic biomarkers			
14	lipocalin-type prostaglandin D2 synthase	↑ PTB risk	[102]

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15	ILs	↑ PTB and PPROM risk	[60], [61]
Metabolomics biomarkers			
16	↑ Glutamate, dulcitol, urocanic acid, N-acetyl glutamine, 1-methyladenine, salicylamide, oleic acid, diglyceride	↑ PTB risk	[36,96,99]
	↓ Glutamine, pyruvate, inositol, alanine, pyroglutamic acid, glutamine, galactose, hexose cluster 5 and 3, inositol, urea, phosphatidylcholines, phosphatidylinositol, ceramides	↑ PTB risk	[36,96,99]
Multi-omics studies			
1	metabolomic (e.g., arabitol, xylitol, etc.), proteomic (e.g., VEGF 121, activin-A, MMPs, etc.), and immunome (e.g., CD56, INF-α, etc.) markers	combine metabolome, proteome, and immunome	[100]
2	IL-6 polymorphisms and MMP-9	combine genomics and proteomics	[101]
3	TLR4 and TNF-α genes with TLR4 mRNA level	combine transcriptomics and genetics	[57]
PTB: preterm birth; sPTB: spontaneous preterm birth; PPROM: preterm premature rupture of membrane; TNF: tissue necrosis factor; TLR-4: toll-like receptor 4; INF-α: Interferon α; VEGF 121: Vascular endothelial growth factor;			

2.4. AI/ML Methods

The current methods for predicting PTB are mainly based on hypothesis-based identification of the risks under a controlled set-up, which may include age at pregnancy, multiple gestations, smoking, drugs or alcohol consumption, infections, and chronic illnesses (diabetes, hypertension, obesity, anemia, asthma, and thyroid disease).[103] However, the results are often misleading, as PTB is also seen in first-time mothers or pregnant women without a known risk factor. Risk prediction can be improved by considering a combination of risks instead of a single one.

AI/ML utilizes the electronic health record (EHR) or pre-defined clinical risk factors for better predictive performance. Deep learning techniques can also be used to analyze high-dimensional EHR, MRI, and ultrasound data; nevertheless, their predictive efficiency is often limited to 59–75%. Most PTB prediction models are either ineffective at managing the sequential high-dimensional EHR data or lack an appropriate interpretation mechanism that allows them to pick the most significant predictors from the listed variables automatically. Medical data mining is essential for extracting relevant data on diagnosis, prognosis, monitoring, and public health management from a large data

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pool. It begins with data collection, then classifies them through algorithms and details the dataset (**Figure 2b**).[104] Description (clustering and association analysis) and prediction (classification and regression) are the two fundamental techniques of data mining used to link PTB with the related risk factors.

Medical data mining is operated in three stages.[105] In stage I, data collection is done through patient surveys, maternal and neonatal records, EHR, or literature surveys. The patient survey includes the background and medical history of the patient, previous and current pregnancy details, baby details, medical disorders in current pregnancy, etc. Stage II involves data discretization, where continuous data attributes are converted into a finite set of intervals with minimum data loss with the help of a minimum information loss (MIL) discretizer. During data pre-processing, the string, continuous, outliers, and missing data present in continuous attributes are first converted into discretized values (i.e., normalization) to improve the performance of classifiers. For example, a binary class dataset can be built by assigning the label "1" to the PTB occurring between the 28th and 37th weeks and the label "0" to the term birth (TB) after the 37th week. In other words, a large amount of data is reduced to a smaller size for easy data handling and makes data compatible with the ML algorithm. The binary class dataset is then tabulated in MS Excel and examined manually or using tools like the WEKA toolbox, Python, and Scikit-Learn library. The next crucial stage is feature selection, which selects the most relevant feature by eliminating extraneous elements from the input dataset to enhance the prediction model's performance. Three standard approaches, namely filter, wrapper, and embedded, are used for this purpose. The former method utilizes information gain, variance thresholds, the chi-square test, etc., to determine the significance of features much faster and more precisely. The wrapper method uses genetic algorithms, forward selection, backward elimination, etc., to find the optimal features. Though its accuracy is high, it takes longer data processing time. The embedding method combines the benefits of the filter and wrapper methods, involving lasso regression, ridge regression, elastic net, etc.; however, it exhibits medium-time complexity.

