Accuracy of fetal fibronectin for assessing preterm birth risk in asymptomatic pregnant women: a systematic review and meta-analysis

Running headline: Fetal fibronectin in asymptomatic women

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Abstract

Introduction

Fetal fibronectin (fFN) is a validated test for assessing risk of preterm birth (PTB) for women presenting with symptoms. Our aim was to evaluate the accuracy of fFN to detect the risk of PTB in asymptomatic women.

Material and methods

Searches were conducted to identify studies where fFN was performed in asymptomatic women beyond 22 weeks' gestation. EMBASE, MEDLINE, CINHAL, AMED and BNI were searched between 2005 and 2017. Studies before 2005 were identified from a published systematic review. Women were grouped as singleton pregnancies, with and without risk factors for PTB, and multiple pregnancy. Quality assessment was performed using QUADAS-2. When possible, data were pooled using a hierarchical, bivariate random effects model.

Results

Fifteen studies met the inclusion criteria: six studies of singleton pregnancies in women without risk factors (1,236 women), four in women with risk factors for PTB (2,628 women) and five studies were of multiple pregnancy (1,427 women). The pooled sensitivity and specificity of fFN in 'no risk factors singletons' were 0.48 (95% CI 0.20–0.77), and 0.96 (95% CI 0.86–0.99) respectively. The likelihood ratio of a positive test result was 12 (95% CI 4.70-30.68). The pooled sensitivity and specificity of fFN in 'risk factors singletons' were 0.34 (95% CI 0.24–0.43), and 0.91 (95% CI 0.88–0.93). Accuracy of fFN in multiple pregnancies was inconclusive.

Conclusion

Our findings suggest in asymptomatic singleton pregnancies without risk factors a positive fFN result indicates a large shift from pre to post-test probability, possibly identifying women at increased risk of PTB.

Keywords: fetal fibronectin, test accuracy, preterm birth, asymptomatic, meta-analysis

List of Abbreviations

PTB preterm birth

fFN fetal fibronectin

Key Message

Fetal fibronectin is an onsite test available evaluating the risk for preterm birth. Its performance in asymptomatic women is undetermined. A positive result in women carrying a singleton pregnancy without risk factors for preterm birth may predict early delivery.

Introduction

Preterm birth (PTB) is defined by the World Health Organisation as 'birth occurring prior to 37 completed weeks of gestation' (1) and is the leading cause of perinatal morbidity and mortality worldwide, in both singleton and multiple pregnancies (2, 3). Spontaneous preterm births occurs in 60-70% of all preterm births (4) with approximately 15 million babies born preterm in 2010 (1). The incidence of PTB varies widely ranging from 5-13% (3, 5). The neonatal morbidity, its long-term sequelae and the mortality associated with PTB have huge implications for clinical resources and convey a significant economic burden (4). Although the rates of survival have improved (3), the rate of disability for survivors has remained unchanged (4, 6).

None of the available scoring systems accurately identify asymptomatic women at risk of spontaneous PTB (4, 7). Identifying asymptomatic women at risk of PTB could be beneficial, as it would allow for timely interventions to reduce perinatal morbidity, such as the administration of corticosteroids and access to neonatal intensive care facilities (5). An objective test for predicting risk of PTB is fetal fibronectin (fFN), an adhesion basement membrane protein found in cervicovaginal secretions (8, 9). fFN levels are routinely found in cervicovaginal secretions up to 20 weeks of gestation but after this, it is rarely found until late in pregnancy and can be an indication of impending labour (9). fFN has been developed as a bedside test for identifying women at risk of preterm labour and it is widely used in high-income settings, such as the United Kingdom (10) for triaging women with symptoms. The aim of this review was to determine the accuracy of fFN for identifying the risk of PTB in asymptomatic pregnant women, as the published data in this area have not been systematically synthesised.

Material and Methods

This review was conducted in compliance with the current standards for test accuracy research (11, 12); reported according to the Preferred Reporting Items for Systematic Reviews (PRISMA) guidelines (13) and recommendations of reporting test accuracy systematic reviews (14). It was prospectively registered (PROSPERO number: CRD42015023779).

