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Biomarkers of spontaneous preterm birth

A systematic review of studies using multiplex analysis

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Biomarkers of spontaneous preterm birth: a systematic review of studies using multiplex analysis

Abstract

Objective: Despite decades of research on risk indicators of spontaneous preterm birth (PTB), reliable biomarkers are still not available to screen or diagnose high-risk pregnancies. Several biomarkers in maternal and fetal compartments have been mechanistically linked to PTB, but none of them are reliable predictors of pregnancy outcome. This systematic review was conducted to synthesize the knowledge on PTB biomarkers identified using multiplex analysis.
Materials and methods: Three electronic databases (PubMed, EMBASE and Web of Science) were searched for studies in any language reporting the use of multiplex assays for maternal biomarkers associated with PTB published from January 2005 to March 2014.

Results: Retrieved citations (3631) were screened, and relevant studies (33) were selected for full-text reading. Ten studies were included in the review. Forty-two PTB-related proteins were reported, and RANTES and IL-10 (three studies) followed by MIP-1β, GM-CSF, Eotaxin, and TNF-RI (two studies) were reported more than once in maternal serum. However, results could not be combined due to heterogeneity in type of sample, study population, assay, and analysis methods.
Conclusion: By this systematic review, we conclude that multiplex assays are a potential technological advancement for identifying biomarkers of PTB, although no single or combination of biomarkers could be identified to predict PTB risk.

Keywords: Biological markers; cytokines; multiplex assay; prematurity.

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Introduction

Preterm birth (PTB; birth before 37 weeks' gestation) is the leading cause of neonatal morbidity and mortality worldwide [1–5], yet little is known about its underlying etiology. PTB is a complex syndrome with multifactorial etiologies that can be broadly classified into two groups: (1) spontaneous PTB, which accounts for approximately 70% of the preterm deliveries, and is further referred to, in this review, as PTB; and (2) provider-initiated PTB, also named iatrogenic PTB, which accounts for the remaining 25% of preterm deliveries. The pathways leading to PTB are redundant and overlapping between etiologies; thus, understanding PTB initiators and effectors is difficult, precluding their use as risk predictors for this condition [6, 7]. Development of new technologies and methodological approaches over the past decade has provided an impetus in the search for predictive and diagnostic PTB biomarkers; many of these biomarkers have been identified in maternal-fetal compartments, including maternal blood, amniotic fluid, and cervicovaginal fluids. Still, there is no clear consensus on which biomarker(s) should be considered the best predictor(s) of PTB. The Preterm Birth International Collaborative (PREBIC; www.prebic.org) performed a systematic review of the literature on single maternal biomarkers for PTB [8]. The report identified 116 different biomarkers in 217 studies published in the last four decades. Three other reviews on PTB biomarkers reached similar conclusions, supporting the concept that there are no single biomarkers able to reliably predict PTB [9–11]. As multiple and intricate pathways result in PTB, it is unlikely that a single biomarker will predict the risk of PTB.

These findings led to additional work by the PREBIC Biomarker Working Group, and a second systematic review examined panels of biomarkers identified through proteomic technologies [12], specifically through protein electrophoresis and mass spectrometry [matrix-assisted laser desorption/ionization (MALDI) or surface-enhanced laser desorption/ionization (SELDI)] protein profiling. From a total of 64 dysregulated proteins identified, none of them were reproducible or capable of predicting PTB. As observed in single-biomarker studies, the use of technologically different assay platforms and analytical approaches contributed to a lack of reproducible data for identifying reliable PTB biomarkers, as did heterogeneity in study design, sampling, assay methods, and analysis. However, as an unbiased approach, a well-designed proteomic study is still promising to yield novel biomarkers.

Technological advancements in the past decade have further modernized biomarker discovery. Multiplexed assays and quantitative protein microarrays have led to the publication of a large number of biomarker studies related to PTB. Advantages of multiplex technology include simultaneous analysis of a broad panel of biomarkers chosen by investigators based on known biomolecular pathways of preterm labor, use of a small sample volume, cost effectiveness, and minimal technological challenges compared to proteomics [13]. Several reports have been published in the past decade using multiplex platforms to report different PTB-related end phenotypes including intra-amniotic infection and inflammation, histological chorioamnionitis, and early onset neonatal sepsis in PTB [14–20]. Samples tested included maternal blood, amniotic and cervicovaginal fluid, and umbilical cord blood.

PREBIC conducted this third systematic review to synthesize the knowledge on PTB biomarkers identified using multiplex approaches. We reviewed the literature published in the last decade on studies using multiplex technologies in identification of PTB biomarkers (search limited to biomarkers of spontaneous PTB only) in various biological samples collected from asymptomatic and symptomatic women with PTB and intact membranes.

Methods

Study identification and selection

We sought studies that used multiplex assay techniques to document potential biomarkers associated with PTB published between January 2005 and March 2014. The search was conducted without language restriction in three electronic databases (PubMed, EMBASE and Web of Science) via OVID. All citations identified were downloaded into Mendeley electronic reference software (Mendeley Ltd., London, UK), and duplicates were excluded. Two independent reviewers screened the titles and abstracts of unique citations, and potentially relevant studies were selected for full-text reading; those that fulfilled the selection criteria were included in the review. Discrepancies between the reviewers were discussed until consensus was reached; in case of persistent disagreement, a third reviewer was consulted.

The search was complemented by screening the reference lists of included studies. A search for unpublished studies was not performed. The search strategy used the following terms and synonyms, adapted according to each database: (*preterm birth OR preterm labor OR preterm delivery*) AND (*Multiplex OR protein array OR immunoassay OR antibody array OR antibody microarray OR protein microarray OR biochip OR array OR microarray OR quantibody array OR multi analyte detection OR multi analyte profiling OR protein profiling OR biochip immunoassay OR Luminex OR xMAP OR ELISA array OR protein macroarray*).

Type of study

Studies that used any multiplex assay in search of multiple biomarkers for PTB and provided qualitative and/or quantitative information about biomarkers were considered eligible for inclusion. All types of study designs were considered acceptable, including cross-sectional, case-control, case-only, and cohort studies.

Population

Studies enrolling symptomatic or asymptomatic women at high or low risk for PTB of any gestational age <37 weeks, parity, race, age, or socioeconomic background were eligible for inclusion in the review.

Definition of multiplex assay

Multiplex assay was defined for the purpose of this review as simultaneous detection of more than one analyte in a single biological reaction using a combination of specific antibodies with no documented cross-reactivity but compatible in combined assays.

Types of outcome

The target outcome was spontaneous preterm labor with intact membranes leading to preterm delivery. The comparison groups included either term labor or preterm-matched gestational age.

Exclusion criteria

Studies were excluded if any of the following applied: (1) pregnancies with other associated complications, such as preeclampsia, intrauterine growth restriction, stillbirth, miscarriage, diabetes, abruptio placentae, or preterm premature rupture of membranes; (2) infection or chorioamnionitis associated with PTB; (3) animal-model studies; (4) genetic syndromes or diseases; (5) *in vitro* studies; (6) gene polymorphisms studies; (7) microbial detection as main objective of the study (microbiologic studies); (8) clinical trials, and (9) multiple pregnancies.

