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Systematic review

Novel biomarkers for the prediction of the spontaneous preterm birth phenotype: a systematic review and meta-analysis

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Background Being able to predict preterm birth is important, as it may allow a high-risk population to be selected for future interventional studies and help in understanding the pathways that lead to preterm birth.

Objective To investigate the accuracy of novel biomarkers to predict spontaneous preterm birth in women with singleton pregnancies and no symptoms of preterm labour.

Search strategy Electronic searches in PubMed, Embase, Cinahl, Lilacs, and Medion, references of retrieved articles, and conference proceedings. No language restrictions were applied.

Selection criteria Observational studies that evaluated the accuracy of biomarkers proposed in the last decade to predict spontaneous preterm birth in asymptomatic women. We excluded studies in which biomarkers were evaluated in women with preterm labour.

Data collection and analysis Two reviewers independently extracted data on study characteristics, quality, and accuracy. Data were arranged in 2 × 2 contingency tables and synthesised separately for spontaneous preterm birth before 32, 34, and 37 weeks of gestation. We used bivariate meta-analysis to estimate pooled sensitivities and specificities, and calculated likelihood ratios (LRs).

Main results A total of 72 studies, including 89 786 women and evaluating 30 novel biomarkers, met the inclusion criteria. Only three biomarkers (proteome profile and prolactin in cervicovaginal fluid, and matrix metalloproteinase-8 in amniotic fluid) had positive LRs > 10. However, each of these biomarkers was evaluated in only one small study. Four biomarkers had a moderate predictive accuracy (interleukin-6 and angiogenin, in amniotic fluid; human chorionic gonadotrophin and phosphorylated insulin-like growth factor binding protein-1, in cervicovaginal fluid). The remaining biomarkers had low predictive accuracies.

Conclusions None of the biomarkers evaluated in this review meet the criteria to be considered a clinically useful test to predict spontaneous preterm birth. Further large, prospective cohort studies are needed to evaluate promising biomarkers such as a proteome profile in cervicovaginal fluid.

Keywords Biomarker, meta-analysis, prediction, preterm birth, systematic review.

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Introduction

Preterm birth is the leading cause of perinatal morbidity and mortality around the world.[1] It accounts for approximately 70% of neonatal deaths and almost half of long-term neurological disabilities.[2] Recently, it was estimated that 12.9 million births, or 9.6% of all births worldwide, were preterm,[3] of which approximately 11.9 million (92.3%) were in Africa, Asia, Latin America and the Caribbean. In most high-income countries, the preterm birth rate has increased predominantly because of a rising number of medically indicated preterm births in singletons, and the preterm delivery of multiple pregnancies conceived with assisted reproductive technology (ART).[1]

Approximately 40–45% of preterm births follow spontaneous preterm labour, 25–30% follow preterm prelabour rupture of the membranes (PPROM), and 30–35% are attributed to medically indicated or elective preterm

deliveries.[1] Births that follow spontaneous preterm labour and PPROM are usually designated together as spontaneous preterm births. There is some evidence that the rate of spontaneous preterm birth is also increasing, and the exact cause remains unclear.[4]

The prediction of spontaneous preterm birth is important because it could allow for the identification of women at high risk of preterm birth, in whom a specific intervention could be tested. Being able to predict which women are likely to have a preterm birth is a prerequisite for the effective use of most interventions aimed at preventing preterm birth. In addition, studies of predictors of spontaneous preterm birth may improve our understanding of the mechanisms of biological and disease pathways leading to spontaneous preterm birth, and possibly to better interventions. A third reason for predicting spontaneous preterm birth is that being able to identify women at low risk would avoid the use of unnecessary and sometimes costly interventions.

Biomarkers have been defined as 'parameters, which can be measured in a biological sample, and which provide information on an exposure, or on the actual or potential effects of that exposure in an individual or in a group'.[5] Based on the known risk factors and pathways of preterm birth, several biomarkers have been tested to see if they predict spontaneous preterm birth. Among those evaluated to date, fetal fibronectin in cervicovaginal fluid has been most strongly and consistently associated with subsequent spontaneous preterm birth. The test is most accurate in predicting spontaneous preterm birth within 7–10 days of a woman presenting with symptoms of threatened preterm birth.[6] However, this biomarker has limited accuracy in predicting spontaneous preterm birth in women without symptoms and signs of preterm labour. No other biomarkers have been consistently found to be useful in clinical settings to predict spontaneous preterm birth in asymptomatic women.

The objective of this systematic review and meta-analysis was to identify and determine the accuracy of biomarkers proposed in the last decade for the prediction of spontaneous preterm birth in women with singleton pregnancies and no symptoms of preterm labour.

Methods

The systematic review followed a prospectively prepared protocol, and is reported using widely recommended guidelines for systematic reviews of diagnostic test accuracy.[7,8]

Literature search

We searched PubMed, Embase, Cinahl, Lilacs, and Medion (all from inception to 31 October 2010), using an existing search strategy.[9] Proceedings of the Society for Maternal–Fetal Medicine and international meetings on preterm birth, reference lists of identified studies, textbooks, previously published systematic reviews, and review articles were also searched. In addition, we contacted investigators involved in the field to locate unpublished studies. There were no language restrictions. All searches were carried out independently by the authors, and the results were merged. For studies that resulted in multiple publications, the data from the publication with the largest sample size were used and supplemented if additional information appeared in the other publications.

Study selection

The systematic review focused on observational studies (cohort, cross-sectional, and case–control) that evaluated the accuracy of biomarkers to predict spontaneous preterm birth in asymptomatic pregnant women with singleton pregnancies. The studies had to provide the necessary information to construct 2×2 tables.

