Development and validation of a predictive tool for spontaneous preterm birth incorporating cervical length and quantitative fetal fibronectin in asymptomatic high-risk women

Katy KUHRT; Elizabeth SMOUT; Natasha HEZELGRAVE, Paul T SEED; Jenny Carter, Andrew H SHENNAN

Woman's Health Academic Centre, Kings College London, London, UK

Corresponding author: Andrew Shennan, Division of Women's Health, 10th Floor North Wing, St Thomas' Hospital, London, SE1 7EH, UK (e-mail: andrew.shennan@kcl.ac.uk)

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Abstract

Objective Preterm birth rates are rising but clinical prediction models are limited. Accurate prediction enables interventions to be targeted to at risk women. Single threshold (50ng/ml) fetal fibronectin (fFN) and cervical length (CL) have been the best predictors of spontaneous preterm birth (sPTB) but quantification of fFN improves prediction and has not yet been included in a prediction model with other clinical factors in asymptomatic women.

Methods Blinded prospective data from 1249 high-risk women attending preterm surveillance clinics were analysed. Parametric survival models, with time-updated covariates for sPTB, were developed and the best selected using the Akaike and Bayesian Information Criteria. The model was developed on the first 624 women and validated on the remaining 625. Fractional polynomials accommodated possible non-linear effects of quantitative fFN (qfFN) and CL. Probability of delivery before 30, 34 or 37 weeks' gestation and within 2 or 4 weeks of test were compared to actual event rates. Predictive statistics were calculated to compare training and validation sets.

Results The final model used a lognormal survival curve with CL, √fFN and previous sPTB/ PPROM as predictors. Predictive statistics were similar for training and validation sets. Areas under the ROC curves ranged from 0.77-0.99 indicating accurate prediction across all 5 outcomes.

Conclusions sPTB in high-risk asymptomatic women can be accurately predicted using a model combining qfFN and CL which supersedes the single threshold fFN test, demographic information and obstetric history. This algorithm has been incorporated into an app for widespread use.

Introduction

Every year 15 million babies are born prematurely worldwide, (before 37 weeks' completed gestation)[1] and 1.1 million die from associated complications.[2] Survivors face greater risk of serious health problems.[2]

Neonatal intensive care and ongoing medical and educational support is expensive – $26 billion annually in the US [3] and, despite advances in prenatal care, the rate of preterm birth has not declined.[2]

Prediction of preterm birth in women at risk should allow targeted intervention. Both cerclage and progesterone have shown promise in reducing spontaneous preterm birth (sPTB) in asymptomatic women, particularly in those with a short cervix.[4, 5, 6, 7] More recently fetal fibronectin (fFN) and cervical length (CL) assessment have superseded previous risk assessment, not only in women symptomatic of preterm birth, but in asymptomatic women where prophylactic preventative therapies can be attempted.[8, 9] Cervicovaginal fluid (CVF) quantitative fFN (qfFN) concentration measurement adds value over the qualitative test in both symptomatic and high-risk asymptomatic women,[10, 11 ,12]and is now available in a simple point of care test in 10 minutes (Hologic 10Q).

We prospectively evaluated clinical risk factors, qfFN and CL measurements taken from high-risk women attending a preterm surveillance clinic to create an optimal risk algorithm. This was then validated on a separate data set of high-risk women.

Methods

This was a prospective blinded secondary analysis of a population of women enrolled in the ongoing EQUIPP (Evaluation of Fetal Fibronectin with a Quantitative Instrument for the Prediction of Preterm Birth) involving 1249 consecutive asymptomatic women deemed to be

at high risk of spontaneous preterm birth, defined as one or more of: previous sPTB or previous preterm rupture of membranes (PPROM) <37 weeks', previous late miscarriage (16-23+6), previous cervical surgery or cervical length measuring <25 mm in the current pregnancy.

Asymptomatic women attending high-risk preterm surveillance clinics were offered longitudinal fetal fibronectin testing using a novel quantitative rapid fFN analyser (Hologic) every 2-4 weeks in addition to routine transvaginal ultrasound length measurement. All high-risk women between 22^{+0}-30^{+0} weeks' gestation asymptomatic of threatened sPTB were considered. From this group, exclusions were sequentially performed to provide the most uniform population for comparative analysis. Women with a blood stained swab or sexual intercourse within the last 24 hours were excluded from the study due to known interference with fFN measurement.[13] Women with multiple pregnancy, those without CL measurement, or those with insufficient or absent qfFN sample, incomplete outcome data or offspring from the current pregnancy with a congenital abnormality were also excluded.