Data extraction

Two independent reviewers extracted data from the eligible studies using a standardized electronic data extraction form prepared for this review. The following data were collected: author; journal; year of publication; country where the study was conducted; study objective and design; total number of participants; number of cases and controls; patient characteristics at baseline including age, race, clinical symptoms, and risk of PTB; outcome phenotype definition; antenatal steroid administration; gestational age at the time of sample collection; source of biological sample; type of multiplex assay; method of validation of the results; and data analysis approach.

Additional relevant assay-related information, such as processing, storage, and dilution of samples, was also collected, if available. The data collected were compared and discussed by the reviewers, and disagreements were resolved by consensus.

Assessment of the quality of the studies

Two independent reviewers assessed the quality of the included studies according to a set of parameters defined for this review using the QUADAS tool [21] and based on our group's previous experience with this type of study [8, 12]. Differences in opinions between reviewers were resolved by consensus; in case of persistent disagreement, a third senior reviewer was consulted.

Grading criteria were as follows:

1. Grading of population characteristics

   Well defined Detailed description of study group(s) (cases, controls), ethics and consent procedure, and inclusion and exclusion criteria. Inclusion criteria should have the definition of phenotype, estimation of gestational age, and gestational age at recruitment and at sample collection. Exclusion criteria should be explained in detail.

   Adequately defined Brief description of study groups (cases, controls). Inclusion criteria should have the definition of phenotype, information as to how gestational age was assessed, and gestational age at recruitment and at sample collection. Exclusion criteria should be explained in detail.

   Poorly described or not reported Difficult to understand study design, study groups, or inclusion and exclusion criteria, and does not provide details listed above.

2. Grading quality of biomarker sampling

   Well defined Detailed description of the sample type, sample collection protocol, timing of sample collection (gestational age), clinical status at sampling (symptomatic vs. asymptomatic), pre-analytical processing of the sample, and storage conditions.

   Adequately defined Brief description of the sample type, sample collection, and pre-analytical processing of the sample, but some aspects are missing or unclear.

   Poorly described or not reported Most of the details are missing and/or difficult to understand as to how the sample collection and pre-analytical processing of the sample were performed, mainly regarding storage and dilution.

3. Grading quality of multiplex analysis

   Well defined Detailed description of sample processing and assay. Details included multiplex assay platform and its manufacturer, number of analytes, source of antibodies and their cross-reactivity and compatibility, lower and upper levels of detection, standards used and methods for calculation of observed concentrations, details on assay performance (intra-assay and inter-assay variabilities), and statistical analysis approaches, including control for multiple comparisons.

   Adequately defined Brief description of the sample processing, multiplex assay, and analytical details as described above.

   Poorly described or not reported Most of the details are missing and/or it is difficult to understand how the samples were prepared and proteomic analyses were performed.

Data synthesis

Biomarkers were grouped according to the name of the identified protein of interest retrieved from the UniProt database, type of sample, and multiplex approach. We planned to pool the results of similar studies in a meta-analysis to assess the results of different multiplex analyses in biological samples from women with and without PTB.

Results

The search of three electronic databases yielded 3631 unique citations, 3598 of which were excluded as irrelevant after title and abstract screening; 33 were selected for full-text reading. From those selected, 22 studies [19, 22–42] that did not fulfill the selection criteria and one study [26] for which the full text could not be obtained after repeated attempts was excluded. Ten studies that used multiplex assay for identification of PTB biomarkers in maternal and/or fetal samples were included in the review. A process flowchart of study identification, selection, and inclusion is presented in Figure 1.

Main characteristics of the ten studies included in the present review are summarized in Table 1. These studies were conducted in seven developed countries and published between 2006 and 2013 in North American or European journals of obstetrics and gynecology or immunobiology, reflecting both clinical and basic science

readership. There were four cohort studies [15, 20, 44, 46], five case-controls [14, 16, 17, 43, 45], and one cross-sectional [18]. Although race/ethnicity plays a major role in biomarker disparity associated with PTB, only two studies reported on the ethnicity of the studied populations [17, 20]. Gestational age (GA) at sample collection, an important factor influencing biomarker association with PTB, was reported in all studies except one [43]; however, the missing information was obtained for this study through personal communication with the authors (Table 1). All studies reported a calculation of GA based on the last menstrual period corroborated by ultrasound dating in the second trimester. In nine out of the ten studies, the samples were collected from patients with symptomatic preterm labor, while in the remaining study, the samples were collected from asymptomatic pregnant women [45].

Biological samples

Fourteen sets of biological samples were analyzed. Three studies analyzed more than one biological sample [14, 17, 20]. The majority of the samples were maternal blood (57.1%), either plasma or serum, followed by amniotic fluid (14.1%), fetal plasma, placenta, choriondecidua, and cervical fluid (7.2% each). Samples were collected during labor (69.2%), after delivery (18.1%), and, in one prospective cohort, between 24 and 26 weeks of gestation before the
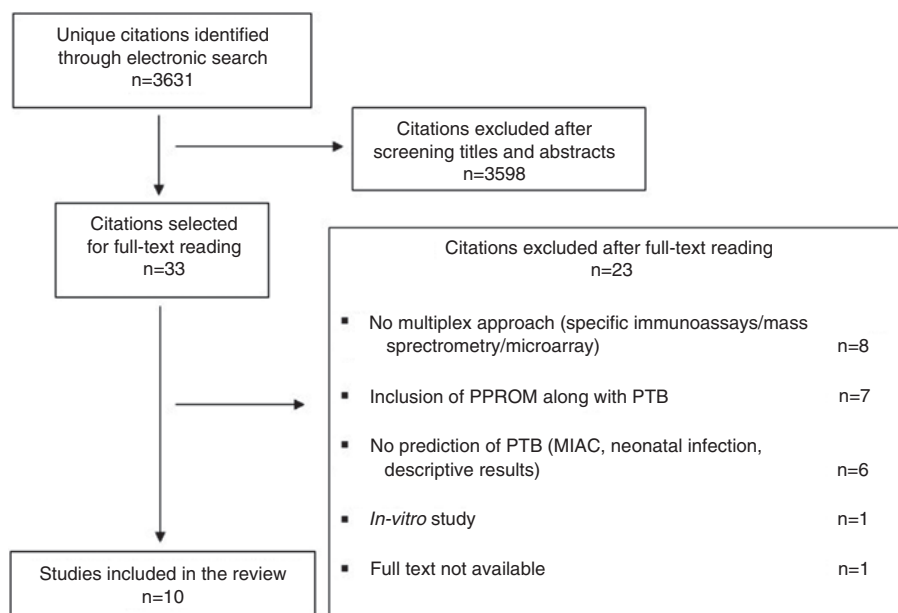

Figure 1: Flowchart of identification and selection of studies on preterm labor-related biomarkers using a multiplex protein assay. (PTB=Preterm birth, MIAC=microbial invasion of the amniotic cavity, defined as positive PCR and/or a positive amniotic fluid culture; PPROM=preterm premature rupture of membranes).

Table 1: Main characteristics of ten studies included in the review.

