The predictive value of quantitative fibronectin testing in combination with cervical length measurement in symptomatic women

The predictive value of quantitative fetal fibronectin testing in combination with cervical length measurement in symptomatic women; a European multicentre cohort study (EUFIS).

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Met opmerkingen [MB1]: I added Sven Schulzke as he was one of the investigators in the protocol.

Key words: Preterm labour, prediction, quantitative fetal fibronectin, cervical length, pregnancy

Word count abstract: 306

Word count manuscript: 3638

Running title: quantitative fetal fibronectin in the short term prediction of preterm delivery

Abstract

Objective: To evaluate whether quantitative fetal fibronectin testing improves the prediction of spontaneous preterm delivery within seven days in symptomatic women undergoing cervical length measurement as compared to qualitative fetal fibronectin testing.

Methods: We performed a European multicentre cohort study in ten centres in five countries. Women with threatened preterm labour between 24 and 34 weeks gestational age and intact membranes underwent fetal fibronectin testing and cervical length measurement. Qualitative fibronectin test results (threshold 50 ng/mL) were immediately available for clinical management, while quantitative test results remained blinded until the end of the study. We used logistic regression to construct a model including quantitative fibronectin and cervical length, and one including qualitative fibronectin and cervical length. We compared the models' capability to identify women at low risk (<5%) for delivery within seven days using a reclassification table. We assessed the risk of preterm delivery within seven days in predefined fibronectin and cervical length strata.

Results: Out of 502 included women, 49 (10%) delivered spontaneously within seven days. The risk of preterm delivery within seven days ranged from 2% in the lowest fFN group (<10ng/mL) to 37% in the highest group (>500ng/mL). Use of quantitative fibronectin instead of qualitative fibronectin resulted in reclassification of 20 women from high risk to low risk, of whom four (20%) delivered within seven days. Using a threshold of 10 ng/mL in women with a cervix <15mm, 25 women at low risk (4%) of delivery within seven days could be identified.

Conclusion: Quantitative fibronectin testing adds discriminative capacity across the risk range in symptomatic women. In terms of reclassification, quantitative fibronectin does not improve the prediction of preterm delivery within seven days compared to the qualitative test.

However, a threshold of 10 ng/mL in women with a short cervix improves identification of women at low risk (<5%) of delivery within seven days.

Introduction

Preterm birth, defined as birth before 37 weeks' gestation, occurs worldwide in 15 million babies and accounts for 11% of all live births.(1) In 1.1 million of these births, prematurity results in deaths, thus making preterm birth the leading cause of neonatal mortality and also morbidity.(2) A large majority of women who present with symptoms of preterm labour will not deliver within seven days and 50% will deliver at term.(3-5) Accurate identification of women who will deliver in short time allows targeted interventions such as corticosteroids, magnesium sulphate, tocolysis and in utero transfer to a perinatal centre, thus avoiding unnecessary interventions with potential side effects for women at low risk.

Among various methods to assess the risk of preterm delivery, fetal fibronectin testing and cervical length measurement are widely used. Fetal fibronectin, a glycoprotein found at the choriodecidual interface, is traditionally used as a binary bedside test providing a positive or negative result based on a threshold of 50 ng/mL.(6) Combining cervical length measurement and fetal fibronectin testing has a high negative predictive value (>98%) for delivery within seven days, but positive prediction is poor.(7) The best strategy to combine the two tests is additional fibronectin testing in women with a cervical length between 15 and 30 mm, reducing the number of unnecessary referrals and admissions to perinatal centres.(8) This approach would also be cost saving without compromising neonatal health outcomes.(9)

A new bedside quantitative fetal fibronectin test showed added value over the conventional qualitative test with an increase of the positive predictive value for preterm delivery on short term by increasing the threshold from 50 ng/mL to 200 ng/mL or 500 ng/mL.(10) We recently demonstrated in a post-hoc analysis on frozen fibronectin samples, that the quantitative fibronectin test has added value in the risk assessment of preterm delivery within seven days across the risk range in combination with cervical length, but it does not improve discrimination between low (<5%) and high risk as compared to qualitative

fibronectin testing and cervical length. Quantitative fibronectin testing has, however, not prospectively been evaluated in combination with cervical length measurement.