Participants were recruited from 5 high-risk antenatal clinics in the UK between 5th October 2010-July 2014. The study was approved by South East London Research Ethics Committee, and all participating centres' local research ethics committees. Written informed consent was obtained from all participants. Gestational ages were confirmed with standard early ultrasound scans.

The fFN sample was obtained prior to CL measurement, because cervical manipulation may cause false release of fFN, during a sterile speculum examination where a polyester swab was used to collect cervicovaginal secretions from the posterior fornix of the vagina. One aliquot (200µl) of the sample contained in a buffer solution was analysed with the conventional qualitative fFN TLI_{IQ} analyser (Hologic) and another aliquot (200µl) of the same sample using the quantitative Rapid fFN 10Q analyser (Hologic). Clinicians trained in the use of

both fFN analysers ran the two tests concurrently. Clinicians were made aware of the Categoric TL_{IQ} result (positive/negative), but 10Q results remained double-blinded to both patient and clinician until after delivery (a random result code was generated by the analyser).

Participants' demographic characteristics, risk factors and obstetric and gynaecological history were entered into a secure online database (www.medscinet.net/PTBstudies). Three CL measurements were taken at each visit using transvaginal ultrasonography and the minimum cervical length was used.

Women were managed in the clinic as per unit protocols. History indicated cerclage was inserted when a woman had experienced 3 or more late miscarriages or previous sPTB <34 weeks and ultrasound indicated cerclage was inserted if a short cervix was detected on ultrasound (n=140; training set = 79; validation set = 61). Progesterone (or placebo) was used only as part of an ongoing double-blinded randomised controlled trial (n = 63; training set = 41; validation set = 22 i.e. half received 200 mg of vaginal progesterone daily from 22-34 weeks' gestation)

Any model performs optimally on the data set used to generate the model. In order to provide a true assessment of the usefulness of the model it should be validated on another data set from a similar population. To this end the study population was split to form a training set, consisting of 624 women with 1245 visits, which was used to develop the model and a validation set, which included 625 women with 1243 visits. A predictive model was run using the validation set to assess the performance of the algorithm.

Statistical methods

Data sets

Statistical analysis was performed with Stata software (version 11.2; StataCorp LP, College Station, Texas). The data was randomly split 1:1 into training and validations sets, each

woman providing qfFN and CL measurement on at least one occasion. All test results from all women were included in the analysis.

Model generation

Survival analysis with time-updated covariates was used to identify the principal predictors of premature delivery with premature onset of labour or PPROM. For each set of results, women were considered at risk of an event only from the time of the visit to the earliest of; their next test, delivery or 37 weeks' gestation. Deliveries after 37 weeks' gestation or preceded by induction of labour or elective caesarean section were regarded as censored. Fetal fibronectin, CL, gestation of test and previous preterm delivery/ PPROM were considered as possible predictors. We performed an initial analysis where other predictive variables including BMI, smoking, ethnicity, previous cervical surgery and previous late miscarriage, were excluded as not significant.

Six parametric survival models were compared for each combination of predictors: exponential, Gompertz, loglogistic, Weibull, lognormal, and gamma.

The best survival function was determined by having the lowest values of the Akaike and Bayesian Information Criteria (AIC and BIC).[14,15] A lognormal survival function with terms for qfFN, CL and previous sPTB/ PPROM was selected. The continuous measures qfFN and CL were then investigated for non-linearity using fractional polynomials[16] and a series of qfFN cutpoints at standard values; >10, 20, 50, 100, 200 and 500.

The best polynomial model was linear in CL and the square root of fFN concentration, outperforming those using cutpoints. These findings were confirmed by consistent improvement in AIC and BIC.

Therefore the final model included linear CL, the square root of fFN concentration and previous sPTB/ PPROM as predictors.

Model validation

The probabilities of delivery before 5 clinically important gestations (30, 34, 37 weeks of pregnancy and within 2 and 4 weeks of testing) were compared to actual event rates in both training and validation sets: ROC curves were drawn, and areas under the curve (AUROC) calculated.