Literature search

A systematic search of primary studies in EMBASE, MEDLINE, CINHAL, AMED and BNI was undertaken prospectively and limited to studies published between 2005 and 2015. The initial search was performed on 29 January 2015 and then updated on 01 February 2017 with no language restrictions. The following terms, associated synonyms and right-hand truncation were used for the searches: 'pregnancy', 'antenatal', 'fetal proteins' and 'fibronectins' (Supporting Information, Table S1). The database search was complemented by the comprehensive reference check of the included studies. Relevant studies published prior to 2005 were identified from a previous comprehensive search published in a Health Technology Assessment report in 2009 (4). Studies of cross-sectional, longitudinal and case-control design studies were included.

Study selection and data extraction

Two reviewers (FDS and JD) independently reviewed titles and abstracts and identified citations fulfilling the pre-determined selection criteria. Full text versions of the selected citations were reviewed for their eligibility. Any disagreements were resolved through consensus. The inclusion criteria were: i) pregnant women carrying either singleton or multiple pregnancies without symptoms of PTB; ii) fFN sampling (index test) undertaken after 22 weeks of gestation using a validated method (15, 16); and, iii) the use of a threshold of ≥50 ng/ml for a positive test, as per the manufacturer's instructions (15). When studies used serial testing, a woman was considered to have a positive fFN test if a level of ≥50 ng/ml was documented at any point during the testing period. If a study reported multiple thresholds, the data for the ≥50 ng/ml cut-off were used.

Studies including women identified as having symptoms of preterm labour (such as uterine contractions, preterm premature rupture of membranes or insertion of a rescue cervical cerclage) were excluded. The reference standard for PTB was defined as birth prior to 37 weeks of gestation (3).

Data were extracted onto a piloted data extraction sheet independently and in duplicate by two reviewers (FDS and JD). Data were collected on population characteristics (age, ethnicity, education, parity, smoking status, risk factors for PTB), description of the index test including the threshold used for a positive test, how the gestational age at testing was

defined, the definition of PTB used by the study authors and the results of the index test (true positives, true negatives, false positives and false negative) to allow creation of a 2x2 table. Women with a singleton pregnancy were grouped according to the presence of risk factors for PTB (with or without). The third group comprised women with multiple pregnancies (twins or triplets). The considered risk factors for PTB were: previous PTB, smoking or other risk factors as defined in the primary studies. All data were tabulated, crosschecked and any discrepancies were discussed between reviewers, involving a third reviewer (ER) when necessary. The risk of bias and applicability of the included studies were assessed independently by two reviewers (FDS and JD) using the QUADAS-2 tool (12). The applicability is determined by how similar or different are the population, the index test and the reference standard to that of the review question (12).

Data synthesis

The data were plotted using RevMan (*Version 5.3. Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014*) and, where possible, pooled using STATA software (*StataCorp. 2011. Version 12. College Station, TX: StataCorp LP*) within the predefined subgroups. When a sufficient number of studies were available, we pooled the accuracy parameters using a bivariate, hierarchical model (random effect) (17). If fewer than four studies were available, a univariate model was used (18). We calculated sensitivity, specificity and likelihood ratios for positive and negative test results with 95% confidence intervals (CIs). Heterogeneity was investigated visually from forest plots of sensitivity and specificity estimates derived from individual studies. Sensitivity analysis concerning the type of used analyser was conducted as a part of a post-hoc exploration. We did not attempt to assess publication bias due to the lack of consensus over the reliability of currently available methods (19, 20). Moreover, we did not have a sufficient number of studies in order to run a meaningful assessment of publication bias (21).

Results

There were 2,020 studies identified following the electronic search and 18 studies identified from the Health Technology Assessment report (4). After duplicates removal, 516 studies were identified and screened for their eligibility. Ninety full-text papers were reviewed, and 15 studies were subsequently included (Figure 1) (16, 22-35). The inter-rater reliability for the study selection was good (κ=0.8).