Studies were excluded: if they were case series or reports, editorials, or comments or reviews without original data; if data for biomarkers were reported only as mean or median values; if accuracy test estimates were not published, and sufficient information to calculate them could not be retrieved, despite writing to the corresponding author; if biomarkers were evaluated in women with preterm labour, PPROM, or multiple gestation, or if the outcome measure included non-spontaneous preterm birth. When a study included women with singleton and multiple pregnancies or women with symptoms and without symptoms of labour, it was not considered for inclusion in the review unless data for asymptomatic women with singleton pregnancies were extractable separately.

We excluded biomarkers, such as cervicovaginal fetal fibronectin, serum alpha-fetoprotein, human chorionic gonadotrophin, and corticotrophin-releasing hormone, and salivary or serum oestriol, for which predictive values for spontaneous preterm birth have already been clearly established.[6,10,11] Overall, the predictive accuracy of these biomarkers is moderate (cervicovaginal fetal fibronectin) to minimal (serum alpha-fetoprotein, human chorionic gonadotrophin, and corticotrophin-releasing hormone, and salivary or serum oestriol). The transvaginal sonographic cervical length was also excluded, as it is not considered to be a biomarker. We did not include genetic biomarkers in this review; however, we identified existing systematic reviews on this topic to generate a list of potential candidate genes associated with spontaneous preterm birth.

All published studies that were deemed suitable were retrieved and reviewed independently by the authors to determine whether or not they should be included. Disagreements were resolved through consensus.

Quality assessment

The methodological quality of included studies was assessed independently by the reviewers using a modified Quality Assessment of Diagnostic Accuracy Studies (QUADAS) tool.[12] Each item was scored as 'yes', 'no', or 'unclear'. The items evaluated in studies included in the systematic review, and their interpretation, were as follows:

1 Study design: cohort study ('yes') compared with case–control ('no').
2 Type of data collection: prospective ('yes') compared with retrospective ('no').
3 Sampling method: women consecutively or randomly selected ('yes') compared with convenience sampling, such as arbitrary recruitment or non-consecutive recruitment ('no').
4 Description of the test: adequate description of the execution of the biomarker test ('yes') compared with inadequate description ('no').
5 Blinding of test results: clinicians managing the patient did not have any knowledge of the biomarker test result ('yes') compared with clinicians unmasked to test results ('no').

If there was insufficient information available to make a judgment on these items, then they were scored as 'unclear'. We did not calculate a summary score to estimate the overall quality of each article as the interpretation of such summary scores is problematic, and is potentially misleading.[13]

The methodological quality of included studies was assessed individually by the reviewers who were not associated with any of the studies. When differences of opinion existed, the decision was based on consensus.

Data extraction

Potentially relevant articles were acquired and data were extracted in duplicate from all reports, and recorded on a form designed independently by the reviewers. There was no blinding of authorship. Information was extracted on study characteristics (recruitment of women, prospective or retrospective data collection, blinding of test results, completeness of follow-up, and reporting of withdrawals), participants (inclusion and exclusion criteria, sample size, demographic characteristics, and country and date of publication), and description of the biomarker test used (gestational age at sampling, frequency of test, sampling site, analytical method used, and cut-off level).

For each study, for all reported cut-off values, and for all categories of spontaneous preterm birth, we then extracted numbers of true-positive, false-positive, true-negative, and false-negative results. Corresponding authors were contacted by email to obtain additional predictive accuracy data. Only two authors supplied additional data.

In studies where serial biomarker samples were collected, we considered predictive values from samples collected at the earliest gestational age. However, we also analysed predictive values separately for each gestational period, which were then compared. Studies reporting spontaneous preterm birth before 35 weeks of gestation were included in the group of studies with spontaneous preterm birth before 34 weeks of gestation in our data synthesis because of the relatively similar neonatal outcomes. In the same way, studies reporting spontaneous preterm birth before 33 weeks of gestation were considered alongside studies reporting spontaneous preterm birth before 32 weeks of gestation.

Disagreements in data extraction were resolved by discussion among the authors.

Statistical analysis

Data were synthesised separately for spontaneous preterm birth before 32, 34, and 37 weeks of gestation.

Data extracted from each study were arranged in 2×2 contingency tables. When these tables contained cells for which the value is 0, we added 0.5 to those cells to calculate variances.[14] Sensitivity and specificity were calculated separately for different biomarkers and cut-off values. To visualise data, we plotted sensitivities and specificities in receiver operating characteristic (ROC) plots using spontaneous preterm birth as the outcome measure at <32, at <34, and at <37 weeks of gestation. A bivariate, random-effects, meta-regression model was used to calculate pooled estimates of sensitivity and specificity with 95% confidence intervals (95% CIs).[15] The bivariate model incorporates and calculates the correlation between estimates of sensitivity and specificity within studies, which produces more valid results. Thereafter, we derived likelihood ratios (LRs) with 95% CIs from the pooled sensitivities and specificities for each outcome reported.[16] LRs indicate by how much a given test result raises or lowers the probability of having the disease: they therefore permit the interpretation of the clinical significance of the results.[17] The LR of a positive test is the ratio of the probability of a positive biomarker test result in women who subsequently have a spontaneous preterm birth to the probability of the positive biomarker test result in women who subsequently do not have a preterm birth (sensitivity/1 − specificity). The LR of a negative test is the ratio of the probability of a negative biomarker test result in women who subsequently have a spontaneous preterm birth to the probability of the negative biomarker test result in women who subsequently do not have a preterm birth [(1 − sensitivity)/specificity]. LRs for a positive test result above 10 and LRs for a negative test result below 0.1 are recognised as convincing predictive evidence. Moderate prediction can be achieved with LR values of

5–10 and 0.1–0.2, whereas values below 5 and above 0.2 give only minimal prediction.[17]

We used LRs generated from meta-analyses to determine the post-test probabilities of spontaneous preterm birth before 32, 34, and 37 weeks of gestation, for positive and negative biomarker test results, as follows:[17]

$$\text{post-test probability of preterm birth} = \frac{\text{likelihood ratio} \times \text{pre-test probability}}{[1 - \text{pre-test probability} \times (1 - \text{likelihood ratio})]}.$$

Estimates of pre-test probabilities of preterm birth before 32, 34, and 37 weeks of gestation were obtained from the global prevalence of these outcomes across the studies.