Here, we prospectively evaluated whether, in combination with cervical length measurement, quantitative fibronectin testing has better predictive accuracy than qualitative fibronectin testing.

Methods

We conducted a European multicentre cohort study in ten centres in five countries (four centres in The Netherlands, two centres in Switzerland, two centres in Belgium, one centre in Germany and one centre in Austria). All centres were perinatal centres that served as tertiary referral centres for high-risk obstetric patients with annual delivery rates ranging from about 1000 in the Antwerp University Hospital to about 4000 in the University Hospitals of Geneva Medical Centre. The study protocol was approved by the ethics committee of the Amsterdam Medical Centre, the University Hospitals of Geneva Medical centre and the University Hospital Basel as well by the board of directors of the participating centres. Written informed consent was obtained from all participants

Women presenting with symptoms of preterm labour (more than three contractions per 30 minutes, vaginal blood loss, abdominal or back pain) or asymptomatic cervical length shortening (< 30 mm), who were between 24 and 34 weeks of gestation, and who had intact membranes were eligible for participation in the study. Gestational age was based on first trimester ultrasound assessment. The women either presented themselves directly at one of the participating centres or were referred by their primary gynaecologist from a general hospital (secondary care) or by a general practitioner or midwife (primary care). Women who had received tocolytic treatment for more than 18 hours were excluded from the study. Women with contraindications for tocolysis such as a lethal congenital abnormality, suspected

intrauterine infection, nonreassuring fetal status or maternal distress requiring immediate intervention such as placental abruption or severe vaginal blood loss were excluded. Other exclusion criteria were cervical dilatation of more than three cm and triplet or higher order pregnancies. Also women who had iatrogenic delivery within seven days after study entry for hypertensive disorders, fetal distress, or other reasons for immediate delivery were excluded. We intended to include 500 women, i.e. 100 women per country.

After study enrolment, all women underwent cervical length measurement and fetal fibronectin (fFN) testing. Cervical length was measured by transvaginal ultrasound. fFN testing was performed using the quantitative Rapid fFN 10Q analyser (Hologic®) according to manufacturer's instructions. A 50 ng/mL cut-off was used for the qualitative result of the fFN test (positive, negative). The qualitative fFN result was available immediately for the use in patient care, but the quantitative fFN result remained blinded until after delivery (the analyser generated a random 3-letter result code). When the study started, both cervical length measurement and the qualitative fFN test were standard care in all participating centres for risk stratification in women with symptoms of preterm labour. Clinicians were trained in transvaginal ultrasonography and performed cervical length measurements themselves. Furthermore, all involved personnel was trained in the use of the specimen collection and the fFN analyser. Preferably, a specimen had to be collected before the vaginal examination or cervical length measurement. Furthermore, additional information on the maternal condition was obtained including vaginal digital examination, maternal serum for CRP and leukocytes, urine and vaginal culture, and blood pressure. In addition, data collection included information on factors that might influence the fFN result, such as the use of vaginal soap or sexual intercourse within 24 hours before performance of the fFN test and vaginal bleeding during testing.

Treatment recommendations were based on previous research. We recommended starting tocolysis and steroids for fetal lung maturation in high-risk women, defined as women with a cervical length below 15 mm and in women with a cervical length between 15 and 30 mm with a positive fFN result. For low-risk women with a cervical length above 30 mm, we recommended to withhold steroids and tocolysis. For women with a cervical length between 15 and 30 mm in combination with a negative fFN test, the clinician on call decided whether to start tocolysis and steroids or not. Tocolytic drugs that could be prescribed were beta-sympathomimetics, nifedipine, atosiban, indomethacin. ~~or magnesium sulphate.~~ Magnesium sulphate for neuroprotection and a~~A~~ntibiotics were given on consideration of the attending physician.

Met opmerkingen [MB2]: Was magnesium sulphate only given for neuroprotection in your centre? Or also as tocolytic. Please let me know!