Author, country	Maternal age in years	Ethnicity	Participant characteristics	Study design/ number of samples analyzed	Biological sample	Time of sampling collection [weeks (w), days (d)]	Approach	Number of biomarkers analyzed	PTL-related biomarker
Hamilton et al. [14], UK	NR	NR	TNL TL Idiopathic PTL PTLI PTNL	Case-control TNL (n=14) TL (n=14) PTL (n=10) PTLI (n=10) PTNL (maternal plasma only) (n=10)	Maternal plasma Choriodecidual tissue	Term (37–42 weeks) Preterm labor (24–35 weeks) Within 30 min of delivery	Bioplex (BioRad®)	07	Increased CXCL8 (vs. TL) Increased CCL5 (vs. PTNL) Decreased CCL2 and CXCL10 (vs. TL) Decreased CCL5 (vs. TL)
Tency et al. [16], Belgium	Cases: 29 (25–33) Controls: 30 (27–33) Median (interquartile range)	NR	PTB<34 weeks (n=47): PTL (n=15) PPROM (n=32) Gestational age matched controls not in PTL TL TNL	Case-control PTB (n=34) Gestational age matched controls (n=34) TL (n=27) TNL (n=21)	Maternal serum	At term (37–42 weeks); PTB (24–34 weeks) Gestational age matched controls (24–34 weeks)	Human Matrix Metalloproteinases 3-Plex Panel (Invitrogen®) Human TIMP Multiplex Kit (R&D systems®)	07	No changes in MMP-3, MMP-9 or TIMP-1, 2, 3 or 4 (vs. PPROM)
Tsiartas et al. [15], Sweden	Cases: 30.5 (28–33.5) Controls: 29 (24–33) Median (25th–75th percentiles)	NR	PTL with intact membranes delivered spontaneously within 7 days (n=57) PTL with intact membranes not delivered within 7 days (n=85)	Cohort Threatened PTL (n=142)	Maternal serum	PTL with intact membranes delivered spontaneously within 7 days 30 weeks (22 weeks 3 days–33 weeks 5 days) PTL with intact membranes not delivered within 7 days 30 weeks 5 days (22 weeks 2 days–33 weeks 5 days) Median (min–max)	Luminex xMAP Technology	27	Increased IL-10, sIL-6Rα, TNF-β, MIP-1β, MMP-9, BDNF, sTNF-RI, GM-CSF, MIF and RANTES
Brou et al. [17], USA	Cases: 27 (10) Controls: 28 (11) Median (interquartile range)	Cases: African-American (n=59) European-American (n=46) Controls: African-American (n=40) European-American (n=46)	Spontaneous PTB (n=105) Term delivery (n=86)	Case-control PTB (n=88) TL (n=77) Case-control PTB (n=90) Control (TL) (n=85) Case-control PTB (n=90) Control (TL) (n=80)	Amniotic fluid Fetal plasma Maternal plasma	At term (37 weeks 0/7 days–42 weeks 0/7 days) PTB (24 weeks 0/7 days–36 weeks 0/7 days)	FAST Slide protein microarray platform (Whatman laboratories®)	44	Increased ANGPT2 (vs. TL) Increased ICAM-1, IGF-1, IL-1β, IL-1Rα, IL-8, MCP-3, MIP-1α, PDGF-BB, TGF-α, TGF-β1, TIMP1, TNF-α, TNF-RI, TNF-RII and VEGF (vs. TL) Increased ANG, Eotaxin, ICAM-1, IL-1β, TNF-RI (vs. TL) Decreased ANGPT2, Fas-ligand, FGF basic, IGF-1, IL-10, IL-13 (vs. TL)

Table 1 (continued)

Author, country	Maternal age in years	Ethnicity	Participant characteristics	Study design/ number of samples analyzed	Biological sample	Time of sampling collection [weeks (w), days (d)]	Approach	Number of biomarkers analyzed	PTL-related biomarker
Laudanski et al. [43], Poland	NR	NR	PTL at 26–36 weeks and PTB (n=51) PTL at 26–36 weeks but term birth (n=23)	Case-control PTL (n=51) False PTL (n=23)	Maternal serum	PTL delivered PTB or at term (26–36 weeks) Term>37 weeks[a]	Raybiotech quantibody	13	Increased IGFBP-1, BDNF, BLC, Eotaxin-1 (vs. false PTL)
Faupel-Badger et al., [44], USA	NR	NR	Severe preeclampsia (n=9) PPROM (n=10) PTL with intact membranes (n=11)	Cohort PE (n=09) PTL (n=11) PPROM (n=10)	Placenta	Within 20 min of delivery (23 weeks 0/7 days–27 weeks 6/7 days)	Meso Scale Discovery (MSD)	15	No changes in inflammatory profile (vs. PPROM)
Kramer et al. [45], Canada	Cases: <20: 1.9% 20–34: 83.1% ≥35: 15.0% Controls: <20: 2.7% 20–34: 78.4% ≥35: 18.9%	NR	Low-risk pregnancy Term (n=441) PTB (n=207)	Case control PTL (n=81) PPROM (n=186) Term (n=441)	Maternal plasma	Prior to labor onset (24–26 weeks)	Luminex xMAP Technology	27	No changes (vs. term)
Holst et al. [20], Sweden	Cases: 29 (19–36) Controls: 29 (17–44) Median (min–max)	White (83%); Asian (10%); Hispanic (4%); Black African (2%)	PTL (n=89)	Cohort PTL (n=89)	Amniotic fluid Cervical fluid	(22 weeks 0/7 days–33 weeks 6/7 days)	Luminex xMAP Technology	27	Increased IL-1β, IL-6, IL-10, IL-17, IL-18, TNF-α, MCP-1, MIP-1α, MIP-1β, GM-CSF, RANTES Increased IL-1β, IL-4, IL-5, IL-6, IL-10, IL-17, sIL-6Rα, IFN-γ, TNF-α, MCP-1, MIP-1α, MIP-1β, TREM-1, BDNF, GM-CSF, neutrophin-4, neutrophin-3, MIF-1, RANTES
Ekelund et al., [46], Denmark	18–43 Median (min–max)	NR	Women with symptoms of threatening PTD (n=93): PTL (n=46) PPROM (n=35) Cervical ripening (n=12)	Cohort PTL (n=7) PPROM (n=10) PTNL (n=13) TL (n=8)	Maternal serum	(24 weeks 0/7 days–33 weeks 6/7 days)	Luminex xMAP Technology	02	IL-18 level of <4.2 pg/mL (17th percentile) and delivery before 34 weeks of gestation

Table 1 (continued)

Author, country	Maternal age in years	Ethnicity	Participant characteristics	Study design/ number of samples analyzed	Biological sample	Time of sampling collection [weeks (w), days (d)]	Approach	Number of biomarkers analyzed	PTL-related biomarker
Laudanski et al. [18], Poland	PTL and PTB: 28.4±6.4 PTL and term birth: 30.1±7.6 Mean±SD	NR	PTL at 26–36 weeks and PTB (n=17) PTL at 26–36 weeks but term birth (n=13) Women at term (n=8)	Cross-sectional PTL (n=17) PTNL (n=13) TL (n=08)	Maternal serum	PTL who delivered PTB or at term: (26–36 weeks) At term (>37 weeks)	Multiplexed mini-array (Pierce®)	09	Decreased MIP-3β(CCL19) (vs. PTNL; vs. TL)