Heterogeneity of the results among studies was investigated through a visual examination of forest plots of sensitivities and specificities, and ROC plots. In addition, heterogeneity was assessed by means of the quantity I^2, which describes the percentage of total variation across studies resulting from heterogeneity rather than chance.[18] A substantial level of heterogeneity is defined as an I^2 statistic value of 50% or more.[18] We explored potential sources of heterogeneity by performing meta-regression analysis of subgroups defined *a priori*.[19] These were study setting, patient characteristics, sample size, sampling frequency, and study quality.

We assessed publication and related biases visually by examining the symmetry of funnel plots (log diagnostic odds ratios versus the inverse of variance), and statistically by using the Egger's regression test;[20] $P < 0.1$ indicated significant asymmetry.

The bivariate models were fitted using the NLMIXED procedure (SAS 9.1 for Windows; SAS Institute, Inc., Cary, NC, USA). The summary ROC curves were constructed using REVMAN v5.0.20. The remaining analyses were performed using SPSS v15.0 (SPSS Inc, Chicago, IL, USA).

Results

Box 1 lists novel biomarkers proposed for the prediction of spontaneous preterm birth. The searches yielded 3522 citations, of which 384 were considered to be relevant (Figure 1). Three hundred and twelve studies were excluded, mainly because there were insufficient data to construct 2×2 tables (22%), biomarkers were evaluated in women with preterm labour (20%), non-spontaneous preterm birth was included in the outcome measures (17%), and because there was a lack of original data (15%). References for excluded studies can be obtained from the authors.

A total of 72 studies (41 cohort and 31 case–control studies), including 89 786 women, and evaluating a total of 30 novel biomarkers, met the inclusion criteria.[21–92] The main characteristics of the studies included in the systematic review are presented in Table S1. Twenty-five studies (35%) were conducted in the USA, and the remaining 47 took place in 20 countries worldwide. The sample size in the cohort studies ranged from 18 to 50 092 (median, 141 women).[66,85] The number of case participants enrolled in case–control studies ranged from 5 to 882,[55,91] and the corresponding number of controls ranged from 5 to 1372.[55,91] Thirty-nine studies ($n = 18\,368$ women) reported on 18 inflammation-related biomarkers,[21–59] 28 ($n = 71\,609$ women) reported on eight placental proteins/hormone-related biomarkers,[60–87] three ($n = 359$ women) reported on two angiogenesis-related biomarkers,[88–90] two ($n = 971$ women) reported on one coagulation-related biomarker,[58,91] and one ($n = 10$ women) reported on proteomics-related biomarkers.[92] Eleven studies evaluated more than one test.[22,25,26,34,35,38,50,54,55,58,59] One study reported on both inflammation- and coagulation-related biomarkers.[58] The median number of studies per biomarker was two (range 1–14).

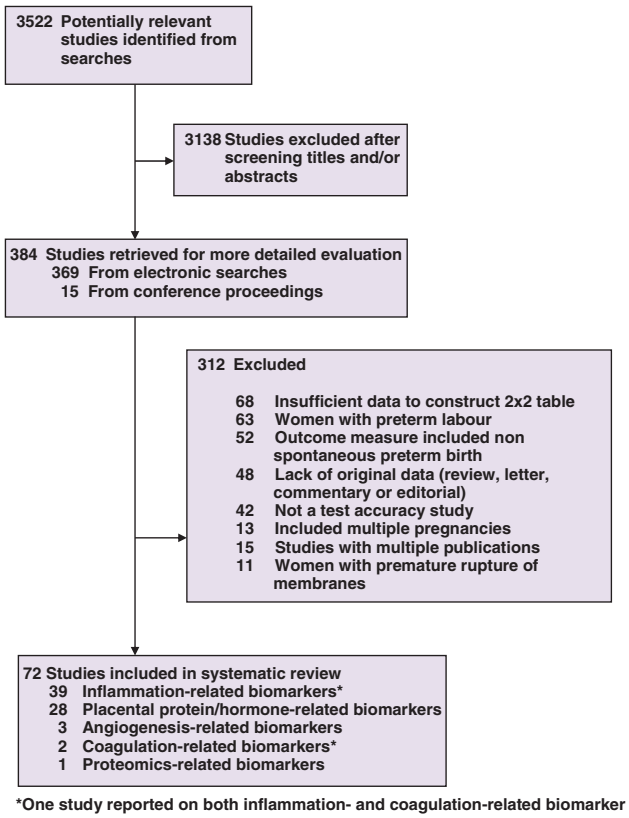

Figure 1. Study selection process.