Finally, an ML-based prediction model is built to accurately predict PTB in stage III. ML is an automated method of data analysis for classification, prediction, and diagnosis of PTB using ML algorithms. Model fitting is performed to select the best classifier among the logistic regression, Naive Bayes, C4.5 decision trees, support vector machines, and neural networks. For instance, logistic regression estimates the probability of an event occurring based on a given dataset of dependent binary variables bounded between 0 and 1 against an independent variable. Support vector machine (SVM) uses a binary classifier and statistical learning theory to locate the most significant gap between two classes, thus simplifying complex and nonlinear patterns with increased classification accuracy and reduced generalization error. Different metrics are used to assess the performance of the classifier, including precision, recall, F1 score, false positive rate (FPR), false negative rate (FNR), apart from true positive rate (TPR), true negative rate (TNR), and correct classification rate (CCR) representing sensitivity, specificity, and accuracy, respectively.[106]

Several data mining, statistical analysis, and ML approaches are available for predicting PTB. For instance, Mercer et al. used a multivariate logistic regression technique to explore various risk factors using 23- and 24-week gestation data.[107] Goodwin et al. developed several rules for predicting PTB using ML and data mining.[108] Gao et al. used deep learning models for predicting PTB using existing electronic medical records (EMRs) of mothers available in healthcare centers.[109] Weber et al. used classifiers like K-nearest neighbors, lasso regression, and random forests for spontaneous preterm prediction while considering demographic, race-ethnicity, and maternal profile.[110] Mailath-Pokorny et al. used a multivariate logistic regression model considering features like maternal history, age at maternity, gestational age during admission, vaginal bleeding, cervical length, preterm history, and PPROM.[111] Son et al. attempted to find the best cut-off values of cervical length for PTB prediction in women with a singleton gestation.[112] Elaveyini et al. used artificial neural networks to predict PTB based on the feed-forward backpropagation algorithm.[113] On the other hand, Włodarczyk et al. employed ML algorithms based on a convolutional neural

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network to simultaneously segment and classify the cervix on transvaginal ultrasound images (**Figure 2c**).[114]