Met opmerkingen [g3]: In Antwerp we give it only for neuroprtection, so never for tocolysis, meaning that for this study it was always associated with a 'real" tocolytic"

The primary outcome was spontaneous preterm delivery within seven days after study entry. We used logistic regression analyses with an interaction term to assess the relationship between potential influencing factors and the quantitative fFN result, such as transvaginal ultrasound, vaginal examination, the use of vaginal soap or sexual intercourse within 24 hours before performance of the fFN test and vaginal bleeding during testing. Quantitative fFN was analysed as a continuous variable, all other factors as dichotomous variables. Linearity of the association between quantitative fFN and the risk of preterm delivery was assessed using cubic spline analyses. A confounding factor was excluded from the analyses in case of a significant interaction. Descriptive statistics were calculated for baseline demographics and obstetric characteristics.

We used logistic regression analyses to construct two multivariable prediction models to predict preterm delivery within seven days: one model including the variables cervical length and qualitative fFN and one model including the variables cervical length and quantitative fFN. Both cervical length and quantitative fFN were analysed as continuous variables. Linearity of the association between the variables cervical length and quantitative

fFN and the risk of preterm delivery was assessed using cubic spline analyses. We used bootstrapping techniques for internal validation of the models to correct for overfitting. Two hundred samples with the same size of the original data set, were drawn from the original data set with replacement. A shrinkage factor was derived from these analyses and used to adjust regression coefficients.(11, 12) We compared the models in terms of overall fit using Nagelkerke R^2. We used the area under the receiver operating characteristics curve (AUC) to compare the models' ability to discriminate between women who delivered within and after seven days. To determine whether quantitative fFN as compared to qualitative fFN in combination with cervical length improves the capability of the model to identify low-risk women (<5% risk), we compared the two models in terms of reclassification. A reclassification table summarizes the number of women who were correctly reclassified from high risk in the model with cervical length and qualitative fFN to low risk in the model with cervical length and quantitative fFN, and vice versa.

For risk stratification, thresholds of 10, 50, 200 and 500 ng/mL for the quantitative fFN test were predefined before analysis and cervical length was divided into groups of <15 mm, 15-30 mm and 30-50 mm. The rates of preterm delivery within seven days were calculated within the corresponding strata. We considered a risk of delivery less than 5% as low risk. This threshold has been derived from the interpretation of cervical length, because this is currently used in clinical decision-making in women with signs of preterm labour. C~~A e~~ervical length measurement with a threshold between 20 and 30 mm corresponds to a post-test probability ~~risk~~ of delivery within seven days of 4-5%, compared to 11% without cervical length information.(5) To assess if more low-risk women could be identified using the quantitative fFN test with various thresholds, we compared the accuracy to that of the qualitative fFN test (threshold 50 ng/mL) in combination with cervical length measurement.

Met opmerkingen [AL4]: Do you mean : a cervical length between 15 and 30 in stead of 20 and 30 mm? What do you mean by without cervical length information?

MB: This is based on the literature, not in this study. Using cervical length measurement with a threshold between 20-30 mm as a predictor women have a risk of 4-5 % to deliver < 7 days, compared to a risk of 11% without CL measurement. I hope that it's more understandable now?

We were interested in the predictive accuracy of the quantitative fFN test in a symptomatic population, but we also included women with asymptomatic cervical length shortening. To assess whether this could have influenced our results, we performed sensitivity analyses on the group of women with symptoms of preterm labour only, leaving those with asymptomatic cervical length shortening out.

Data analyses were performed in SPSS version 20.0 (Chicago, IL, USA) and R version 2.10.0 (The R foundation for Statistical Computing, 2009).

Results

Between January 2013 and May 2014, a total of 532 women were eligible for study enrolment, of whom five women did not meet the inclusion criteria, 11 had one or more exclusion criteria and seven did not give their consent to participate in the study. In the remaining 509 participating women, we performed fFN testing and cervical length measurement. The fFN result and cervical length were both not recorded in three women. We excluded one woman who had an elective caesarean section within seven days after enrolment. In total, 502 women were available for analysis (Figure 1); 197 included in the Netherlands, 162 in Switzerland, 61 in Belgium, 49 in Germany and 33 in Austria.