Box 1. Novel biomarkers for the prediction of the spontaneous preterm birth phenotype identified in the literature

1. Inflammation-related biomarkers
Interleukins-2, -6, -8, and -10
Tumor necrosis factor-α
Granulocyte colony-stimulating factor
Stromal cell-derived factor-1α
Interferon-γ
Matrix metalloproteinase-8
A disintegrin and metalloprotease-8 and -12
Elastase
Secretory leukocyte proteinase inhibitor
Soluble vascular cell adhesion molecule-1
Soluble intercellular adhesion molecule-1
C-reactive protein
Ferritin
Alkaline phosphatase
2. Placental proteins/hormone-related biomarkers
Human chorionic gonadotropin
Phosphorylated insulin-like growth factor binding protein-1
Prolactin
Relaxin
Pregnancy-associated plasma protein A
Placental protein 13
Activin A
Pregnancy-specific β-1-glycoprotein
3. Angiogenesis-related biomarkers
Angiogenin
Soluble endoglin
4. Coagulation-related biomarkers
Thrombin-antithrombin III complex
5. Proteomics-related biomarkers
Desmoplakin isoform-1
Stratifin
Thrombospondin-1 precursor
6. Genetic biomarkers

Biomarkers in the following biological samples were included in the review: serum and/or plasma (34 studies), cervicovaginal secretions (23 studies), and amniotic fluid (16 studies). Biomarkers were evaluated at the following gestational ages: before 15 weeks of gestation (11 studies); 15–20 weeks of gestation (22 studies); 20–36 weeks of gestation (30 studies); serially at two gestational periods (six studies); serially at three gestational periods (one study); serially at five gestational periods (one study); and unreported (one study). Fifty-nine studies provided data on preterm birth before 37 weeks of gestation, eight provided data on preterm birth before 35 weeks of gestation, 19 provided data on preterm birth before 34 weeks of gestation, one provided data on preterm birth before 33 weeks of gestation, and 11 provided data on preterm birth before 32 weeks of gestation.

The methodological quality of studies included in the review is summarised in Figure 2. Only 19 studies (26%) met all five criteria. Data were collected prospectively in 60% of the studies and retrospectively in 26%; in the other 14%, the type of data collection was not described. In 57% of the studies, women were included consecutively. The great majority of studies (96%) reported sufficient details regarding the execution of the test, whereas in 47% of the studies it was unclear whether test results were provided to caregivers.

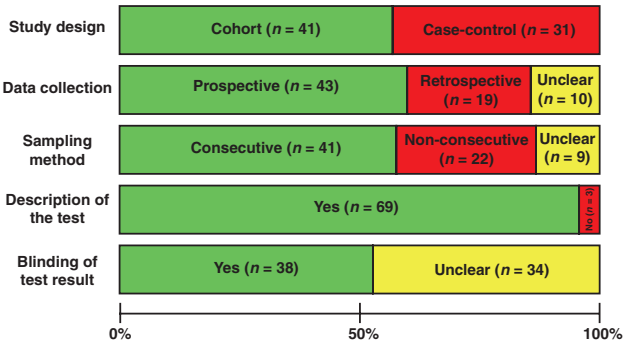

Figure 2. Methodological quality of studies included in the systematic review.

Inflammation-related biomarkers

Fourteen studies evaluated C-reactive protein,[21,26,34,36,37,45–48,50,52–54,58] 13 studies evaluated interleukin-6,[22,23,27,29,33–35,38,44,49,55,56,59] 10 studies evaluated ferritin,[25,26,28,31,34,38–41,54] four studies evaluated tumour necrosis factor-α (TNF-α),[22,24,55,59] three studies evaluated interleukin-8,[32,42,43] and eight studies evaluated several other inflammatory biomarkers, including granulocyte colony-stimulating factor (G-CSF),[30,55] soluble intercellular adhesion molecule-1 (sICAM-1),[34,50] alkaline phosphatase,[25,34] interleukin-2,[55] interleukin-10,[34] stromal cell-derived factor-1α (SDF-1α),[57] interferon-γ,[55] matrix metalloproteinase-8 (MMP-8),[35] a disintegrin and metalloprotease (ADAM)-8 and -12,[50,51] elastase,[50] secretory leukocyte proteinase inhibitor (SLPI),[50] and soluble vascular cell adhesion molecule-1 (sVCAM-1).[50]

Table S2 presents pooled estimates of predictive accuracy of inflammation-related biomarkers for spontaneous preterm birth. For serum levels of interleukins-2, -6 and -10, TNF-α, G-CSF, interferon-γ, ADAM-12, sICAM-1, C-reactive protein, ferritin, and alkaline phosphatase, the pooled sensitivities, specificities, and positive and negative LRs for predicting preterm birth before 32, 34, and 37 weeks of gestation ranged between 3 and 49% (median, 24%), between 51 and 97% (median, 77%), between 0.4 and 4.5 (median, 1.1), and between 0.6 and 1.3 (median, 1.0), respectively. For cervicovaginal levels of interleukins-6 and -8, TNF-α, and ferritin, the pooled sensitivities, specificities, and positive and negative LRs varied from 24 to 44% (median, 35%), from 75 to 93% (median, 83%), from 1.1 to 4.0 (median, 2.1), and from 0.6 to 1.0 (median, 0.8),

respectively. For amniotic fluid levels of interleukin-6, TNF-α, SDF-1α, MMP-8, ADAM-8, elastase, SLPI, sVCAM-1, sICAM-1, and C-reactive protein, the pooled sensitivities, specificities, and positive and negative LRs ranged from 12 to 86% (median, 54%), from 43 to 99% (median, 83%), from 0.9 to 40.0 (median, 2.8), and from 0.2 to 1.1 (median, 0.6), respectively. Preterm birth before 37 weeks of gestation was best predicted by amniotic fluid levels of interleukin-6 (four studies; 313 women; pooled positive and negative likelihood ratios of 6.1 and 0.2, respectively) and MMP-8 (one study; 114 women; positive and negative likelihood ratios of 40.0 and 0.6, respectively).