The sensitivity and specificity of the algorithm was investigated using a probability above 10% as indicating a positive test. This value was chosen as being practically useful for separating low-risk women (probability around 5%) from high-risk women (probability around 20%) for purposes of clinical management.

Results

After exclusions, illustrated in figure 1, the final high-risk population studied consisted of 1249 women and, including longitudinal sampling, 2488 fFN measurements were recorded. This final group was split into a training set, consisting of 624 women with 1245 visits, and a validation set of 625 women and 1243 visits. The mean gestational age of visit was 23.35 in the training set and 23.39 in the validation set.

The rate of sPTB was 16%, 8%, 4%, 1% and 2% for delivery at <37, <34 and <30 weeks' and within 2 and 4 weeks of test respectively in the training set and 15%, 8%, 4%, 1% and 2% respectively in the validation set.

The proportion of women in each fFN concentration category and CL values were similar between training and validation sets (table 1). The demographics for both sets were comparable. See table 2. 143 (17%) women in the total study population received ultrasound indicated cerclage as per preterm surveillance clinic management protocols and 67 were part

of an ongoing randomised controlled trial where half of the women were in the treatment arm and received 200 mg of vaginal progesterone daily from 22-34 weeks' gestation.

Model generation (using the training set only).

Of the predictors considered, qfFN, CL and previous sPTB/ PPROM were significant in the stepwise model at $p < 0.01$. The best parametric survival model (determined by AIC and BIC) was a lognormal survival function with terms for qfFN, CL and previous sPTB or PPROM. The best fractional polynomial model was linear in CL and the square root of fFN concentration.

Model validation

Table 3 summarizes predictive statistics calculated using a probability >10% as indicating a positive test, to allow comparison of training and validation sets for prediction of delivery at each of the 5 clinically important gestations; <30, <34, <37 weeks' gestation and delivery within 2 or 4 weeks of qfFN test.

Overall, predictive statistics including sensitivity, specificity, LR+, LR- are similar for training and validation sets across all 5 gestations.

Furthermore the prediction of sPTB using the prediction model is good. The NPV is $\geq$90 for all 5 gestations which means that <10 % of women with a negative test will deliver preterm. This is clearly in line with the definition of a negative test (calculated probability < 10%), and confirms that the calculated probabilities are behaving as intended. The LR+ and LR- are all much greater than 1 or less than 1 respectively which indicates that positive test results are strongly associated with sPTB and negative tests results with its absence.[17] LR + in the validation set are highest for delivery at <30 weeks and within 2 and 4 weeks of test at 5.7 (3.6-8.9), 33.3 (15.5-71.6) and 15.0 (8.4-27.0) respectively meaning that women with a

positive test are approximately 6, 33 and 15 times more likely to deliver at these three gestations than women without a positive test.

The ROC curves in figure 2 are an indication of overall test performance regardless of fetal fibronectin threshold. The AUROC, ranging from 0.77-0.99 in the validation set, indicate that the model accurately predicts sPTB across all 5 gestations investigated.

Discussion

We have created a highly accurate prediction model, incorporating quantifiable fFN, CL and history of sPTB/ PPROM which remains accurate when tested on a validation set.

The ROC curves are an indication of overall test performance regardless of qfFN or CL threshold. Previous models have been constrained by limiting prediction into categories above and below a threshold. This model will give the optimal prediction throughout the range of values for a clinician to act on, and therefore can be more appropriately applied to an individual. The AUROC show that overall prediction for the model is extremely good with ROC areas ranging from 0.77-0.99 in the validation set – a marked improvement on ROC curves in previously published literature for fFN testing to predict sPTB at <34 and <37 weeks in asymptomatic women where AUROC areas of 0.61 (0.59-0.63) and 0.65 (0.63-0.66) respectively are reported.[18]

It has consistently been shown that additional information is available when fFN results are quantified.[10, 11,12,19] In addition combined qfFN testing and CL measurement has been shown to lead to improved prediction of sPTB.[20] This could explain improved ROC areas for the new predictive model which incorporates information obtained from the measurement of qfFN and CL compared to qfFN testing alone. Combining CL and qfFN is likely to be best, and this is consistent with our data and probably supersedes any other variables.