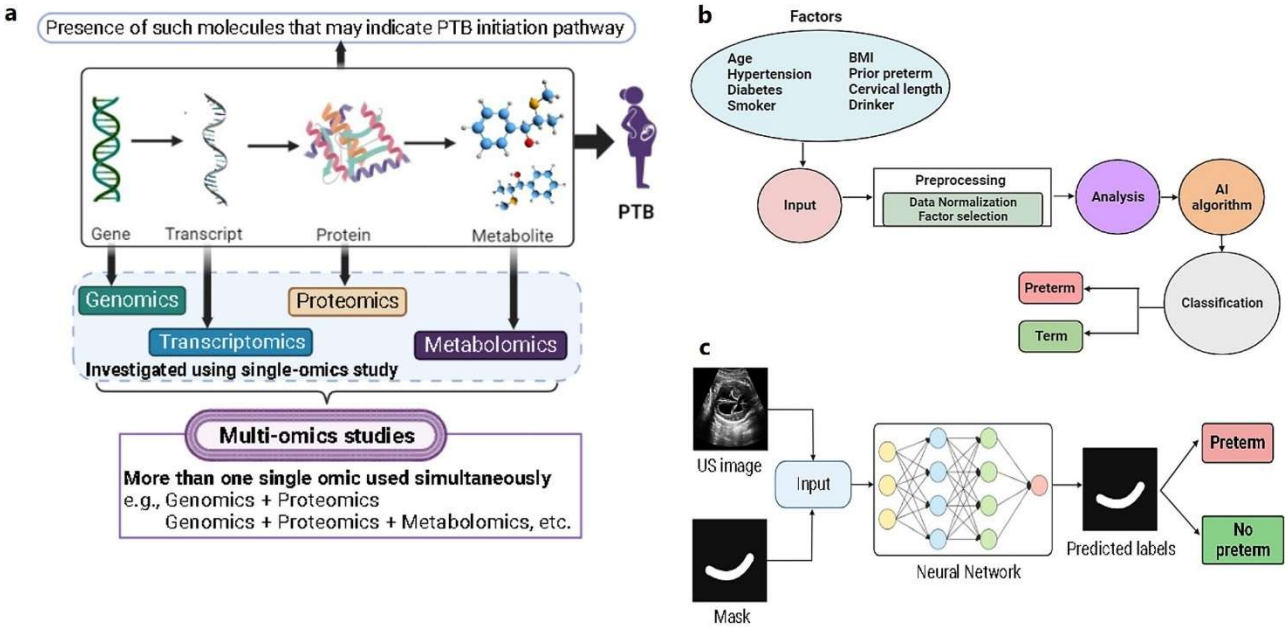

Figure 2. Multi-omics and AI/ML-based approaches for the early detection of PTB. (a) The diagram shows that single or multi-omic studies can identify PTB biomarkers containing genes, proteins, and metabolites. (b) The workflow shows different stages of AI/ML-based prediction of PTB, data input from electronic health records (EHR), pre-processing, and classification by machine learning (ML) algorithm. (c) PTB classification using transvaginal ultrasound image with the mask as an input for the neural network resulting in preterm or control as output. (inspired from [115]).

3. Principles of biomarker detection

Lateral flow and microfluidic techniques are used to detect different biomarkers.

3.1. Lateral flow immunoassay (LFIA):

LFIA, also known as immunochromatographic or rapid diagnostic test (RDT), is a paper-based analytical technique for the on-site detection of target analyte in a complex mixture within a few minutes after adding samples to the sample pad.[116] A variety of biological samples can be tested using LFAs, including blood, urine, saliva, sweat, serum, plasma, vaginal secretion, etc.[117] LFIAs fulfill all the criteria of an ideal POCT that must be "ASSURED" (affordable, sensitive, specific, user-friendly, rapid, robust, equipment-free, and delivered). First, the sample is added to the sample pad on a test strip. Due to capillary flow, it migrates to the conjugation pad immobilized with particulate label-conjugates, preferably with colored or fluorescent colloidal gold or paramagnetic nanoparticles (**Figure 3a**). The target analyte is captured as the sample-conjugate mixture migrates to the test membrane (porous nitrocellulose), where antigen, protein, antibody, aptamer, etc., are immobilized on test and control lines. Excess samples and reagents continue to flow and get entrapped in the absorbent pad. Positive results are indicated by a colored line on the conjugation pad, which can be interpreted visually or quantitatively by fluorescence-based optical test strip readers. [118]

3.2. Microfluidic devices:

Microfluidic devices are portable, affordable, fast, and sensitive point-of-care test (POCT) analytical systems frequently combined with a "lab-on-a-chip." They are made of microscopic channels that transfer fluids from µl to nl in volume. Preconcentration (electrokinetic manipulation, solid-phase extraction, and size-selective membranes), purification, and labeling are the basic steps for on-chip sample preparation. Polymethyl methacrylate (PMMA) and polydimethylsiloxane