NR=Not reported, TNL=term not in labor, TL=normal term labor, PTL=preterm labor, PTLI=PTL with infection, PTNL=preterm not in labor, PPROM=preterm prelabor rupture of membranes, PTB=preterm birth, PE=preeclampsia, PTNL=preterm not labor, w=weeks, d=days. [a]Personal communication. Reported protein names (For UniProt database recommend names, see Table 2): CXCL8=chemokine (C-X-C motif) ligand 8, interleukin-8, CCL5=C-C motif chemokine 5, RANTES, CCL2=monocyte chemotactic protein 1 (C-C motif chemokine 2), CXCL10=interferon γ-inducible protein-10, IL-10=interleukin (IL)-10, sIL-6Rα=soluble IL-6 receptor subunit alpha, TNF-β=tumor necrosis factor beta, MIP-1β=macrophage inflammatory protein-1β, MMP-9=matrix metalloproteinase-9, BDNF=brain-derived neurotrophic factor, TNF-RI=tumor necrosis factor receptor 1, GM-CSF=granulocyte macrophage colony-stimulating factor, MIF=macrophage migration inhibitory factor, RANTES=C-C motif chemokine 5, RANTES, ANGPT2=angiopoetin-2, FGF basic=basic FGF, ICAM-1=intercellular adhesion molecule 1, IGF-I=insulin-like growth factor-binding protein 1, IL-1β=interleukin-1 beta, IL-1Rα=interleukin-1 receptor antagonist protein, IL-8=interleukin-8, MCP-3=monocyte chemotactic protein 3, MIP-1α=macrophage inflammatory protein 1 alpha, PDGF-BB=platelet-derived growth factor receptor beta, TGF-α=transforming growth factor-alpha, TGF-β1=transforming growth factor-beta, TIMP1=tissue inhibitor of metalloproteinasas 1, TNF-α=tumor necrosis factor-alpha, TNFR-II=tumor necrosis factor receptor 2, VEGF=vascular endothelial growth factor A, IL-10=interleukin-10, IL-13=interleukin-13, IGFBP-1=insulin-like growth factor-binding protein 1, BLC=B lymphocyte chemoattractant, IL-6=interleukin-6, IL-17=interleukin-17, IL-18=interleukin-18, MCP-1=monocyte chemotactic protein 1, CCL2, IL-4=interleukin-4, IL-5=interleukin-5, IFN-γ=interferon gamma, TREM-1=triggering receptor expressed on myeloid cells-1, MIP-3β(CCL19)=macrophage inflammatory protein 3 beta.

onset of labor [45]. Seventy percent of the studies provided details on sample collection, preparation, and storage.

Details about sample size calculation were provided only by one paper [14]. An acceptable power calculation was not provided by any of the studies, likely due to either a lack of prior data for power calculation or the inability to generate power that would be able to detect significant changes in multiple analyte concentrations among various groups. Sample size varied from 30 to 648 participants, with most of the studies (76.9%) including fewer than 100 subjects comprising both cases and controls.

Type of multiplex assays used

The assays used in the selected studies are listed in Table 1. Forty percent of the studies used multiplexed assays based on Luminex technology (LabMAP, Luminex Corp, Austin, TX, USA) [47, 32, 24, 15], while FAST Slide protein microarray platform (Whatman Laboratories, Little Chalfont, Buckinghamshire, UK), Meso Scale Discovery (MSD; Gaithersburg, MD, USA), Searchlight assay (Pierce Biotechnology Laboratory, Rockford, IL, USA), and Raybiotech assay (Norcross, GA, USA) were used by Brou et al. [17], Faupel-Badger et al. [44], Laudanski et al. [18], and Laudanski et al. [43], respectively. Only one study performed validation of the multiplex assay [16], while the others referenced previous work or provided no details. The rationale for the assay choice was not explicitly provided in any of the studies.

Biomarkers associated with PTB

The panel of biomarkers analyzed between studies varied widely. The number of analytes reported ranged from two [46] to 44 [17]. From the total of 78 different biomarkers analyzed, 42 were associated with PTB (Table 2). The majority of the studied biomarkers were cytokines, chemokines, cytokine agonists or antagonists, receptors and soluble receptors, metalloproteinases and their inhibitors, growth and angiogenic factors, and adhesion molecules known to be involved in inflammatory pathways associated with PTB. Of the 31 PTB-related biomarkers assessed in maternal serum (see Table 1), the ones most frequently identified were RANTES [14, 15, 20] and IL-10 [15, 17, 20] followed by MIP-1β, GM-CSF [15, 20], Eotaxin [17, 43], and TNF-RI [15, 17]. Increased amniotic fluid ANGPT2 was associated with PTB [17], and the levels of ICAM-1, IGF-I, IL-1β, IL-1Rα, IL-8, MCP-3, MIP-1α, PDGF-BB, TGF-α, TGF-β1, TIMP1, TNFα, TNFR-I, TNFR-II, and VEGF were

Table 2: List of spontaneous preterm birth-related proteins extracted from the multiplex assay studies according to UniProt database (www.uniprot.org; April 3, 2013).

Recommended protein name	Reported protein name(s)	Gene	Acc. no.	Sample	References
Angiopoetin-2	Angiopoetin-2	ANGPT2	O15123	Amniotic fluid, maternal plasma	Brou et al. [17]
Basic FGF-repressed zinc binding protein isoform a variant	FGF basic	FGF basic	Q59FR0	Maternal plasma	Brou et al. [17]
Brain-derived neurotrophic factor	Brain-derived neurotrophic factor	BDNF	P23560	Maternal serum, maternal plasma, cervical fluid	Tsiartas et al. [15], Laudanski et al. [43], Holst et al. [20]
C-C motif chemokine 2	Monocyte chemotactic protein 1	CCL2	P13500	Maternal plasma, amniotic fluid, cervical fluid	Hamilton et al. [14], Holst et al. [20]
C-C-motif chemokine 3	Macrophage inflammatory protein 1α	CCL3	P10147	Fetal plasma, amniotic fluid, cervical fluid	Brou et al. [17], Holst et al. [20]
C-C motif chemokine 4	Macrophage inflammatory protein 1β	CCL4	P13236	Maternal plasma, amniotic fluid, cervical fluid	Tsiartas et al. [15], Holst et al. [20]
C-C motif chemokine 5	C-C motif chemokine 5, RANTES	CCL5	P13501	Maternal plasma, choriondecidua, maternal serum, amniotic fluid, cervical fluid	Hamilton et al. [14], Tsiartas et al. [15], Holst et al. [20]
C-C motif chemokine 7	Monocyte chemotactic protein 3	CCL7	P80098	Fetal plasma	Brou et al. [17]
C-C motif chemokine 19	Macrophage inflammatory protein 3β	CCL19	Q99731	Maternal serum	Laudanski et al. [18]
C-X-C motif chemokine 10	Interferon γ-inducible protein-10	CXCL10	P02778	Maternal plasma	Hamilton et al. [14]
C-X-C motif chemokine 13	B lymphocyte chemoattractant	CXCL13	O43927	Maternal serum	Laudanski et al. [43]
Eotaxin	C-C motif chemokine 11, Eotaxin 1	CCL11	P51671	Maternal serum, fetal plasma, maternal plasma	Brou et al. [17], Laudanski et al. [43]
Granulocyte macrophage colony-stimulating factor	Granulocyte macrophage colony-stimulating factor	CSF2	P04141	Maternal plasma, amniotic fluid, cervical fluid	Tsiartas et al. [15], Holst et al. [20]
Insulin-like growth factor	Insulin-like growth factor	IGF1	P05019	Fetal plasma	Brou et al. [17]
Insulin-like growth factor-binding protein 1	Insulin-like growth factor-binding protein 1	IGFBP1	P08833	Maternal serum	Laudanski et al. [43]
Intercellular adhesion molecule 1	Intercellular adhesion molecule 1	ICAM1	P05362	Fetal plasma, maternal plasma	Brou et al. [17]
Interelukin-1 receptor antagonist protein	Interelukin-1 receptor antagonist protein	IL1RN	P18510	Fetal plasma	Brou et al. [17]
Interferon γ	Interferon γ	IFNG	P01579	Cervical fluid	Holst et al. [20]
Interleukin-1 β	Interleukin-1 β	IL1B	P01584	Fetal plasma, maternal plasma, amniotic fluid, cervical fluid	Brou et al. [17]
Interleukin-4	Interleukin-4	IL4	P05112	Cervical fluid	Holst et al. [20]
Interleukin-5	Interleukin-5	IL5	P05113	Cervical fluid	Holst et al. [20]
Interleukin-6	Interleukin-6	IL6	P05231	Amniotic fluid, cervical fluid	Holst et al. [20]
Interleukin-8	Chemokine (C-X-C motif) ligand 8, interleukin-8	CXCL8	P10145	Maternal plasma, fetal plasma	Brou et al. [17]
Interleukin-10	Interleukin-10	IL10	P22301	Maternal plasma, amniotic fluid, cervical fluid	Tsiartas et al. [15], Brou et al. [17], Holst et al. [20]
Interleukin-17A	Interleukin-17A	IL17A	Q16552	Amniotic fluid, cervical fluid	Holst et al. [20]
Interleukin-18	Interleukin-18	IL18	Q14116	Maternal serum, amniotic fluid	Holst et al. [20], Ekelund et al. [46]
Interleukin-6 receptor subunit α	Interleukin-6 receptor subunit α	IL6R	P08887	Maternal plasma, cervical fluid	Tsiartas et al. [15], Holst et al. [20]
Lymfotoxin-α	Tumor necrosis factor β	LTA	P01374	Maternal plasma	Tsiartas et al. [15]
Macrophage migration inhibitory factor	Macrophage migration inhibitory factor	MIF	P14174	Maternal plasma	Tsiartas et al. [15]
Matrix metalloproteinase-9	Matrix metalloproteinase-9	MMP9	P14780	Maternal plasma	Tency et al. [12], Tsiartas et al. [15]
Metalloproteinase inhibitor 1	Tissue inhibitor of metalloproteinsas 1	TIMP1	P01033	Fetal plasma	Brou et al. [17]