Placental protein/hormone-related biomarkers

Nine studies provided data on cervicovaginal phosphorylated insulin-like growth factor binding protein-1 (phIGFBP-1),[65–67,69,72–74,77,79] seven studies provided data on relaxin,[34,60,61,70,71,75,78] three studies provided data on cervicovaginal human chorionic gonadotrophin (hCG),[63,68,76] two studies provided data on cervicovaginal prolactin,[62,64] four studies provided data on pregnancy-associated plasma protein-A (PAPP-A),[81,84,85,87] two studies provided data on activin-A,[80,82] and one study each provided data on placental protein-13 (PP13)[83] and pregnancy-specific-β1 glycoprotein (SP1).[86] For cervicovaginal levels of hCG, phIGFBP-1, and prolactin, the pooled sensitivities, specificities, positive and negative LRs to predict preterm birth before 32, 34, and 37 weeks of gestation ranged between 33 and 83% (median, 67%), 62 and 97% (median, 79%), 1.6 and 19.0 (median, 3.2), and 0.2 and 0.8 (median, 0.5), respectively (Table 1). Cervicovaginal prolactin (one study; 40 women) and phIGFBP-1 (one study; 104 women) had the best LRs to predict preterm birth before 34 weeks of gestation (positive and negative LRs of 19.0 and 0.5, and 6.4 and 0.2, respectively). These biomarkers were less accurate for predicting preterm birth before 37 weeks of gestation. Serum levels of PAPP-A, PP13, activin A, and SP1 had a minimal predictive accuracy for preterm birth before 32, 34, and 37 weeks of gestation (pooled positive and negative LRs between 0.8 and 4.1, and between 0.7 and 1.0, respectively).

Angiogenesis- and coagulation-related biomarkers

Two studies reported data on angiogenin,[88,89] two studies reported data on the thrombin-antithrombin III complex,[58,91] and one study reported data on soluble endoglin.[90] The predictive accuracy of these biomarkers for preterm birth before 34 and 37 weeks of gestation was minimal to moderate (positive and negative LRs between 1.1 and 6.7, and between 0.4 and 1.0, respectively).

Proteomics-related biomarkers

One small, nested case–control study ($n = 10$ women) identified and quantified, by liquid chromatography/tandem mass spectrometry, an entire set of proteins (proteomic profile) in cervicovaginal fluid collected at 24–28 weeks of gestation, as potential biomarkers of preterm birth.[92] Three proteins (desmoplakin isoform-1, stratifin, and thrombospondin-1 precursor) were significantly elevated in women who delivered between 28 and 32 weeks of gestation, compared with the controls at >37 weeks of gestation. By using a peak area ratio of 0.05 as the cut-off value, we calculated sensitivities and specificities of 100% for both desmoplakin isoform-1 and stratifin to predict preterm birth before 32 weeks of gestation. For thrombospondin-1 precursor, the corresponding values were 60 and 100%, respectively.

Post-test probabilities of preterm birth

Table 2 summarises pooled estimates of pre- and post-test probabilities of having spontaneous preterm birth for biomarkers with a positive LR ≥ 5 or a negative LR ≤ 0.2. The overall prevalence rates (pre-test probabilities) of spontaneous preterm birth before 32, 34, and 37 weeks of gestation were 2.0, 4.3, and 12.4%, respectively. The prevalence of preterm birth before 37 weeks of gestation found in our review was very similar to that from vital USA statistics (12.7%).[93] High concentrations of MMP-8 in amniotic fluid, the biomarker with the highest LR for a positive test result, increased the pre-test probability of preterm birth before 37 weeks of gestation from 12.4 to 85.0%. Conversely, low concentrations of interleukin-6 and TNF-α in amniotic fluid and prolactin in cervicovaginal fluid, the biomarkers with the lowest LRs for a negative test result, decreased the risk to 2.8%. High levels of prolactin in cervicovaginal fluid increased the pre-test probability of preterm birth before 34 weeks of gestation from 4.3 to 46.1%, whereas low levels of phIGFBP-1 in cervicovaginal fluids decreased the risk to 0.8%. Expression at high levels of two proteins (desmoplakin isoform-1 and stratifin) from a proteome profile of cervicovaginal fluid increased the pre-test probability of preterm birth before 32 weeks of gestation from 2.0 to almost 100.0%, whereas expression at low levels decreased the risk to 0.0%.

There was a substantial level of heterogeneity ($I^2 \geq 50\%$) among studies in 12 of 27 meta-analyses performed. Meta-regression analysis showed that heterogeneity was mainly explained by study design, sample size, and study quality. Studies with case–control design, small sample size, and lower methodological quality tended to overestimate the accuracy of the predictive test. All funnel plots showed no asymmetry, either visually or statistically ($P > 0.10$ for all, by Egger test).