The superiority of combined qfFN and CL measurement as predictors of preterm birth was further implied by stepwise regression where all proposed variables, save qfFN, CL and previous sPTB/ PPROM were rejected as non-significant. However, the list of variables was not exhaustive and there may be others which affect prediction of sPTB. For example, there is evidence to suggest that having a cervical cerclage *in situ* may affect fFN result. Duhig et al showed that for delivery at <30 weeks the specificity of FFN testing was significantly lower in asymptomatic high risk women with cervical cerclage compared to those without, (77 vs 90%; $p \leq 0.00001$), and this was attributed to increased false positive results.[21] Further work is required to establish whether cervical cerclage and other variables could be included in the model to improve its performance. Currently, with 17% of the women in this cohort receiving cerclage, the model is good and can be used.

The fact that the data was derived from a rigorously, prospectively collected dataset (used for the EQUIPP study), the largest of its kind, is a strength of this study, which is the first to develop a model for the creation of an algorithm to predict sPTB in asymptomatic high risk women including quantifiable fFN. Furthermore, the parametric method used to generate the model, combined with appropriate censoring, makes full use of the available data, without introducing biases. As a result the sPTB rate in this study, which is similar to that reported in recent studies[9,11,12] analysing qfFN in asymptomatic women, is likely to be a good representation of sPTB in asymptomatic high risk women in the general population. This further suggests that the model generated in this study will be relevant beyond this study population and an accurate predictive tool.

A potential limitation of the study is that predictive statistics were based on results derived from women managed in Preterm Surveillance Clinics as per protocols, which included interventions such as ultrasound indicated cerclage (17%) and progesterone (5%) as part of OPPTIMUM, an on-going randomised controlled trial. The predictive model remains good

even under these circumstances, i.e. women both with and without intervention can rely on it to predict outcome. As interventions are not only to prolong gestation, e.g. steroids and magnesium sulphate to improve neonatal morbidity, the information remains potentially valuable. Further work needs to ascertain if interventions improve outcome when targeted to women identified with the algorithm, but some interventions may be withheld in women with poor history, determined as low risk by the algorithm. Determining risk without these interventions would be unethical, and the algorithm remains pragmatic and valuable for this reason.

Further work has been done to update the model for use in symptomatic as well as asymptomatic women. Ultimately, the algorithm will be made widely available to clinicians through an application accessed from a smart phone or via the internet. It performs significantly better than any previous predictive tool.

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TABLE 1 Fetal fibronectin concentration and cervical length measurements for training and validation sets.

	Training set n=624 Number (%)	**Validation set n=625 Number (%)**	**All n=1249 Number (%)**
<10	400 (64)	401 (64)	801 (64)
10-19.9	63 (10)	67 (11)	130 (10)
20-49.9	51 (8)	65 (10)	116 (9)
50-99.9	26 (4)	29 (5)	55 (4)
100-199.9	41 (7)	29 (5)	70 (6)
>200	43 (7)	34 (5)	77 (6)
Cervical Length (mm)			
<15	37(6)	27 (4)	64 (5)
15-24.9	68 (11)	75 (12)	143 (11)
>25	519 (83)	523 (84)	1 042 (83)

Table 2 Demographic characteristics for women in the training and validation sets

Demographic	Training set n=624 Number (%)	Validation set n=625 Number (%)	All n=1249 Number (%)
Age	33	33	33
Ethnic category			
White	340 (55)	356 (57)	696 (56)
Black	179 (29)	178 (29)	357 (29)
Asian	50 (8)	53 (9)	103 (8)
Other	55 (9)	37 (6)	92 59)
BMI (kg/m^2)			
<20	59 (10)	63 (10)	122 (10)
20-24.9	268 (43)	292 (47)	560 (45)
25-29.9	176 (28)	157 (25)	333 (27)
>30	120 (19)	111 (18)	231 (19)
Smoking			
Never	462 (74)	474 (76)	936 (75)
Current	34 (5)	43 (7)	77 (6)
Stopped before pregnancy	102 (16)	87 (14)	189 (15)
Stopped during pregnancy	26 (4)	21 (3)	47 (4)
Risk Factors			
Previous spontaneous preterm birth	243 (39)	232 (37)	475 (38)
Previous preterm prelabour rupture of membranes (PPROM)	127 (20)	112 (18)	239 (19)
Previous late miscarriage (16^{+0}-	151 (24)	122 (20)	273 (22)

23^{+6})			
Previous cervical surgery	274 (44)	280 (45)	554 (44)
Cervical length measuring <25mm in current pregnancy	95 (15)	90 (14)	185 (15)

Table 3 Predictive statistics for the 3 gestations: <30, <34, <37 weeks' and delivery within 2 or 4 weeks of fFN test. For each outcome, an individual probability of early delivery > 10% is treated as a positive test result.