Logistic regression analysis showed no interaction between quantitative fFN and the performance of transvaginal ultrasound (n=240, p=0.63), vaginal examination (n=114, p=0.57), the use of vaginal soap (n=96, p=0.30) or sexual intercourse (n=20, p=0.74) within 24 hours before performance of the fFN test. Similarly, there was no interaction with vaginal bleeding during testing (n=62, p=0.84). Therefore, women with one of these characteristics were not excluded from the analyses.

Baseline demographics and obstetric characteristics for the study participants are shown in Table 1. Two hundred and fifty-three women (50%) presented directly to one of the

perinatal centres, 125 women (25%) were referred from secondary care centres, while 124 women (25%) were referred from primary care midwifery practices or by a general practitioner. The median gestational age at testing was 29.4 weeks (interquartile range 26.6 – 31.6 weeks). There were 73 women (15%) with a multiple pregnancy, 28 women (6%) with a previous preterm delivery between 34 and 37 weeks of gestation, and 56 (11%) with a previous preterm delivery before 34 weeks of gestation. The mean cervical length was 20 mm (standard deviation ± 9 mm), the fFN result was negative in 291 women (58%) and the median fFN concentration was 32 ng/mL (interquartile range 7 – 200 ng/mL). There were 27 women (5%) with one or more previous invalid test results. Delivery within seven days after study entry occurred in 50 women (10%). The median time to delivery in these 50 women was 2.2 days (interquartile range 0.9 – 4.4 days).

Cubic spline analyses showed a linear relationship between both quantitative fFN and cervical length and the risk of preterm delivery within seven days, and were therefore analysed as continuous variables. In univariable logistic regression analysis, a positive fFN test was associated with a higher risk of preterm delivery within seven days (OR 9.9 (95% CI 4.0 – 19)) as well as a higher fFN concentration (OR 1.006 per ng/mL (95% CI 1.005 – 1.008)). A longer cervix was associated with a lower risk of preterm delivery within seven days (OR 0.9 per mm (95% CI 0.87 – 0.94)). Table 2 shows the two multivariable models. The model with qualitative fFN and cervical length was presented after shrinkage with an average shrinkage factor of 0.98 and the model with quantitative fFN and cervical length with an average shrinkage factor of 0.99. The model with cervical length and qualitative fFN had an AUC of 0.81 (95% CI 0.75 – 0.87) and a Nagelkerke R^2 of 0.24 The model with CL and quantitative fFN had an AUC of 0.83 (95% CI 0.77 – 0.89) and a Nagelkerke R^2 of 0.29.

Table 3 shows the reclassification of participants based on a multivariable model with quantitative fFN and cervical length compared to a model with qualitative fFN and cervical

Met opmerkingen [tejada665]: Which was the preterm birth rate?

MB: Sorry, I don't understand your question..

Met opmerkingen [AL6]: Except when the fibronectine is more than 500 ng/ml). Can we prove if there is infection or other risc (previous, race, or?)

MB: These are the results of univariable logistic regression analysis of CL, so had nothing to do with fFN..

length. Using quantitative fFN and cervical length resulted in 57 women (11%) being reclassified; 37 women (7%) were reclassified as low risk, of whom four (11%) delivered within 7 days. However, as this is above the currently used risk threshold of 5%, these women are incorrectly reclassified as low risk. On the other hand, 20 women were reclassified as high risk, of whom four (20%) delivered within seven days.