Discussion

The ideal clinical biomarker to predict spontaneous preterm birth should identify women who present with all

Table 1. Predictive accuracy of other biomarkers for spontaneous preterm birth

Biomarker	Biological sample	Outcome: spontaneous preterm birth (weeks)	No. of studies	No. of women	Pooled sensitivity (%) (95% CI)	Pooled specificity (%) (95% CI)	Positive likelihood ratio (95% CI)	Negative likelihood ratio (95% CI)	I^2 (%)
Placental proteins/hormone-related biomarkers									
hCG	Cervicovaginal fluid	<37	1[76]	75	70 (48–85)	62 (49–73)	1.8 (1.2–2.9)	0.5 (0.2–1.0)	NA
		<34	3[63,68,76]	656	67 (59–73)	87 (83–89)	5.0 (3.9–6.4)	0.4 (0.3–0.5)	0
phIGFBP-1	Cervicovaginal fluid	<37	9[65–67,69,72–74,77,79]	6003	33 (28–39)	79 (78–80)	1.6 (1.3–1.9)	0.8 (0.8–0.9)	79
		<34	1[72]	104	83 (55–95)	87 (79–92)	6.4 (3.6–11.5)	0.2 (0.1–0.7)	NA
		<32	1[72]	4984	54 (35–71)	76 (74–77)	2.2 (1.5–3.2)	0.6 (0.4–0.9)	NA
Prolactin	Cervicovaginal fluid	<37	1[64]	40	83 (44–97)	74 (57–85)	3.2 (1.6–6.1)	0.2 (0.1–1.4)	NA
		<34	1[62]	40	50 (9–91)	97 (87–100)	19.0 (1.8–205.2)	0.5 (0.1–2.1)	NA
Relaxin	Blood	<37	5[60,61,70,75,78]	1749	38 (31–45)	58 (56–61)	0.9 (0.8–1.1)	1.1 (1.0–1.2)	69
		<34	3[34,71,75]	1249	22 (16–29)	45 (42–48)	0.4 (0.3–0.5)	1.7 (1.5–1.9)	71
		<32	1[34]	100	10 (4–21)	90 (79–96)	1.0 (0.3–3.2)	1.0 (0.9–1.1)	NA
PAPP–A	Blood	<37	4[81,84,85,87]	61 768	11 (10–12)	93 (93–93)	1.6 (1.4–1.8)	1.0 (0.9–1.0)	15
		<34	2[85,87]	55 565	13 (11–15)	94 (93–94)	2.0 (1.7–2.4)	0.9 (0.9–1.0)	61
PP-13	Blood	<37	1[83]	335	28 (17–43)	90 (86–93)	2.8 (1.6–5.0)	0.8 (0.6–1.0)	NA
Activin-A	Blood	<37	2[80,82]	478	15 (11–21)	88 (83–91)	1.2 (0.8–1.9)	1.0 (0.9–1.1)	84
		<34	1[80]	240	14 (9–22)	88 (81–93)	1.2 (0.6–2.4)	1.0 (0.9–1.1)	NA
		<32	1[80]	94	9 (3–20)	89 (77–95)	0.8 (0.2–2.8)	1.0 (0.9–1.2)	NA
SP1	Blood	<37	1[86]	588	41 (31–51)	90 (87–92)	4.1 (2.8–5.9)	0.7 (0.5–0.8)	NA
Other biomarkers									
Angiogenin	Amniotic fluid	<37	2[35,89]	164	50 (31–69)	89 (82–93)	4.4 (2.4–8.1)	0.6 (0.4–0.9)	73
		<34	2[88,89]	93	60 (36–80)	91 (83–96)	6.7 (2.9–15.2)	0.4 (0.2–0.8)	0
Endoglin	Blood	<37	1[90]	259	38 (26–52)	75 (69–81)	1.6 (1.0–2.4)	0.8 (0.6–1.0)	NA
Thrombin-antithrombin III complex	Blood	<37	2[58,91]	971	43 (38–49)	59 (55–63)	1.1 (0.9–1.2)	1.0 (0.9–1.1)	84
		<34	1[91]	248	52 (34–69)	77 (71–82)	2.3 (1.5–3.5)	0.6 (0.4–0.9)	NA
Proteomic profile									
Desmoplakin isoform-1	Cervicovaginal fluid	<32	1[92]	10	100 (57–100)	100 (57–100)	∞ (∞–∞)	0.0 (0.0–0.0)	NA
Stratifin	Cervicovaginal fluid	<32	1[92]	10	100 (57–100)	100 (57–100)	∞ (∞–∞)	0.0 (0.0–0.0)	NA
Thrombospondin-1 precursor	Cervicovaginal fluid	<32	1[92]	10	60 (23–88)	100 (57–100)	∞ (1.4–∞)	0.4 (0.1–1.2)	NA

CI, confidence interval; hCG, human chorionic gonadotropin; NA, not applicable; PAPP-A, pregnancy-associated plasma protein A; phIGFBP-1, phosphorylated insulin-like growth factor binding protein-1; PP-13, placental protein 13; SP1, pregnancy-specific β-1-glycoprotein.

Table 2. Pre- and post-test probabilities of spontaneous preterm birth for biomarkers with a positive likelihood ratio ≥5 or a negative likelihood ratio ≤0.2