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(PDMS) are the most widely used materials for fabricating microfluidic devices. Microchip electrophoresis (μCE) is a high-resolution technique that separates ions based on their electrophoretic mobility under an applied voltage.[119] Initially, ferritin, lactoferrin, defensin, TNF-α1, CRF, and other PTB biomarkers were isolated by liquid-liquid extraction and quantitatively estimated by LC/MS, allowing for PTB risk prediction with 87% selectivity and 81% specificity.[120] Alternatively, FITC-labeled preterm birth peptide 1 (P1) and ferritin were successfully analyzed using a miniature pressure-injected multilayer PDMS μCE device, yielding a peak height that was three times greater than the traditional electrokinetic injection. [121] A similar type of pressure-actuated, multichannel, multilayer integrated microfluidic device was created by integrating μCE with upstream SPE, allowing better resolution of peaks for ferritin and corticotropin-releasing factor in the electropherogram.[28] Using a 3D printed microfluidic device with a multiplexed immunoaffinity monolithic column containing specific monoclonal antibodies against each PTB biomarker under fluorescence imaging, three PTB biomarkers—CRF, TNF, and thrombin-antithrombin III (TAT) complex—were successfully eluted from depleted human blood serum (**Figure 3b**).[122] The FITC-labelled TAT complex combined with six other PTB risk biomarkers, including preterm birth peptides 1 and 2 (P1, P2), ferritin, lactoferrin, TNF, and CRF, could be successfully separated and analyzed using a similar "T-shaped" μCE device fitted with a fluorescence detector.[123]

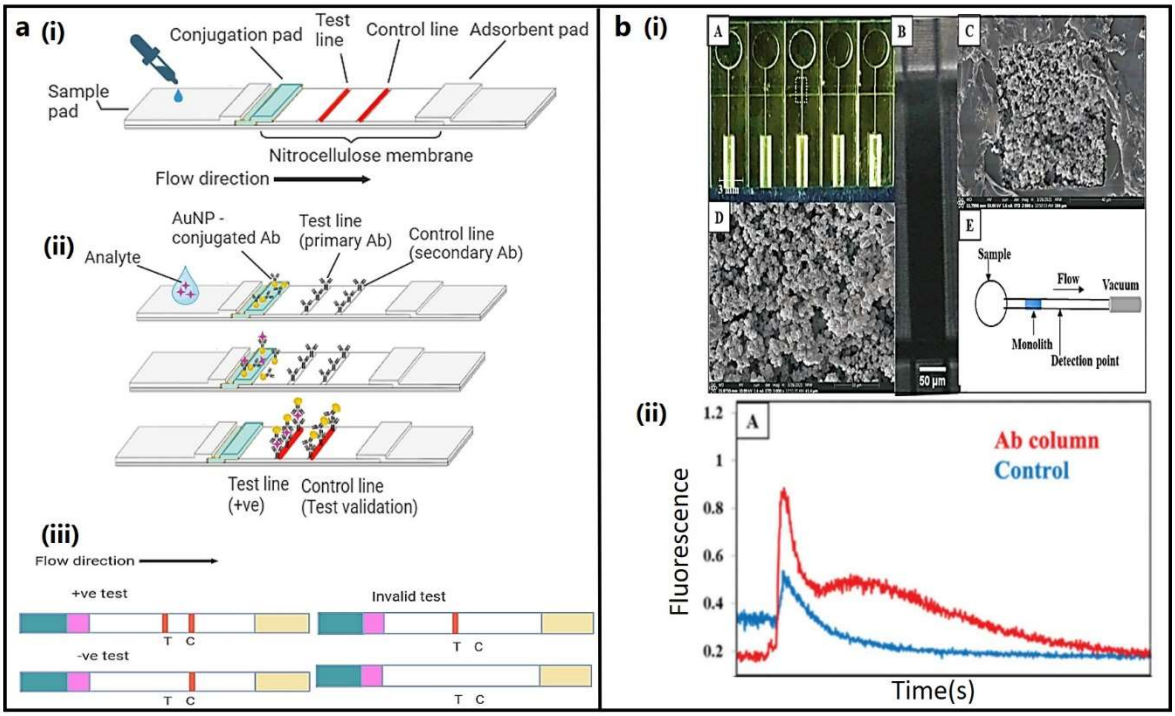

Figure 3. Principles of biomarker detection. (a) lateral flow immunoassay (LFIA) (i) test strip design (ii) representative images of various steps in a sandwich assay (iii) interpretation of test results; (b) 3D printed monolith devices for PTB biomarker extraction (i) 3D printed device (A), monolith within the channel (B), SEM images of monolith (C, D), and schematics of the device (E), (ii) elution of labeled biomarkers from monolith containing antibodies (Ab) against CRF, TNF, and TAT (Ab column) or monolith lacking Ab (control) producing red and blue fluorescence, respectively. [122] Copyright© 2022 Royal Society of Chemistry.