Included studies recruited women with a history of previous PTB (n = 5), (16, 22, 24, 25, 28) women who were smokers (n = 2), (22, 25) women with cervical cerclage or women who were being followed-up in preterm birth surveillance clinics for other risk-factors (n = 6) (16, 22, 24, 25, 28, 29). There was variation in the devices used by studies included in this review for measuring fFN. Seven studies used *Adeza Biomedical, Sunnyvale®,* (16, 26-28, 32-34) five studies used *Hologic®* (22, 25, 30, 31, 35) and three studies (23, 24, 29) did not provide information regarding the device used. Ten studies collected samples for fFN testing according to the manufacturer's instructions ('standard' fFN). Five studies used a blind method for obtaining samples for fFN testing ('blind' fFN) (Table 1).

There was also variation in the timing of sampling. In singleton pregnancy studies, six used serial sampling (timing not specified in one study (28); every 2, 2-3, 2-4 or every 2-6 weeks in the five remaining studies (16, 25, 27, 32, 33) with samples collected between 22 and 36 weeks of gestation. Three studies used a single sample of fFN taken between 22-28, 26-28 or at 28 weeks of gestation (22, 23, 26). For the twin studies, fFN testing was performed every 2-4 weeks between 22-32 weeks of gestation in all three included studies. Two studies included women with a triplet pregnancy both used serial testing every 2-3 weeks from 22-32 weeks of gestation. All the studies except one (22) used a pre-specified threshold of ≥50 ng/ml to denote a positive test result.

The reference standard used was gestation at birth. There was variation in the way the reference standard was calculated. Ten of studies used the last menstrual period to calculate the gestational age, which was confirmed by a first or second trimester ultrasound. The ultrasound result took precedence if there was a discrepancy (22-24, 26, 28, 30-34). Five studies did not specify how the gestational age was calculated (16, 25, 27, 29, 35). Full details of studies included in this systematic review are presented in Supporting Information, Table S2.

Quality Assessment

The risk of bias in all four domains of the QUADAS-2 tool was considered to be low in the majority of the studies. There were also low applicability concerns for most of the studies (Figure 2). Quality assessment for singleton pregnancy studies is presented for those studies included in the meta-analysis only.

In the studies of fFN testing in women carrying singleton pregnancies without risk factors, the risk of bias due to the implementation of the index test and reference standard was assessed as low in the majority of the studies (5/6). In each QUADAS-2 domain, there was one study classified as at high risk of bias. The concern over the applicability of the studies was presented with respect to patient selection, index test and reference standard was assessed as low in the majority of the included studies.

All three studies of women carrying singleton pregnancies with risk factors for PTB were classified as low risk of bias for the index test, reference standard, and flow and timing (Figure 2). In one study (28), the description of patient selection raised concerns; hence the study was classified as high risk of bias in this domain. There was no concern over the applicability in any of the studies with respect to patient selection or how the index test was taken. In two cases (25, 28) the description was insufficient to assess the studies applicability with respect to the reference standard.

The majority of studies with twin or triplet pregnancies were classified as at low risk of bias and their applicability to the review question (Figure 2). In one study (24) the description of patient selection raised concerns leading to its classification as at high risk of bias in this domain. Two out of five studies were labelled as high risk of bias due to the implementation of index test. One study was assessed to be at high risk of bias over applicability of the index test (24).

Accuracy of fFN in asymptomatic singletons without risk factors for PTB

Nine studies reported accuracy data for fFN in singleton pregnancies of which six (1,236 women) (23, 26, 27, 32-34) included women without risk factors for PTB (Figure 3). The pooled sensitivity and the specificity were 0.48 (95% CI 0.20–0.77), and 0.96 (95% CI 0.86–0.99) (Figure 4). The visual inspection of heterogeneity showed a greater variability in the sensitivity measures than the specificity. The 95% prediction region covered over half of the ROC space. The calculated likelihood ratio of a positive test result was 12.01 (95% CI 4.70-30.68), the likelihood ratio of a negative test result was 0.54 (95% CI 0.30-0.97). The sensitivity analysis with studies that used only Adeza Biomedical analyser showed a change in fFN sensitivity 0.60 (95% CI 0.34–0.81) with a minor change in the pooled specificity 0.93 (95% CI 0.83–0.97).