Table 2 (continued)

Recommended protein name	Reported protein name(s)	Gene	Acc. no.	Sample	References
Neurotrophin-3	Neurotrophin-3	NTF3	P20783	Cervical fluid	Holst et al. [20]
Neurotrophin-4	Neurotrophin-4	NTF4	P34130	Cervical fluid	Holst et al. [20]
Platelet-derived growth factor receptor β	Platelet-derived growth factor receptor β	PDGFRB	P09619	Fetal plasma	Brou et al. [17]
Protransforming growth factor α	Transforming growth factor-α	TGFA	P01135	Fetal plasma	Brou et al. [17]
TGF-β receptor type 1	Transforming growth factor-β receptor type 1	TGFBR1	P36897	Fetal plasma	Brou et al. [17]
Triggering receptor expressed on myeloid cells 1	Triggering receptor expressed on myeloid cells 1	TREM1	Q9NP99	Cervical fluid	Holst et al. [20]
Tumor necrosis factor	Tumor necrosis factor-α	TNF	P01375	Fetal plasma, amniotic fluid, cervical fluid	Brou et al. [17], Holst et al. [20]
Tumor necrosis factor receptor superfamily member 1 A	Tumor necrosis factor receptor 1	TNFRSF1A	P19438	Maternal plasma, fetal plasma, maternal plasma	Tsiartas et al. [15], Brou et al. [17]
Tumor necrosis factor receptor superfamily member 1B	Tumor necrosis factor receptor 2	TNFRSF1B	P20333	Fetal plasma	Brou et al. [17]
Tumor necrosis factor ligand superfamily member 6	Fas-ligand	FASLG	P48023	Maternal plasma	Brou et al. [17]
Vascular endothelial growth factor A	Vascular endothelial growth factor A	VEGFA	P15692	Fetal plasma	Brou et al. [17]

higher in fetal plasma from PTB cases than in fetal plasma from term delivery controls [17].

RANTES [14, 15, 20] and IL-10 [15, 17, 20] were found to associate with PTB by three studies. These articles were further analyzed for pooling their results in a meta-analysis. However, we could not perform a meta-analysis because of major differences in the biological samples and study populations. Tsiartas et al. [15] and Holst et al. [20] used the same biomarker panel (27-plex), and the assays were performed by the same laboratory. Both studies reported an association between RANTES in various biological compartments and latency in the onset of preterm labor. Holst et al. [20] reported that RANTES levels in the amniotic and cervical fluids were significantly associated with delivery within 7 days, while Tsiartas et al. [15] found that high levels of maternal serum RANTES and short cervical length were significant to the prediction of spontaneous preterm delivery within 7 days. In the third work, Hamilton et al. [14] reported that RANTES increased in plasma from preterm in-labor women compared to preterm but not in labor; however, no differences were reported between PTB and normal term birth samples. Similarly, there were differences in the types of samples and phenotypes of the outcomes reported by the three studies that measured IL-10 [15, 17, 20], which also prevented us from pooling the results into a meta-analysis. In a retrospective cohort study, Brou et al. [17] reported decreased IL-10 in maternal plasma in PTB compared to normal term births. Tsiartas et al. [15] found higher maternal serum IL-10, whereas Holst et al. [20] reported an increase in both cervical and amniotic fluids IL-10 associated with delivery within 7 days in women with PTB compared to those women who did not deliver within 7 days.

A complete list of the biomarkers, frequency of testing, and the distribution according to the pathophysiologic pathway is provided in Table 1.

Quality assessment

As shown in Figure 2, most of the studies included in the review (8/10) had a well-defined study population [15–18, 20, 46, 44, 45]. Only two studies received a low-quality grading because of poor description of the characteristics of the sample population [14, 43]. In five articles, the sample collection and preparation were well described [15, 17, 18, 20, 45]. Sample collection and management were adequate in four other studies [16, 46, 44, 43], and one article provided only a summary description [14]. The assay methodology was well defined in five studies [15, 17, 18, 20, 45], whereas two studies [16, 46] were graded
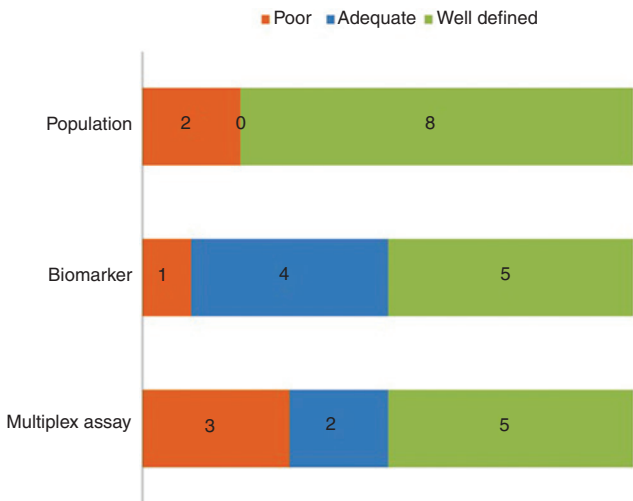
Figure 2: Quality assessment of the ten multiplex assay studies on spontaneous preterm birth-related biomarkers.

of adequate quality, and three were judged to be of poor quality [14, 43, 44].