4. Point-of-care testing (POCT) devices

The market size for PTB diagnostic test kits is predicted to grow from an estimated $419 million in 2022 to $857 million by 2032. Hologic, Medixbiochemica, Qiagen N.V, Paraseng Diagnostics, Inc., Hangzhou AllTest Biotech, Biosynex, etc., are a few of the significant players in the PTB diagnostic market for detecting fFN, PAMG-1, and IGFBP-1, IL-6, etc., in CSV or urine samples; few of them are discussed in detail.

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4.1. PartoSure® test

The CE-approved PartoSure® test kit from Paraseng Diagnostics, Inc. (**Figure 4a**) uses goat anti-mouse monoclonal and anti-immunoglobulin antibodies at the test and control regions to detect PAMG-1 and IgG in CVF, respectively, with a limit of detection (LOD) of 1 ng/ml. CVF from the swab is rinsed using an extraction buffer, and the PartoSure test strip is dipped into it. This process transfers the sample through multiple test kit zones. The test and control lines appear a few minutes later. The sensitivity, specificity, and positive and negative predictive values of the PartoSure® kit recorded in different studies are summarized in **Table 3**.[124]

4.2. QuikCheck™ fFN

The Hologic QuikCheck™ fFN is another CE-approved qualitative fFN detection kit (**Figure 4b**) that uses a mouse monoclonal anti-fFN antibody and an extraction buffer containing gold conjugated-goat polyclonal anti-FN antibody. A swab containing CVF is extracted by the extraction buffer, where the test strip is dipped for 10 minutes for complete migration of the sample via capillary action. When fFN is present in the sample and successfully binds to the anti-hFN gold conjugate, the anti-fFN antibody develops a visible test line, indicating a positive test. The remaining unbound complex migrates further and binds with the immobilized plasma fibronectin, forming the control line. Consequently, a positive test will result in two visible lines, while a negative test will yield a single one. Quantitative fFN estimation can be performed within 20 minutes of development using the Rapid fFN TLi Analyzer (Hologic). The sensitivity, specificity, PPV, NPV, and accuracy of QuikCheck™ fFN were 94.5, 89.1, 89.7, 94.2, and 91.8 %, respectively (**Table 3**).[125]

4.3. HealthcheX® Foetal Fibronectin (fFN) Test

The HealthcheX® fFN quick test cassette from Hangzhou AllTest Biotech measures fFN in vaginal secretions against anti-fFN antibodies immobilized in the test region (**Figure 4c)**. The sample is mixed thoroughly with 80 µl of extraction buffer containing a 3:1 mixture of 2-methyl-2H-isothiazol-3-one and 5-chloro-2-methyl-4-isothiazolin-3-one. This mixture allows the sample to interact with gold-conjugated anti-fFN antibodies and migrate across the membrane through a capillary action. A colored band is formed in the "T" region of the specimen if the concentration of fFN is > 50 ng/ml (detectable limits), indicating a positive result with reported sensitivity, specificity, and overall accuracy of 98.1, 98.7, and 98.4%, respectively (**Table 3**).[126]

4.4. Human Fetal Fibronectin XpressCard

Antagen's Human Fetal Fibronectin XpressCard uses mouse anti-human fFN antibody immobilized on the T-test line to detect fFN in fresh morning urine based on the LIFA principle (**Figure 4d)**. Following 100 µL of sample addition to the sample well, the fFN in the sample binds with the gold-conjugated mouse anti-human fFN antibody, which further complexes with the anti-human fFN antibody immobilized on the test line. A colored band appearing on the test line indicates a positive test result. The reported sensitivity of the device is 10 ng/mL.[127]