		<30 weeks	**<34 weeks**	**<37 weeks**	**2 weeks**	**4 weeks**
Prevalence (%)	Training	4.3 (2.9-6.2)	8.2 (6.1-10.6)	15.1 (12.3-18.1)	0.8 (0.3-1.9)	1.9 (1.0-3.3)
	Validation	3.5 (2.2-5.3)	8.3 (6.3-10.8)	15.0 (12.3-18.1)	0.6 (0.2-1.6)	1.8 (0.9-3.1)
Sensitivity (%)	Training	63.0 (42.4-80.6)	78.4 (64.7-88.7)	77.7 (67.9-85.6)	60.0 (14.7-94.7)	58.3 (27.7-84.8)
	Validation	54.5 (32.2-75.6)	71.2 (56.9-82.9)	74.5 (64.4-82.9)	75.0 (19.4-99.4)	63.6 (30.8-89.1)
Specificity (%)	Training	90.5 (87.8-92.7)	80.1 (76.6-83.3)	64.0 (59.7-68.1)	98.1 (96.6-99.0)	95.1 (93.1-96.7)
	Validation	90.4 (87.7-92.6)	77.7 (74.0-81.0)	63.5 (59.2-67.6)	97.7 (96.2-98.8)	95.8 (93.9-97.2)
ROC Area	Training	0.88	0.83	0.78	0.97	0.91
	Validation	0.84	0.83	0.77	0.99	0.92
Likelihood Ratio (+)	Training	6.6 (4.5-9.7)	3.5 (2.9-4.3)	2.2 (1.8-2.5)	31.0 (12.5-76.8)	11.9 (6.6-21.5)
	Validation	5.7 (3.60-8.9)	3.6 (2.8-4.5)	2.0 (1.7-2.4)	33.3 (15.5-71.6)	15.0 (8.4-27.0)
Likelihood	Training	0.4 (0.3-	0.3 (0.2-	0.4 (0.2-	0.4 (0.1-	0.4 (0.2-

Ratio (-)		0.7)	0.5)	0.5)	1.2)	0.9)
	Validation	0.50 (0.32-0.80)	0.4 (0.2-0.6)	0.4 (0.3-0.6)	0.3 (0.1-1.4)	0.4 (0.2-0.8)
PPV	Training	23.0 (14.0-34.2)	23.8 (17.6-31.0)	27.7 (22.3-33.5)	20.0(4.3-48.1)	18.9 (8.0-35.2)
	Validation	17.1 (9.2-28.0)	24.5 (17.9-32.2)	26.5 (21.3-32.3)	17.6 (3.8-43.4)	21.2 (9.0-38.9)
NPV	Training	98.2 (96.7-99.1)	97.6 (95.7-98.8)	94.2 (91.2-96.4)	99.7 (98.8-100.0)	99.1 (98.0-99.7)
	Validation	98.2 (96.7-99.1)	96.8 (94.8-98.2)	93.4 (90.3-95.7)	99.8 (99.1-100)	99.3 (98.3-99.8)

Figure legends

FIGURE 1: Flow diagram of participants illustrating those excluded. Standards for the reporting of diagnostic accuracy studies (STARD) flow diagram illustrates number of participants involved in the study after exclusions were made according to the defined exclusion criteria.

FIGURE 2: ROC curves to show overall prediction. ROC curves for prediction of delivery <30, <34 and <37 weeks' gestation and within 2 and 4 weeks of testing in the validation set for a model including linear cervical length (CL), the square root of fetal fibronectin (fFN) and previous sPTB or PPROM.

FIGURE 1 Flow diagram of participants illustrating those excluded.