Biomarker (no. of studies; no. of women)	Biological sample	Outcome: spontaneous preterm birth (weeks)	Pre-test probability (%)	Summary likelihood ratios (95% CI)		Post-test probabilities (%)	
				Positive test result	Negative test result	Positive test result	Negative test result
Interleukin-6 (4; 313)	Amniotic fluid	<37	12.4	6.1 (4.4–8.4)	0.2 (0.1–0.3)	46.3 (38.3–54.3)	2.8 (1.4–4.1)
Tumour necrosis factor-α (1; 144)	Amniotic fluid	<37	12.4	3.9 (2.6–5.9)	0.2 (0.1–0.4)	35.6 (26.9–45.5)	2.8 (1.4–5.4)
Matrix metalloproteinase-8 (1; 114)	Amniotic fluid	<37	12.4	40.0 (5.3–301.4)	0.6 (0.4–0.9)	85.0 (42.9–97.7)	7.7 (5.4–10.9)
Human chorionic gonadotropin (3; 656)	Cervicovaginal fluid	<34	4.3	5.0 (3.9–6.4)	0.4 (0.3–0.5)	18.3 (14.9–22.3)	1.7 (1.4–2.1)
Phosphorylated insulin-like growth factor binding protein-1 (1; 104)	Cervicovaginal fluid	<34	4.3	6.4 (3.6–11.5)	0.2 (0.1–0.7)	22.3 (13.9–34.1)	0.8 (0.2–3.0)
Prolactin (1; 40)	Cervicovaginal fluid	<37	12.4	3.2 (1.6–6.1)	0.2 (0.1–1.4)	31.2 (18.5–46.3)	2.8 (1.4–16.5)
		<34	4.3	19.0 (1.8–205.2)	0.5 (0.1–2.1)	46.1 (7.5–90.2)	2.2 (0.6–8.5)
Angiogenin (2; 93)	Amniotic fluid	<34	4.3	6.7 (2.9–15.2)	0.4 (0.2–0.8)	23.1 (11.5–40.6)	1.9 (1.1–3.6)
Proteomic profile: desmoplakin isoform-1 and stratifin	Cervicovaginal fluid	<32	2.0	∞ (∞–∞)	0.0 (0.0–0.0)	~100.0 (~100.0–~100.0)	0.0 (0.0–0.0)

CI, confidence interval.

subtypes of the condition. It should be measurable in a biological sample that is easily obtained from the mother, with minimal risk to her and her fetus. The test should ideally be inexpensive, reproducible, and easy to perform early in pregnancy to allow for potential interventions. It should also have a very high LR to increase the probability of spontaneous preterm birth in women with a positive test result, and a low LR to confidently exclude the disorder with a negative test result. Lastly, the required technological platform should be widely available.[94,95]

None of the 30 novel biomarkers evaluated in this review meets the criteria to be considered a clinically useful test to predict spontaneous preterm birth in women with singleton pregnancies and no symptoms of preterm labour. Three biomarkers (MMP-8 in amniotic fluid, and prolactin and proteome profile in cervicovaginal fluid) had LRs for a positive test result that were greater than 10. However, each of these biomarkers was evaluated in only one small study, with sample sizes ranging from 10 to 114. In addition, MMP-8 in amniotic fluid and prolactin in cervicovaginal fluid had high positive LRs, but at the expense of compromised negative LRs. Four biomarkers (interleukin-6 and angiogenin, in amniotic fluid; hCG and phIGFBP-1, in cervicovaginal fluid) had a moderate predictive accuracy. The remaining novel biomarkers, including all those measured in plasma and serum, had a very low predictive accuracy.

The strength of our review is that, by complying with stringent criteria, we have performed a rigorous systematic review of predictive test accuracy. These criteria included the use of a prospective protocol designed to address a highly specific research question, an extensive search of relevant studies without language restrictions, a strict assessment of the methodological quality of the included studies, the use of techniques recently recommended for meta-analysis of diagnostic and predictive tests, studying sources of heterogeneity, and a quantitative assessment of the evidence. However, our study has some limitations. First, like any systematic review, it is limited by the quality of the studies included. The main cause of reduced quality was the apparent lack of blinding in almost half of the publications reviewed. A lack of blinding of test results is particularly relevant because women with abnormal results might have been followed more carefully or might have received therapy, both of which would have biased an evaluation of the test's predictive accuracy. Second, there were considerable differences in cut-off values for 'abnormal' results between studies evaluating the same test, which limited our ability to make comparisons. Third, there was significant heterogeneity in individual studies in 44% of the meta-analyses performed; therefore, pooled estimates of predictive accuracy should be interpreted very cautiously. Homogeneity is one of the desired prerequisites for

meta-analysis, but it is not an absolute requirement. We explored the sources of heterogeneity as thoroughly as possible: partial explanations were provided by the methodological quality of studies, as well as their design and sample size, as previously reported in other studies of diagnostic tests.[96,97] Fourth, there was no uniform definition of spontaneous preterm birth across the studies included. In some reports, spontaneous preterm birth was defined as birth before 37 weeks of gestation after spontaneous onset of labour or rupture of membranes; in other studies, the definition excluded cases of preterm birth after the prelabour rupture of membranes. This results in differences in the incidence of spontaneous preterm birth, and consequently in the post-test probabilities of the condition. Finally, the statistical power of the great majority of our meta-analyses was limited by the small number of studies evaluating several biomarkers and the relatively small sample size of the studies included.

There are a number of explanations for the relatively poor performance of the novel biomarkers evaluated in this review to predict spontaneous preterm birth adequately. First, this complex phenotype is now thought to be a syndrome initiated by multiple mechanisms, including infection/inflammation, uteroplacental ischaemia or haemorrhage, uterine overdistension, stress, and other immunologically mediated processes.[98] Therefore, given the pathophysiological heterogeneity of the condition, the limited clinical utility of any individual biomarker for predicting spontaneous preterm birth is understandable. Second, the usefulness of any biomarkers as a predictor of preterm birth should be considered relative to the gestational age at which the sample is collected. For example, it has been reported that some biomarkers such as alkaline phosphatase, ferritin, and fibronectin have much less predictive accuracy when measured at earlier gestational ages.[95] In our review, eight studies evaluated biomarkers serially at two or more gestational periods. We analysed data separately for each gestational period in these studies, and did not find significant differences in predictive values (data not shown). Third, there is some evidence that the predictive accuracy of some biomarkers differs with ethnicity. For example, amniotic fluid interleukin-1β and TNF-α concentrations were significantly higher in African American preterm birth cases than African American term controls, but there were no differences between term and preterm birth samples in European Americans.[99,100] In fact, the concentrations of interleukins-6 and -8 in the amniotic fluid were similar between preterm and term samples in African Americans, whereas they were significantly higher in European American cases compared with European American controls.[99,101] If these findings are confirmed, it would indicate that predictive biomarkers in this condition perform differently across ethnic populations and/or geographical locations. Fourth, novel biomarkers could have different predictive ability depending upon the gestational age at birth. For example, salivary oestriol has a better predictive accuracy for late compared with earlier preterm births.[102] Other factors that could affect the predictive accuracy of any marker include the baseline risk for spontaneous preterm birth in the population being studied and the frequency of sampling.