4.5. Actim® Partus

Actim® Partus (Medix Biochemica, Finland) is another CE-marked dipstick test device for detecting phIGFBP-1 in cervical secretions for accurate sPTB prediction in symptomatic women. Two monoclonal antibodies against human IGFBP-1 are used in the dipstick; one is conjugated with blue latex particles, and the other is immobilized as a test line on the membrane. After inserting the dipstick into the extraction solution containing the speculum from the swab, the sample migrates down the strip via capillary flow, binds to the latex-conjugated anti-pIGFBP-1 antibody, and then bound with anti-pIGFBP-1 antibody in the test region. [128] A blue-colored band on the test line signifies a positive test result. In contrast, a blue band on the control line is formed by the free antigen-antibody complex binding with the immobilized antibody, indicating a valid test. [129] The device's

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claimed sensitivity, specificity, NPV, PPV, and diagnostic efficiency are 60, 67.7, 23, 91.3, and 66%, respectively, with LOD of 10 ng/ml LOD.[130]

4.6. Premaquick©

Premaquick® (Biosynex, France) enables a combined detection of IL-6 and native (non-fragmented) and total (fragmented and native) IGFBP-1. The presence of native IGFBP-1 in CVF signifies cervical ripening and the cervix's decidual cells lysis without ruptured membranes. In contrast, a fragmented form of IGFBP-1 indicates local proteolytic activity, leakage of amniotic fluid, and fetal stress due to contraction. IL-6 signifies infection or inflammation in the amniotic and cervicovaginal zones. Premaquick® offers a comprehensive method for detecting myometrium activity, cervical ripening, and inflammation/infection biomarkers for PTL risk assessment. The triple marker combination (IL-6/native phIGFBP-1/total IGFBP-1) provided an accurate prediction of sPTB in threatened singleton pregnancies with sensitivity, specificity, PPV, NPV, and accuracy of 95.1, 97.5, 97.5, 95.2, and 96.3%, respectively (**Table 3**).[131]

Table 3. Performance metrics of POCT devices for analyzing the PTL/PTB proteomic biomarkers.

Sr. no.	Device	Biomarker	Sample	LOD (ng/mL)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)	Ref.
1.	PartoSure® test	PAMG-1	CVS	1.0	80 (<7 d)	95	96	76	-	[124]
					63 (<14 d)	96	89	91	-	
2.	Quikcheck fFN test	fFN	CVS	≥ 50	94.5	89.1	89.7	94.2	91.8	[85]
3.	healthcheX fFN test	fFN	CVS	>50	98.1	98.7	-	-	98.4	[126]
4.	Antagen fFN XpressCard	fFN	Urine	10	-	-	-	-	-	[127]
5.	Actim® Partus	ph IGFBP-1	CVS	10	60	67.7	23	91.3	66	[130]
					95	92	86	97	-	[132]
					80	94	57	98	-	[133]
6.	Premaquick©	IL-6/ phIGFBP-1/ IGFBP-1	CVS	-	95.1	97.5	97.5	95.2	96.3	[131]

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Abbreviations: CVS: cervicovaginal secretion; PAMG-1: Placental alpha macroglobulin-1; fFN: fetal fibronectin; ph IGFBP-1: phosphorylated Insulin like growth factor binding protein-1; PPV: positive predictive value; NPV: negative predictive value