Discussion

PTB is a complex syndrome characterized by pathways with interacting biomarkers [47, 48]. In this review, we examined multiplex assays where multiple biomarkers were studied simultaneously in the same sample. We identified ten eligible studies that analyzed 74 biomarkers and identified 42 dysregulated biomarkers associated with PTB. Most of these studies recruited European-American and African-American populations. Several biomarkers were found to be associated with PTB in maternal plasma/serum samples in more than one study, and RANTES and IL-10 emerged as potential candidates for further evaluation and validation studies with similar methodological approaches. However, criteria for performing a meta-analysis were not met, as none of the biomarkers were replicated in additional samples/studies by the same investigators or by other investigators following the same approach, population, and outcome. We have chosen not to discuss biomarkers reported in tissue-based studies as their usefulness in predicting PTB in clinical specimens has yet to be evaluated. Accordingly, this systematic review also concludes that, at present and based on reported findings, no single or combination of biomarkers can be used to predict PTB risk.

Unlike the findings from our two previous systematic reviews [8, 12], the multiplex studies included in this review had an overall better design and quality of reporting. Most of these studies were published fairly recently (between 2006 and 2013), and it is encouraging that a follow-up or replication study can be performed easily as the limitations are fewer and easier to address. However, many of the methodological issues reported in the aforementioned prior reviews still persist, especially with respect to study design, hindering PTB biomarker discovery. We highlight below some of these concerns.

All reports included in this review defined the studied outcome phenotype as spontaneous PTB. However, definition of this phenotype still remains as an enigma in PTB research due to the lack of standard and established definitions used in biomarker discovery and clinical trials. Recent reports highlighted the urgent need for defining PTB phenotypes [7, 49, 50]. A gold standard is yet to be developed, and investigators are still using their own criteria for phenotype definition.

One key area identified by this report is the heterogeneity in the selection of biomarkers. Two major criteria used by investigators in biomarker panel selection led to a high diversity of biomarkers evaluated in a relatively small number of studies: (1) investigation of a specific pathophysiologic pathway of PTB and (2) convenience in using a predefined assay. Just like any other "candidate" marker studies, bias is introduced when selecting the panel by either of these methods. Although there are no proper solutions for eliminating bias in candidate marker selection, incorporating biomarkers from all known and reported pathways, rather than skewing the selection of markers by choosing from "desired" pathways, is not the best approach to minimize such biases. Selection of panels based on convenience and without proper rationale or biological relevance is unlikely to yield reproducible data.

Multiplex assays have emerged as important tools for screening disease-related biomarkers. Numerous types of multiplex arrays are available on the market from different manufacturers, differing by number and type of cytokines included in the array, sensitivity in detection, the variety of antibodies and standards used in the kits, and incubation time [51]. In our review, 40% of the studies used the Luminex technology assay (LabMAP) [52]. Using a multiplex bead array, Luminex assays facilitate simultaneous cytometric quantitation of multiple immune mediators (i.e. cytokines) in solution by capturing these to spectrally distinct beads with the distinct advantages of higher throughput, smaller volume (as little as 12 μL per sample) of various biological samples (e.g. maternal serum, cervical or vaginal fluid), and lower costs [53]. However, one of the included studies applied the FAST Slide protein microarray platform [17]. The main advantage of this technology

is that the matrix retains arrayed proteins in a near-quantitative fashion that translates into antibody arrays with sensitivity down to antigen concentrations of 1 pg/mL. Faupel-Badger et al. [44] used MSD multiplex microarray, which is based on MULTI-ARRAY® technology, a proprietary combination of electrochemiluminescence detection and patterned arrays. The instrument (Sector Imager 2400) that is used to detect signals from the assay uses highly efficient custom-designed optics and ultrasensitive photo detectors to collect and quantitatively measure light emitted from the microplates. In 2006, Laudanski et al. [18] used a Searchlight assay which is a multiplexed sandwich enzyme-linked immunosorbent assays (ELISAs) with imaging system based on a cooled charge-coupled device camera which detects the chemiluminescent signal at each spot in the array. In 2012, Laudanski et al. [43] used a multiplex-based platform from Raybiotech for simultaneous quantitative measurement of different proteins from one sample, which has the advantage of being read by most of the conventional array fluorescent plate readers available on the market. Considering the diversity in described assays, it appears that the choice in using one particular assay may be driven by several decision factors, such as a researcher's familiarity with a specific PTB pathway, a particular research question, the type of biological sample available, convenience, and financial feasibility.

Other areas of heterogeneity among studies included study design, type of sample used for biomarker discovery, timing of sample collection, and inclusion of asymptomatic or symptomatic women. The methodology of statistical analysis employed introduced yet another field of heterogeneity. Many of these studies lacked reporting on confounding variables and control for multitudes of covariates. Therefore, results may also have been impacted by association by chance due to analysis confounded by multiple testing or lack of adequate power. Many of these issues were identified and discussed in detail in our prior systematic reviews. Readers are encouraged to review the guidelines and checklists in our previous publications that apply to studies using multiplex assays [8, 12]. Moreover, based on the above-detailed issues in identifying PTB biomarkers through multiplex approaches, we suggest a few guidelines to consider prior to initiation of future studies.

Suggested approaches for multiplex assay discovery and future validation strategies

Success of a biomarker study is directly dependent on the quality of specimen collection and how it is processed until assay, considering appropriate preservatives and storage conditions to avoid any variations in the biomarker level and integrity. Reporting should include a detailed description of these procedures, as well as sensitivity and specificity of the assay, and inter- and intra-assay variations should be considered and reported. Sampling is dependent upon study design and other rationale established prior to collecting, and biological specimens that are easy to obtain should be chosen to assess the risk of PTB. The appropriate sample size of required cases and controls should be calculated to properly evaluate the association of biomarkers alone and in combination, and this parameter depends on the number of analytes under investigation. Thus, sample size calculations for exploratory factor analysis should be employed. While no optimal cases-to-controls ratio exists, a higher ratio of controls to cases would result in greater study power. Subject-to-variable ratios from 20:1 to 100:1 are not uncommon. When the number of biomarkers is large compared to the number of cases and controls, chance associations may be uncovered and hinder progress. Statistical analysis and proper selection of analytical methods should address the hypothesis and objectives established prior to the study and the study design. We recommend the following steps: (1) perform statistical assessment of distribution of biomarker concentration (normal or non-normal); (2) select appropriate form of presenting data based on distribution [i.e. mean±standard deviation (SD), median (interquartile range)]; (3) identify potential confounding factors (e.g. clinical and demographic) and adjust for confounders; and (4) adjust for multiple testing. If the discovery set has sufficient power to project biomarker combinations, the interference on quantitation should be evaluated as well. For example, if the discovery multiplex assay evaluated a large number of potential markers and the results indicate that only a small number are associated with PTB risk, it might make sense to only test the smaller number in future validation efforts. In this context, a prospective validation using a new, independent set of specimens is also required to confirm the performance of the biomarker panel. During this validation, it is not appropriate to add additional biomarkers or adjust the predictive algorithm; if such modifications are required to improve the prediction, then a new prospective effort utilizing an independent specimen set is required to validate the modifications.