Table 4 shows the risk stratification. The risk of preterm delivery within seven days increased with increasing fFN concentration from 2% in the lowest fFN group (<10 ng/mL) to 37% in the highest fFN group (>500 ng/mL) and with shortening of the cervix from 2% in the group with the longest cervices (30-50 mm) to 25% in the group with the shortest cervices (<15 mm). In women with a cervical length below 15 mm the risk of preterm delivery within seven days ranged from 4% in the lowest fFN group to 60% in the highest fFN group. In women with a cervical length between 15 and 30 mm the risk ranged from 2% to 18%. No women with a cervical length between 30 and 50mm delivered within seven days except two out of six women (33%) in the highest fFN group (>500 ng/mL). Using the conventional threshold of the qualitative fFN test (50 ng/mL) in combination with a cervical length between 15 and 30 mm 183 women were identified, of whom three (2%) delivered within seven days. Changing the threshold from 50 to 200 ng/mL in women with a cervical length between 15 and 30 mm, 51 women were identified, of whom four (8%) delivered within seven days. Changing the threshold from 50 to 10 ng/mL in women with a cervical length of 15 mm, 25 women were identified, of whom one (4%) delivered within seven days.

We included 71 women (14%) with asymptomatic cervical length shortening, of whom 4 (6%) delivered within seven days. Sensitivity analyses showed that, leaving these women out, the results of both the risk stratification and the reclassification did not differ from the results in the study population including women with asymptomatic cervical length

Met opmerkingen [tejada667]: To me you identify 25 (ffn 1<10) + 26 (ffn 10-<50)

MB: We are interested in the 10 ng/mL threshold instead of 50 to identify 25 women with a low risk (4%) of PTD. 26 women with fFN 10-50 have a risk > 5% (12%) so we're not interested in this group.

Met opmerkingen [tejada668]: 4 women PTD

shortening as well. These results are available as supplementary information (Supplementary Table 1 en Table 2).

Discussion

This study assessed the diagnostic accuracy of the quantitative fFN test in combination with cervical length measurement, as compared to the qualitative test, in the prediction of spontaneous preterm delivery within seven days in symptomatic women. The results demonstrate that quantification of fFN provides additional information on risk stratification as the risk of preterm delivery increases with increasing fFN concentrations. In terms of reclassification, quantitative fFN does not improve the identification of women with a low risk (<5%) of preterm delivery within seven days compared to the qualitative fFN test. However, if we look more in detail using the risk stratification, we see that a threshold of 10 ng/mL in women with a short cervix (<15 mm), who are in current practice treated as high risk without additional fFN testing, could identify more low-risk women.

The major strength of this study is that data were derived from a well-described, large, prospectively collected cohort of symptomatic women, representing a European population with mostly Caucasian women. The qualitative fFN test was collected according to protocol and the result was used to determine further management, whereas the quantitative result remained blinded until the end of the study. It is the first study, to our knowledge, to evaluate the quantitative fFN test in combination with cervical length measurement with prospectively collected data.

An important question is whether tocolysis has influenced our results by reducing the number of women who delivered within seven days after study entry. Tocolytics have a short half-life and are in general only given for the first 48 hours after admission in order to delay

delivery long enough to administer antenatal corticosteroids causing improved neonatal outcomes.(13, 14) Good evidence that tocolysis, given during the first 48 hours after admission, delays delivery after seven additional days have passed, is lacking.(15) The World Health Organization recommends not to use tocolytic treatments, other than to prolong pregnancy (up to 48 hours) to provide a window for administration of antenatal corticosteroids and/or in-utero transfer, because of the potential risks and lack of evidence of the efficacy.(16) Therefore, we think that it is unlikely that tocolysis have influenced the results of this study.

A second limitation is the inclusion of singleton and twin pregnancies as these entities have different pathways a different cut off value for a short cervix in twin. It is not clear whether thresholds for fFN differ between singleton and twin pregnancies. Unfortunately, the number of twin pregnancies in this study was too low to perform the analyses in this subgroup.

In this study, also asymptomatic women with cervical length shortening (14%) were included, even though we were interested in a symptomatic population. The preterm birth rate in this subgroup was 6%, indicating a lower risk than in the total study population. Sensitivity analyses showed that including these asymptomatic women did not influence the results. There is an ongoing discussion about general versus selected cervical length screening. It is mentioned that universal screening could lead to over-diagnosis of cervical length shortening and unnecessary interventions such as bed rest, cervical cerclage, and hospitalization.(17) Further studies should evaluate the combination of cervical length measurement and fFN in a universal screening for prevention of preterm birth.