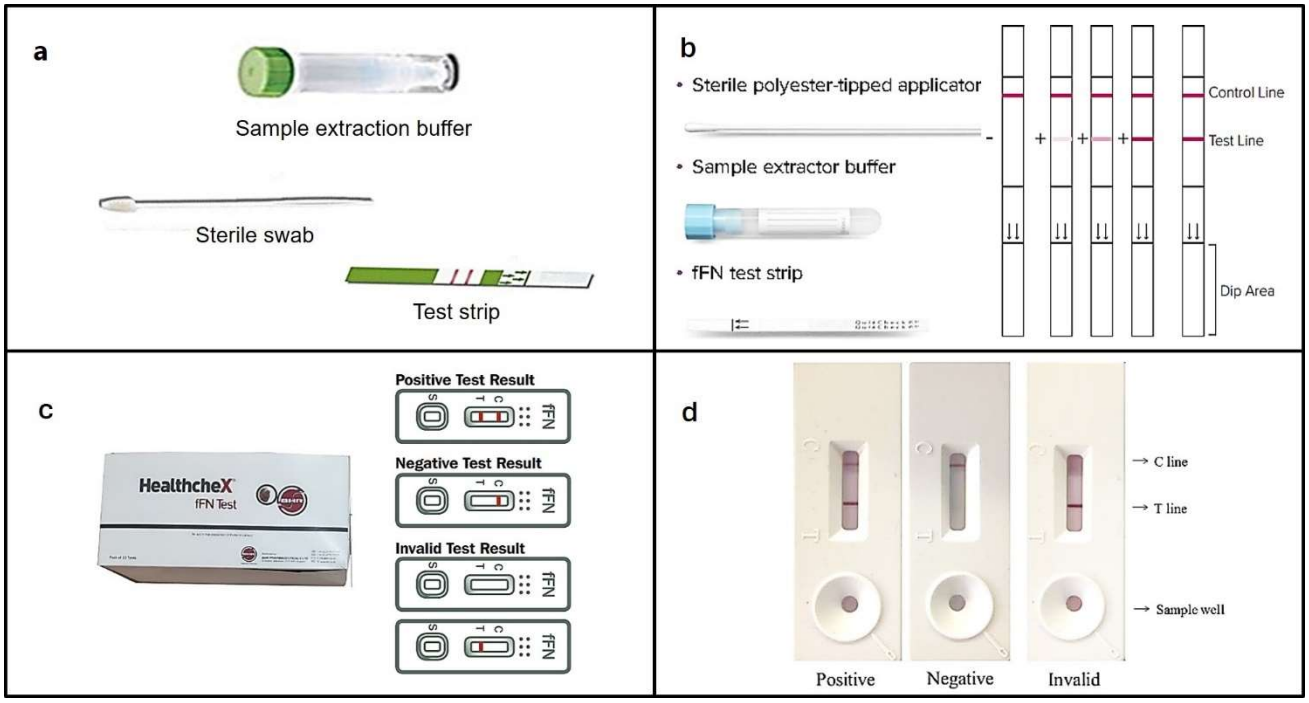

Figure 4. Marketed point-of-care testing (POCT) devices (a) PartoSure® [134] for the detection of PAMG-1, whereas (b) Hologics® QuikCheck fFN test [135] (c) HealthcheX® fFN test [126] and (d) Antagen® fFN Xpresscard [127] are used for the of detection of fetal fibronectin (fFN).

5. Challenges

The early prediction of the sPTB is the major challenge that can help to reduce miscarriage cases and the associated PTB complications later in life for premature babies. Half of the women with PTB do not show known clinical risk factors. The current PTB detection method, such as TVUS examination, nitrazine test, etc., fails to detect PTB accurately, which makes PTB a complicated and life-threatening condition for both the mother and fetus. The TSUV often uses a cut-off value of ≤ 25 mm for CL measurements, but women with a CL of 25–30 mm are still at risk of giving birth within seven days. In that case, combining CL assessment with quantitative fFN testing may improve the predictive capacity.[136]

The usage of diagnostic markers has several drawbacks, one of which is the potential for false negative diagnoses, which can increase the fetus's risk of morbidity and death. The fFN test is frequently interfered with by vaginal bleeding, and it is also inappropriate to screen asymptomatic or multiparous women [137]. Likewise, the cervical phIGFBP-1 test has poor predictive accuracy in asymptomatic patients, and it is frequently impacted by vaginal bleeding, antibiotic use, and sexual activity [138].