We identified a recent systematic review and meta-analysis in which the objective was to identify genetic associations with preterm birth.[103] The authors included data on 189 polymorphisms in 84 genes, and performed 36 meta-analyses. Of the 22 polymorphisms in 15 genes studied in meta-analyses for maternal DNA variants, four variants, including an 86-bp variable number of tandem repeats (VNTR) in the interleukin-1 receptor antagonist and three single nucleotide polymorphisms (SNPs) in the β-2-adrenergic receptor, interferon-γ, and coagulation factor-2 showed nominally significant summary odds ratios (1.4–1.8) with modest P values in the main (allele-based) analyses (0.01–0.04). Of the 14 polymorphisms in eight genes studied in meta-analyses of newborn DNA variants, the same SNP in coagulation factor-2 yielded a significant odds ratio of 1.8 ($P = 0.03$). Nevertheless, all five associations were graded as having only weak epidemiological credibility. Insufficient data were available to examine paternal genetic associations.

As no single biomarker has yet been shown to fulfil all the requirements of a clinically reliable predictive test for spontaneous preterm birth in women with singleton pregnancies and no symptoms of preterm labour, multiple-marker tests based on combinations of several independent markers have been proposed. Goldenberg et al.[34] evaluated a five-marker test (cervicovaginal fetal fibronectin, cervical length, and serum α-fetoprotein, G-CSF, and defensins or alkaline phosphatase) in the Preterm Prediction Study. Any two 'positive' tests had sensitivities and specificities of 59 and 98%, and 43 and 94%, to predict spontaneous preterm birth before 32 and 35 weeks of gestation, respectively. The combined use of markers showed better predictive accuracy than when used alone. In addition, the overlap between the markers was small, supporting the notion that there are several heterogeneous pathways that lead to spontaneous preterm birth. Further prospective, large-scale, longitudinal studies are needed to determine whether the combined use of novel biomarkers has more predictive accuracy than the use of individual biomarkers.

There tends to be variability in biomarker levels over time and/or between individuals, making it difficult to determine the threshold level at which the risk for spontaneous preterm birth is increased. By contrast, genetic susceptibility (if it genuinely exists in this condition) would be more stable, although it would be influenced by

environmental factors, such as infection. The systematic review by Dolan et al.[103] on the genetic associations with preterm birth revealed a paucity of research in the field, problems in phenotype definition, large variability in the gestational cut-off used to define preterm birth, a lack of power to detect modest effect sizes, and inconsistency in the exclusions of preterm births because of maternal or fetal indications. The combination of data in the meta-analyses performed did not yield large enough sample sizes, and all of the five nominally significant associations that were identified were graded as having weak credibility. More systematic and unbiased genetic approaches using consistent phenotypic definitions and involving low-resource countries are needed in the future to examine the predictive accuracy of genetic markers for spontaneous preterm birth.

Proteomics is a newly developed field of research that studies the global set of proteins and their expression, function, and structure in a tissue, cell, or organism at a given moment.[104,105] In recent years, proteomics has been extensively used to search for biologically relevant biomarkers and to generate protein profiles characteristic of intra-amniotic infection/inflammation and preterm birth.[106–108] Nevertheless, up to now, only one study has evaluated proteomics as a potential biomarker to predict spontaneous preterm birth. Shah et al.[92] randomly selected samples of cervicovaginal fluid collected at 24–28 weeks of gestation from five women who delivered preterm between 28 and 32 weeks of gestation following spontaneous preterm labour, and five women who delivered after 37 weeks of gestation. A proteome profile was then generated to characterise and quantify the proteins present in cervicovaginal fluid. The identified proteins were screened to provide a set of 15 candidates that had either previously been proposed as potential biomarkers for spontaneous preterm birth or could be linked mechanistically to preterm birth. Three proteins (desmoplakin isoform-1, stratifin, and thrombospondin-1 precursor) were significantly elevated in women who later experienced preterm birth. From a scatter-plot graph for the peak area ratios computed for these proteins, we calculated specificities of 100% for the three proteins, and sensitivities of 100% for desmoplakin isoform-1 and stratifin, and 60% for thrombospondin-1 precursor. Although these preliminary results are encouraging, they need to be confirmed in a large, prospective cohort study.

In conclusion, none of the novel biomarkers evaluated in this review are clinically useful for predicting spontaneous preterm birth. It should be stressed that only when the use of a biomarker and subsequent treatment have been shown to result in a significant reduction in spontaneous preterm birth should any single or multiple marker test be introduced into routine clinical practice.[95]

Disclosure of interests
None.

Contribution to authorship
All authors contributed to the planning and design of the study. AC-A and JV were involved in the acquisition of data, and undertook the initial analysis and interpretation of data. All authors subsequently assisted in drafting and revising the manuscript. The final version of the manuscript was approved by all authors.

Details of ethics approval
No ethics approval was required.

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Supporting information

The following supplementary materials are available for this article:

Table S1. Characteristics of studies included in the systematic review.

Table S2. Predictive accuracy of inflammation-related biomarkers for spontaneous preterm birth.

Additional supporting information may be found in the online version of this article.

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