The mathematical algorithm, which combines the assay results of each biomarker into a single output predictive of PTB, is as important as the biomarkers of a predictive biomarker combination. Prior to the validation studies using this algorithm, a sample set independent of those used in discovery phase can be tested for the optimization of the algorithm. Indeed, even if the biomarker set includes

numerous proteins, these measurements should be combined into a single risk estimate (similar to combination of multiple markers to provide a single estimate for the risk of other diseases). Multi-marker assays developed in this way are amenable to traditional validation methods.

The rationale behind biomarker research assumes that multiple biomarkers perform better than a single biomarker for assessing PTB risk. Success of biomarker studies depends on the ability to identify those biomarkers that predict PTB from within the larger panel of biomarkers studied. These biomarker sets may be difficult to identify unless statistical approaches allow for the possibility that each biomarker of the set is by itself a poor predictor, and perform well only in combination. Negative results of studies to date could have been hindered by a lack of this type of analysis.

Finally, appropriate reporting of data involves following all the listed guidelines throughout the study. Reporting should be based on the hypothesis tested and match the studied objectives. Inclusion of study limitations will help the reader better interpret reported findings and address such limitations in future studies.

In summary, multiplex assays provide promising technological advancement in identifying biomarkers of PTB over other approaches that our group has previously systematically reviewed. Understanding the limitations of existing reports and designing studies, following the criteria detailed above may facilitate the identification of better biomarkers for screening and prediction of PTB.

References

[1] Saigal S, Doyle LW. An overview of mortality and sequelae of preterm birth from infancy to adulthood. Lancet. 2008;371:261–9.
[2] Platt MJ. Outcomes in preterm infants. Public Health. 2014;128:399–403.
[3] Howson CP, Kinney MV, Mcdougall L, Lawn JE. Born Too Soon: Preterm birth matters. Reprod Health. 2013;10:1–9.
[4] Blencowe H, Cousens S, Oestergaard MZ, Chou D, Moller AB, Narwal R, et al. National, regional, and worldwide estimates of preterm birth rates in the year 2010 with time trends since 1990 for selected countries: a systematic analysis and implications. Lancet. 2012;379:2162–72.
[5] Goldenberg RL, Culhane JF, Iams JD, Romero R. Epidemiology and causes of preterm birth. Lancet. 2008;371:75–84.
[6] Romero R, Espinoza J, Kusanovic JP, Gotsch F, Hassan S, Erez O, et al. The preterm parturition syndrome. Br J Obstet Gynaecol. 2006;113:17–42.
[7] Barros FC, Papageorghiou AT, Victora CG, Noble JA, Pang R, Iams J, et al. The distribution of clinical phenotypes of preterm birth syndrome: implications for prevention. JAMA Pediatr. 2015;169:220–9.
[8] Menon R, Torloni MR, Voltolini C, Torricelli M, Merialdi M, Betrán AP, et al. Biomarkers of spontaneous preterm birth: an overview of the literature in the last four decades. Reprod Sci. 2011;18:1046–70.
[9] Conde-Agudelo A, Papageorghiou AT, Kennedy SH, Villar J. Novel biomarkers for the prediction of the spontaneous preterm birth phenotype: a systematic review and meta-analysis. Br J Obstet Gynaecol. 2011;118:1042–54.
[10] Wei SQ, Fraser W, Luo ZC. Inflammatory cytokines and spontaneous preterm birth in asymptomatic women. Obstet Gynecol. 2010;116:393–401.
[11] Honest H, Hyde CJ, Khan KS. Prediction of spontaneous preterm birth: no good test for predicting a spontaneous preterm birth. Curr Opin Obstet Gynecol. 2012;24:422–33.
[12] Kacerovsky M, Lenco J, Musilova I, Tambor V, Lamont R, Torloni MR, et al. Proteomic biomarkers for spontaneous preterm birth: a systematic review of the literature. Reprod Sci. 2014;21:283–95.
[13] Ellington AA, Kullo IJ, Bailey KR, Klee GG. Antibody-based protein multiplex platforms: technical and operational challenges. Clin Chem. 2010;56:186–93.
[14] Hamilton SA, Tower CL, Jones RL. Identification of chemokines associated with the recruitment of decidual leukocytes in human labour: potential novel targets for preterm labour. PLoS One. 2013;8:e56946.
[15] Tsiartas P, Holst RM, Wennerholm UB, Hagberg H, Hougaard DM, Skogstrand K, et al. Prediction of spontaneous preterm delivery in women with threatened preterm labour: a prospective cohort study of multiple proteins in maternal serum. Br J Obstet Gynaecol. 2012;119:866–73.
[16] Tency I, Verstraelen H, Kroes I, Holtappels G, Verhasselt B, Vaneechoutte M, et al. Imbalances between matrix metalloproteinases (MMPs) and tissue inhibitor of metalloproteinases (TIMPs) in maternal serum during preterm labor. PLoS One. 2012;7:e49042.
[17] Brou L, Almli LM, Pearce BD, Bhat G, Drobek CO, Fortunato S, et al. Dysregulated biomarkers induce distinct pathways in preterm birth. Br J Obstet Gynaecol. 2012;119:458–73.
[18] Laudanski P, Lemancewicz A, Pierzynski P, Akerlund M, Laudanski T. Decreased serum level of macrophage inflammatory chemokine-3beta/CCL19 in preterm labor and delivery. Eur J Obstet Gynecol Reprod Biol. 2006;124:23–6.
[19] Cobo T, Tsiartas P, Kacerovsky M, Holst RM, Hougaard DM, Skogstrand K, et al. Maternal inflammatory response to microbial invasion of the amniotic cavity: analyses of multiple proteins in the maternal serum. Acta Obstet Gynecol Scand. 2013;92:61–8.
[20] Holst RM, Hagberg H, Wennerholm UB, Skogstrand K, Thorsen P, Jacobsson B. Prediction of spontaneous preterm delivery in women with preterm labor. Obstet Gynecol. 2009;114:268–77.

[21] Whiting P, Rutjes AW, Reitsma JB, Bossuyt PM, Kleijnen J. The development of QUADAS: a tool for the quality assessment of studies of diagnostic accuracy included in systematic reviews. BMC Med Res Methodol. 2003;3:25.

[22] Taylor BD, Holzman CB, Fichorova RN, Tian Y, Jones NM, Fu W, et al. Inflammation biomarkers in vaginal fluid and preterm delivery. Hum Reprod. 2013;28:942–52.

[23] Park MR, Park JY, Kwon DN, Cho SG, Park C, Seo HG, et al. Altered protein profiles in human umbilical cords with preterm and full-term delivery. Electrophoresis. 2013;34:493–500.

[24] Hsu TY, Lin H, Lan KC, Ou CY, Tsai CC, Cheng BH, et al. High interleukin-16 concentrations in the early second trimester amniotic fluid: an independent predictive marker for preterm birth. J Matern Fetal Neonatal Med. 2013;26:285–9.

[25] Gervasi MT, Romero R, Bracalente G, Erez O, Dong Z, Hassan SS, et al. Midtrimester amniotic fluid concentrations of interleukin-6 and interferon-gamma-inducible protein-10: evidence for heterogeneity of intra-amniotic inflammation and associations with spontaneous early (<32 weeks) and late (>32 weeks) preterm delivery. J Perinat Med. 2012;40:329–43.