Accuracy of fFN in asymptomatic singletons with risk factors for PTB

Three studies included women carrying singleton pregnancy with risk factors for PTB contributed to our meta-analyses (22, 25, 28). One study (16), was excluded from the meta-analyses due to the definition of the reference standard used (PTB prior to 34 weeks of gestation). The studies all included women with a previous history of PTB, two included women who were smokers and all included women with other risk factors such as previous second trimester miscarriage, previous cervical surgery, women with an incidental short cervix and women with a cervical cerclage in situ or history of a previous cerclage. The pooled sensitivity and the specificity were 0.34 (95% CI 0.24-0.43), and 0.91 (95% CI 0.88-0.93). The likelihood ratio of a positive test result was 3.47 (95% CI 2.84-4.24). The likelihood ratio of a negative test result was 0.75 (95% CI 0.68-0.82).

Accuracy of fFN in asymptomatic women with multiple pregnancy

None of the studies that evaluated fFN in women with multiple pregnancies used a reference standard defined as birth before 37 weeks. The majority of included studies used less than 32 weeks as the definition of PTB in multiple pregnancy, therefore this definition was adopted post hoc. Three studies (1,332 women) included women with twin gestations (24, 29, 31). The prevalence of PTB in these studies ranged from 6.9 - 30.0%. Sensitivity ranged from 0.29-0.41 and specificity ranged from 0.92-0.96. Two studies (95 women) were conducted in women carrying triplet pregnancies (30, 35). The prevalence of PTB in this population ranged from 17.9-23.2%. Sensitivity ranged from 0.60 - 0.63 and specificity ranged from 0.92-0.96 (Table 1).

Discussion

Summary of results

This systematic review is the first to assess the performance of fFN in asymptomatic pregnant women without risk factors for preterm delivery. In singleton pregnancy without risk factors for PTB, a positive result can be indicative of an increased risk of premature delivery. Based on the findings of this review the test is unlikely to provide clinically useful information when used in women with documented risk factors for PTB (e.g. previous second-trimester miscarriage, previous cervical surgery). The accuracy of fFN in women with multiple

pregnancy was inconclusive.

Strengths and Limitations

The main strengths of this review include the use of strict published criteria for performing test accuracy systematic reviews, using a prospective protocol and conducting a comprehensive literature search. We contacted authors of included studies where needed for clarifications and included responses within our assessment processes.

The main limitations of this review are related to the heterogeneity of the data within the included studies. We included studies undertaken in the context of preterm birth surveillance clinics. Women in these clinics are a heterogeneous population, including some women having a history of PTB or having a cervical cerclage in situ and others with a different spectrum of risk factors. Including data collected in these clinics may have introduced selection bias into our analyses (4). Despite the risk of bias, we were keen not to exclude these data, as they reflection the current models of antenatal care for women with known risk factors for PTB in high income settings (5).

In this review, we limited our inclusion criteria to studies where fFN was tested after 22 weeks of gestation, based on the manufactures' instructions (15). We chose 22 weeks gestation as the lowest gestational age for testing, based on how a positive result would be managed clinically (5). There are a number of recently published studies where fFN testing has occurred before 22 weeks of gestation, however the performance of fFN in this context and the clinical management protocols for a positive result are not well established. We included studies where a 'blind method' for collecting fFN was used, where samples for fetal fibronectin measurement were taken without the insertion of a speculum and visualization of the posterior fornix (16). This technique has been validated and its performance deemed similar to the direct visualisation method (16) hence our decision to include primary studies using this technique in our review. However, we cannot exclude the possibility that inclusion of this alternative method alongside traditional sampling techniques may have introduced bias into the estimates of test performance in our meta-analyses.