Met opmerkingen [MB9]: I asked you all to evaluate the cases with asymptomatic CL shortening to know why the CL measurement was performed. As soon as I've received this information from you, I could also describe these reasons…

Met opmerkingen [g10]: I don t think we included any of these cases in Antwerp?

Our results support previous studies that reported improved prediction of preterm delivery using quantification of fFN in both symptomatic and high-risk asymptomatic women.(10, 18) Abbott et al. reported increasing spontaneous preterm birth rates within two

weeks with increasing fFN concentrations, ranging from 2% in the lowest fFN group (<10 ng/mL) to 46% in the highest fFN group (>500 ng/mL), in symptomatic women. Changing the threshold from the conventional 50 ng/mL to 200 ng/mL led to an increase in the positive predictive value from 20% to 37% for the prediction of delivery within two weeks with minimal effect on the negative predictive value.(10) However, in this study, the quantitative fFN test was not evaluated in combination with cervical length measurement.

We recently described similar analyses performed on data of a Dutch prospective nationwide cohort study (ref Bruijn et al BJOG in press). In this study we evaluated the quantitative fFN test on frozen fFN samples obtained from symptomatic women with a cervical length below 30 mm. The results were in line with the results of the current study, showing an increasing risk of delivery within seven days with increasing fFN concentrations. However, using a threshold of 10 ng/mL in women with a short cervix (<15 mm), our previous study showed that no more women at low risk of preterm delivery within seven days were identified, whereas the present results showed that 25 women were identified of whom 1 (4%) delivered within seven days, indicating that these women could be considered as low risk. This difference could possibly be explained by the smaller sample size in the former study. More stringent prediction of low-risk women (e.g. with a threshold of 10 ng/mL in women with a cervical length below 15 mm) could further reduce the number of referrals and admissions to perinatal centres, as well as medication side effects and maternal stress. Women with a positive fFN test (>50 ng/mL) and a cervical length between 15 and 30 mm are currently treated as high risk. Changing the threshold from 50 ng/mL to 200 ng/mL in these women to improve the positive predictive value and save more women from unnecessary treatment and referrals, did not lead to identification of more low-risk women.

We were particularly interested in discrimination between low and high risk of spontaneous preterm delivery within seven days using a threshold of 5%, because this is the

cut-off that we currently use in clinical decision-making in women with signs of preterm labour. This threshold is derived from the interpretation of cervical length, as cervical length measurement with a threshold between 20 and 30 mm corresponds to a post-test probability of delivery within seven days of 4-5%, compared to 11% without cervical length.(5) ~~51 women were identified of whom 4 (8%) delivered within seven days. Because this is above the acceptable 5% risk threshold, the use of a higher fFN threshold is not usefulOur results demonstrate that quantification of fFN has added value over the qualitative bedside test in risk discrimination across the risk range, however, improvement of discrimination between low (<5%) and high risk of spontaneous preterm delivery within seven days is limited. Logistic regression analyses showed an incorrect reclassification with quantitative fibronectin of 20 women from high risk to low risk, because 4 of these women (20%) delivered within seven days. We are particularly interested in the 5% threshold, because this is the cut-off that we currently use in clinical decision making in women with signs of preterm labour. This threshold is derived from the interpretation of cervical length, as a cervical length between 20 and 30 mm corresponds to a risk of delivery within seven days of 4-5%, compared to 11% without cervical length information.(5)~~

Unpublished results of the cost-effectiveness analysis of the Apostel-1 study showed that additional fFN testing in women with a cervical length between 15 and 30 mm is a cost-effective strategy, without compromising neonatal health. Our results raise the interesting question whether the use of the quantitative fFN test is cost-effective, since based on our results women with a short cervix should be tested as well. In the future, we will perform a cost-effectiveness analysis to compare different strategies; cervical length measurement alone, quantitative fFN testing alone, qualitative fFN testing in combination with cervical length measurement, and quantitative fFN in combination with cervical length measurement.

Met opmerkingen [g11]: You should make some reference, e g a poster presentation somewhere?

Met opmerkingen [MB12]: Is it possible to mention unpublished results like this?

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Figure 1. Participant flow diagram.