[26] La Sala GB, Ardizzoni A, Capodanno F, Manca L, Baschieri MC, Soncini E, et al. Protein microarrays on midtrimester amniotic fluids: a novel approach for the diagnosis of early intrauterine inflammation related to preterm delivery. Int J Immunopathol Pharmacol. 2012;25:1029–40.

[27] Kaukola T, Ojaniemi M, Tuimala J, Herva R, Saarela T, Kingsmore SF, et al. Cord blood chemokines differentiate between spontaneous and elective preterm births in singleton pregnancies. Cytokine. 2011;54:85–91.

[28] Holst RM, Hagberg H, Wennerholm UB, Skogstrand K, Thorsen P, Jacobsson B. Prediction of microbial invasion of the amniotic cavity in women with preterm labour: analysis of multiple proteins in amniotic and cervical fluids. Br J Obstet Gynaecol. 2011;118:240–9.

[29] Wilke C, Renz H, Tekesin I, Hellmeyer L, Herz U, Schmidt S, et al. Suppression of IL-2 and IFN-gamma production in women with spontaneous preterm labor. J Perinat Med. 2006;34:20–7.

[30] Yang Q, Sayed YE, Shaw GM, Fu J, Schilling J, Madan A. Second-trimester serum cytokines in women who develop spontaneous preterm labor at <28 weeks' gestation versus term labor. Am J Perinatol. 2010;27:31–6.

[31] Dubicke A, Fransson E, Centini G, Andersson E, Byström B, Malmström A, et al. Pro-inflammatory and anti-inflammatory cytokines in human preterm and term cervical ripening. J Reprod Immunol. 2010;84:176–85.

[32] Curry AE, Thorsen P, Drews C, Schendel D, Skogstrand K, Flanders WD, et al. First-trimester maternal plasma cytokine levels, pre-pregnancy body mass index, and spontaneous preterm delivery. Acta Obstet Gynecol Scand. 2009;88:332–42.

[33] Matoba N, Yu Y, Mestan K, Pearson C, Ortiz K, Porta N, et al. Differential patterns of 27 cord blood immune biomarkers across gestational age. Pediatrics. 2009;123:1320–8.

[34] Gargano JW, Holzman C, Senagore P, Thorsen P, Skogstrand K, Hougaard DM, et al. Mid-pregnancy circulating cytokine levels, histologic chorioamnionitis and spontaneous preterm birth. J Reprod Immunol. 2008;79:100–10.

[35] Chow SSW, Craig ME, Jones CA, Hall B, Catteau J, Lloyd AR, et al. Differences in amniotic fluid and maternal serum cytokine levels in early midtrimester women without evidence of infection. Cytokine. 2008;44:78–84.

[36] Curry AE, Vogel I, Skogstrand K, Drews C, Schendel DE, Flanders WD, et al. Maternal plasma cytokines in early- and mid-gestation of normal human pregnancy and their association with maternal factors. J Reprod Immunol. 2008;77:152–60.

[37] Skogstrand K, Hougaard DM, Schendel DE, Bent N-P, Svaerke C, Thorsen P. Association of preterm birth with sustained postnatal inflammatory response. Obstet Gynecol. 2008;111:1118–28.

[38] Curry AE, Vogel I, Drews C, Schendel D, Skogstrand K, Flanders WD, et al. Mid-pregnancy maternal plasma levels of interleukin 2, 6, and 12, tumor necrosis factor-alpha, interferon-gamma, and granulocyte-macrophage colony-stimulating factor and spontaneous preterm delivery. Acta Obstet Gynecol Scand. 2007;86:1103–10.

[39] Menon R, Merialdi M, Lombardi SJ, Fortunato SJ. Differences in the placental membrane cytokine response: a possible explanation for the racial disparity in preterm birth. Am J Reprod Immunol. 2006;56:112–8.

[40] Jacobsson B, Holst RM, Andersson B, Hagberg H. Monocyte chemotactic protein-2 and -3 in amniotic fluid: relationship to microbial invasion of the amniotic cavity, intra- amniotic inflammation and preterm delivery. Acta Obstet Gynecol Scand. 2005;84:566–71.

[41] Dörtbudak O, Eberhardt R, Ulm M, Persson GR. Periodontitis, a marker of risk in pregnancy for preterm birth. J Clin Periodontol. 2005;32:45–52.

[42] Chandiramani M, Seed PT, Orsi NM, Ekbote UV, Bennett PR, Shennan AH, et al. Limited relationship between cervico-vaginal fluid cytokine profiles and cervical shortening in women at high risk of spontaneous preterm birth. PLoS One. 2012;7:e52412.

[43] Laudanski P, Raba G, Kuc P, Lemancewicz A, Kisielewski R, Laudanski T. Assessment of the selected biochemical markers in predicting preterm labour. J Matern Neonatal Med. 2012;25:2696–9.

[44] Faupel-Badger JM, Fichorova RN, Allred EN, Hecht JL, Dammann O, Leviton A, et al. Cluster analysis of placental inflammatory proteins can distinguish preeclampsia from preterm labor and premature membrane rupture in singleton deliveries <28 weeks of gestation. Am J Reprod Immunol. 2011;66:488–94.

[45] Kramer MS, Kahn SR, Platt RW, Genest J, Chen MF, Goulet L, et al. Mid-trimester maternal plasma cytokines and CRP as predictors of spontaneous preterm birth. Cytokine. 2010;49:10–4.

[46] Ekelund CK, Vogel I, Skogstrand K, Thorsen P, Hougaard DM, Langhoff-Roos J, et al. Interleukin-18 and interleukin-12 in maternal serum and spontaneous preterm delivery. J Reprod Immunol. 2008;77:179–85.

[47] Bhat G, Williams SM, Saade GR, Menon R. Biomarker interactions are better predictors of spontaneous preterm birth. Reprod Sci. 2014;21:340–50.

[48] Menon R, Bhat G, Saade GR, Spratt H. Multivariate adaptive regression splines analysis to predict biomarkers of spontaneous preterm birth. Acta Obstet Gynecol Scand. 2014;93:382–91.

[49] Villar J, Papageorghiou AT, Knight HE, Gravett MG, Iams J, Waller SA, et al. The preterm birth syndrome: a prototype phenotypic classification. Am J Obstet Gynecol. 2012;206:119–23.

[50] Goldenberg RL, Gravett MG, Iams J, Papageorghiou AT, Waller SA, Kramer M, et al. The preterm birth syndrome: issues to consider in creating a classification system. Am J Obstet Gynecol. 2012;206:113–8.

[51] Khan SS, Smith MS, Reda D, Suffredini AF, McCoy JP. Multiplex bead array assays for detection of soluble cytokines: comparisons of sensitivity and quantitative values among kits from multiple manufacturers. Cytometry B Clin Cytom. 2004;61:35–9.

[52] Prabhakar U, Eirikis E, Davis HM. Simultaneous quantification of proinflammatory cytokines in human plasma using the LabMAP assay. J Immunol Methods. 2002;260:207–18.

[53] Dupont NC, Wang K, Wadhwa PD, Culhane JF, Nelson EL. Validation and comparison of luminex multiplex cytokine analysis kits with ELISA: determinations of a panel of nine cytokines in clinical sample culture supernatants. J Reprod Immunol. 2005;66:175–91.