Standards for the reporting of diagnostic accuracy studies (STARD) flow diagram illustrates number of participants involved in the study after exclusions were made according to the defined exclusion criteria

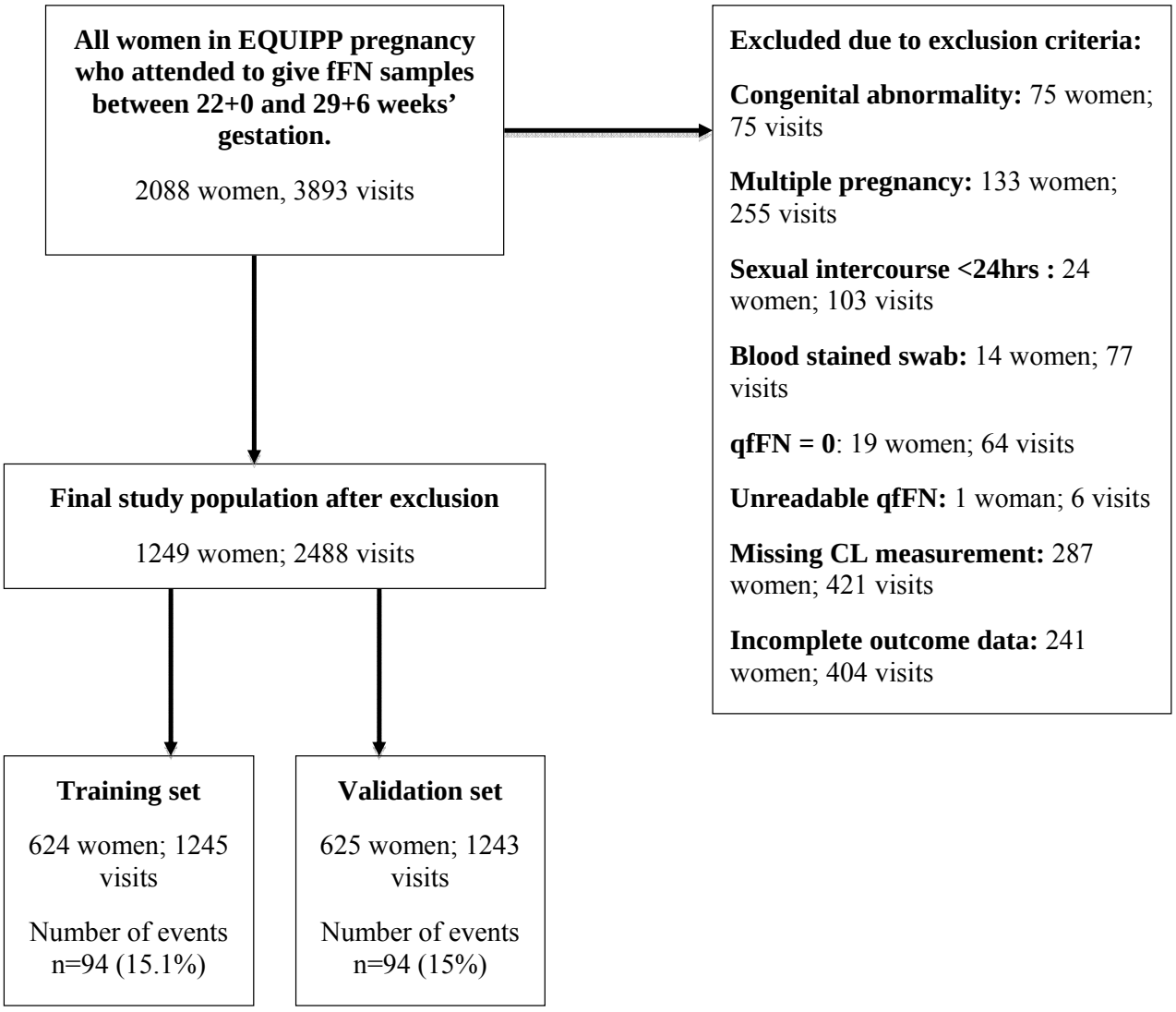

FIGURE 2: ROC curves for prediction of delivery <30, <34 and <37 weeks' gestation and within 2 and 4 weeks of testing in the validation set for a model including linear fetal fibronectin (fFN) and previous sPTB or PPROM.