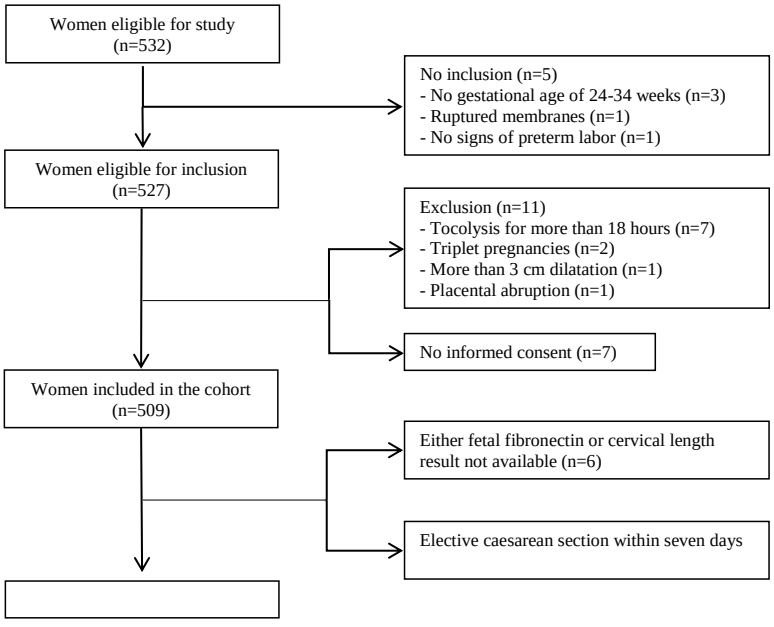

Table 1. Baseline characteristics of the study population (n=502)

Characteristics	Values
Delivery within 7 days after study entrance (n (%))	50 (10)
Maternal age – yr (mean ± SD)	30.0 ± 5.2
Gestational age at study entry (median (IQR))	29.4 (26.6 – 31.6)
Body-Mass Index kg/m2 (median (IQR))* (n=472)	22.2 (20.2 – 25.6)
Caucasian race (n (%))	388 (77)
Maternal smoking (n (%))* (n=457)	49 (10)
Nulliparous (n (%))	279 (56)
Previous preterm birth <37 wks (n (%))	79 (16)
Previous preterm birth <34 wks (n (%))	56 (11)

Multifetal gestation (n (%))	73 (15)
Symptoms of preterm labour:	
Contractions (n (%))	392 (78)
Vaginal blood loss (n (%))	62 (12)
Abdominal or back pain (n (%))	302 (60)
Asymptomatic cervical length shortening (n (%))	71 (14)
Last prenatal visit in:	
Perinatal centre (n (%))	253 (50)
General hospital (n (%))	125 (25)
Primary care practices (n (%))	124 (25)
Fetal fibronectin	
Positive (n (%))	211 (42)
Quantitative result - ng/mL (median (IQR))	32.0 (7.0 – 200)
Cervical length – mm (mean ± SD)	20 ± 9

Table 2. Multivariable models for predicting spontaneous preterm delivery within seven days in symptomatic women, including cervical length and quantitative fetal fibronectin or qualitative fetal fibronectin as predictors.

	Beta*	OR (95% CI)
Intercept	-1.9	
Cervical length (mm)	-0.08	0.92 (0.88 – 0.96)
Qualitative fFN	1.9	6.7 (3.0 – 15)
*Coefficients were shrunken with an average shrinkage factor 0.98 AUC = 0.81 (95% CI 0.75 – 0.88) Nagelkerke R^2 = 0.24		
Intercept	-1.9	
Cervical length (mm)	-0.08	0.93 (0.89 – 0.97)
Quantitative fFN (ng/mL)	0.006	1.006 (1.004 – 1.007)
*Coefficients were shrunken with an average shrinkage factor 0.99 AUC = 0.83 (95% CI 0.77 – 0.89) Nagelkerke R^2 = 0.29		

Table 3. Reclassification table, showing the number of women reclassified from high risk to low risk and vice versa using the model with cervical length and qualitative fetal fibronectin compared to the model with cervical length and quantitative fetal fibronectin.