In twin pregnancies, fFN testing is known to have a low to moderate accuracy in predicting PTB (2), but is likely to be a stronger predictor than cervical length measurement (36). Birth

before 32 weeks was the definition of PTB used in the studies of multiple pregnancies as the reference standard and was adopted by us post-hoc. This was a deviation from our study protocol which was necessary to allow pooling of study findings. We identified three studies meeting our inclusion criteria, however despite our efforts to widen inclusion there was insufficient data to permit meta-analyses in this subgroup. As a result, we cannot comment on the performance of fFN for predicting PTB in twin or triplet pregnancies.

Interpretation

Many primary studies looking into the accuracy of fFN for predicting PTB in asymptomatic pregnant women are inadequately powered to estimate the accuracy of the test. This is largely due to the varying prevalence of PTB in individual study samples (37).

Our results show that fFN testing in asymptomatic women, without any risk factors had the highest likelihood ratio (12.01). As a general rule, a positive likelihood ratio >10 or a negative likelihood ratio <0.1, is associated with a large shift from pre-test to post-test probability (38) demonstrating that the test is useful in predicting the outcome. Likelihood ratios (sensitivity/(1-specificity) are also less dependent on the condition being present or not in the population by attributing equal weights to sensitivity and specificity. (39) When in a certain population there are a significant number of individuals without the condition being tested (such as in a low risk obstetric population), the number of those correctly identified as having the condition or not, is described by the specificity of the test (39). However, using fetal fibronectin as a single screening test in pregnant women without any risk factors for PTB is unlikely to be feasible or acceptable (40). It is more likely that fetal fibronectin could be used in combination with other predictors of PTB (for example cervical length measurement) to increase the odds of detecting women at risk of PTB (41).

There were a number of studies in our review where fFN samples were collected at different gestational ages and many studies employed a serial fFN sampling strategy. In these instances, we used the latest gestational age of testing as our index test. It is beyond the scope of this review to suggest an optimal time for sample collection. Similarly, our review cannot suggest the optimum number of samples required within a serial sampling strategy to identify those women at risk and allow for timely interventions. These questions will require further primary research to be addressed.

A previous report (40) suggested that positive fFN results between 24 and 28 gestational weeks are helpful in identifying women at high-risk of PTB during the a period of time where the neonatal morbidity and mortality is high. Our systematic review did not look at testing of fFN within these gestational ages as a subgroup and therefore we cannot make any recommendations as to whether testing of women with risk factors at these gestations affects outcomes. A study by Jwala et al. in 2016 (42) suggested that quantitative fFN and and cervical length measurement in asymptomatic women without risk factors for PTB increase the sensitivity of fFN, but whether this is useful clinically has not been determined. A recent prospective study comparing women with previous history of PTB to women without a history of PTB found that a positive fFN was the best predictor for recurrence of PTB, increasing the recurrence risk between two to four-fold compared to women without a positive fFN result (43). There are however, many conflicting data on whether fFN is useful for care delivery in women with risk factors for PTB (44), which we postulate is secondary to poor quality primary research in this area.

Our systematic review showed that fFN is a useful test for identifying women at risk of PTB without any pre-existing risk factors. This is based on the accuracy measure obtained for the likelihood ratio of a positive test rather than the pooled sensitivity. fFN may also be useful in managing women with risk factors for PTB, however, it is likely that these women are cared for in specialist centres, where other strategies of risk identification such as cervical length measurement are employed, which may be more helpful in stratifying risk for women with risk factors for PTB (45).

Conclusion

There is currently no evidence supporting the use of fFN testing in asymptomatic women (3, 46). Our systematic review suggests that in women with singleton pregnancies without risk factors for PTB, a positive fFN may be predictive of preterm birth, but should be used with caution. Further good quality research is needed to determine the usefulness of fFN testing in the pathway of care for women without risk factors who are asymptomatic for PTB.

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