5. Treatment and preventive measures

Once detected early, PTL can be treated with medications like tocolytic agents or labor suppressant drugs, which delay the labor by 24-48 hours by suppressing uterine muscle contractions or relaxing the smooth muscles.[139] Tocolytic agents can be classified as β-agonists, calcium channel blockers, nonsteroidal anti-inflammatory drugs (NSAIDs), oxytocin antagonists, etc.[140] Magnesium sulfate has been used for decades in treating PTL as a uterine smooth muscle relaxant

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due to inhibition of the myosin light chain. β-agonists (e.g., terbutaline, Isoxsuprine) relax uterine muscles through the cAMP/myosin light-chain kinase pathway, whereas calcium channel blockers (e.g., nifedipine) act by blocking L-type channels. NSAIDs (e.g., indomethacin) inhibit the synthesis of prostaglandin, an inflammatory mediator required to initiate parturition. Oxytocin antagonists (e.g., atosiban) act by competitively blocking oxytocin receptors on the myometrial plasma membrane and thus prevent oxytocin-mediated uterine contraction. Cervical cerclage is a surgical procedure in which a suture is placed around the cervix under ultrasound to prevent its shortening and widening.[141] [142] These drugs are contraindicated in the mother and fetus during severe preeclampsia, hemorrhage, and significant cardiac disease, as they may create a greater risk for maternal hemodynamic compromise and collapse. Fetal contraindications include gestational age ≥34 weeks, lethal fetal anomalies, intrauterine fetal demise, chorioamnionitis, and required immediate delivery.[143] Some antenatal corticosteroids, such as betamethasone and dexamethasone, were prescribed to women with a high risk of delivery within two weeks to accelerate fetal lung development and prevention of perinatal complications, such as respiratory distress syndrome, intraventricular hemorrhage, and necrotizing enterocolitis.[144]

In patients at increased risk beginning in the second trimester of pregnancy and not in severe preterm labor, progesterone supplements can also be utilized as preventive treatments for PTB, although the exact mechanism is unclear. After applying vaginal progesterone gel, a 45% decrease in sPTB rate before 33 weeks and better neonatal outcomes were observed in women with a mid-trimester short cervical length.[145] Based on the available data, two mechanisms have been proposed: 1) an increase in the local progesterone level in gestational tissues that counteracts the functional reduction in progesterone, and 2) an anti-inflammatory effect that reduces intrauterine inflammations associated with PTB/PTL. [143] Progesterone supplements don't show any significant adverse effects.

6. Summary and outlook

PTB of babies before 37 weeks of pregnancy is one of the leading causes of neonatal mortality and morbidity. The review summarises the current status of PTB, including epidemiology, etiology, complications, and existing and novel diagnostic methods, including microfluidics, multi-omics, and AI/ML-based detection.

The patient's symptoms (PROM, cervical incompetence, polyhydramnios, infections), clinical findings, and cervical dilation are typically used for PTL diagnosis. Therefore, the risk of PTL/PTB is primarily diagnosed by symptomatic observation, speculum examination, TSUV, ferning test, etc., which are time-consuming and often produce deceptive results. A more accurate prediction can be made using more sensitive techniques, such as multi-omics investigations for biomarker identification and their quantitative detection utilizing an LFIA kit or a microfluidic microchip and AI/ML-based risk evaluation. fFN exhibited the highest ability among predictive biomarkers, followed by alpha-fetoprotein and CRP, whereas IL-6 levels in maternal plasma and CSV showed good diagnostic performance among cytokines. Even though a single biomarker might not help with the early prediction of PTB, a combination of biomarkers can improve the prediction accuracy, for instance, Premaquick©. Nevertheless, CL evaluation appears to be a more practical method for predicting PTB in standard clinical practice, even though biomarkers for PTL may offer a more accurate diagnosis.

There can be challenges and limitations of biomarker-based detection. It is necessary to design suitable strategies for avoiding possible interferences and low predictive accuracy in asymptomatic women, especially false negative diagnoses. For treatment of PTL, cervical cerclage may be helpful; antibiotics may work in case of known etiology, such as PPROM, urinary infection, prostaglandin synthetase inhibitors (e.g., indomethacin), and tocolytic agents may be recommended for a few days to prevent uterine contractions. However, long-term use may produce harmful effects on the fetus. Progesterone supplementation may reduce the risk of PTL/PTB and newborn delivery by relaxing the uterine muscle, raising progesterone levels locally, and decreasing inflammatory activity.

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