	CL & quantitative fFN		
CL & qualitative fFN	**Low risk***	**High risk**	**Total**
Low risk*	250 (3PTD – 1%)	20 (4 PTD – 20%)	270 (7 PTD – 3%)
High risk	37 (4 PTD – 11%)	195 (39 PTD – 20%)	232 (43 PTD – 19%)
Total	287 (7 PTD – 2%)	215 (43 PTD – 20%)	502 (50 PTD – 10%)

CL = cervical length, fFN = fetal fibronectin, PTD = preterm delivery < 7 days
* Low risk is defined as a risk of PTD <5%

Table 4. Risk stratification of preterm delivery within seven days using quantitative fetal fibronectin in combination with cervical length.

	Fetal fibronectin group					
CL group	**<10 ng/mL**	**10-49 ng/mL**	**50-199 ng/mL**	**200-499 ng/mL**	**≥500 ng/mL**	**Total**
<15mm	25 (1 PTD -4%)	26 (3 PTD-12%)	22 (5 PTD-23%)	36 (13 PTD -36%)	15 (9 PTD - 60%)	124 (31 PTD - 25%)
15mm-29mm	91 (2 PTD -2%)	92 (1 PTD -1%)	51 (4 PTD -8%)	45 (7 PTD - 16%)	17 (3 PTD - 18%)	296 (17 PTD - 6%)
30mm-50mm	37 (0 PTD -0%)	20 (0 PTD-0%)	12 (0 PTD -0%)	7 (0 PTD - 0%)	6 (2 PTD - 33%)	82 (2 PTD - 2%)
Total	153 (3 PTD-2%)	138 (4 PTD -3%)	85 (9 PTD-11%)	88 (20 PTD-23%)	38 (14 PTD-37%)	502 (50 PTD -10%)

PTD = preterm delivery within 7 days

Supplementary Table 1. Sensitivity analysis in subgroup without women with asymptomatic cervical length shortening. Reclassification table, showing the number of women reclassified from high risk to low risk and vice versa using the model with cervical length and qualitative fetal fibronectin compared to the model with cervical length and quantitative fetal fibronectin.

	CL & quantitative fFN		
CL & qualitative fFN	**Low risk***	**High risk**	**Total**
Low risk*	205 (3PTD – 1%)	28 (2 PTD – 7%)	233 (5 PTD – 2%)
High risk	30 (3 PTD – 10%)	168 (38 PTD – 23%)	198 (41 PTD – 21%)
Total	235 (6 PTD – 3%)	196 (40 PTD – 20%)	431 (46 PTD – 11%)

CL = cervical length, fFN = fetal fibronectin, PTD = preterm delivery < 7 days
* Low risk is defined as a risk of PTD <5%

Supplementary Table 2. Sensitivity analysis in subgroup without women with asymptomatic cervical length shortening. Risk stratification of preterm delivery within seven days using quantitative fetal fibronectin in combination with cervical length.

	Fetal fibronectin group					
CL group	**<10 ng/mL**	**10-49 ng/mL**	**50-199 ng/mL**	**200-499 ng/mL**	**≥500 ng/mL**	**Total**
<15mm	19 (1 PTD -5%)	16 (1 PTD-6%)	18 (5 PTD-28%)	31 (13 PTD -42%)	13 (8 PTD - 62%)	97 (28 PTD - 29%)
15mm-29mm	75 (2 PTD -3%)	77 (1 PTD -1%)	41 (3 PTD -7%)	42 (7 PTD - 17%)	17 (3 PTD - 18%)	252 (16 PTD - 6%)
30mm-50mm	37 (0 PTD -0%)	20 (0 PTD-0%)	12 (0 PTD -0%)	7 (0 PTD - 0%)	6 (2 PTD - 33%)	82 (2 PTD - 2%)
Total	131 (3 PTD-2%)	113 (2 PTD -2%)	71 (8 PTD-11%)	80 (20 PTD-25%)	36 (13 PTD-36%)	431 (46 PTD -11%)

PTD = preterm delivery within 7 days