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The value of cervical electrical impedance spectroscopy to predict spontaneous preterm delivery in asymptomatic women: the ECCLIPPx prospective cohort study

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Contribution.

What are the novel findings of this work?

Existing tools for predicting spontaneous premature birth have limited clinical utility. We show that cervical Electrical Impedance Spectroscopy can predict spontaneous preterm birth in asymptomatic women as a standalone test as well as to improve current risk assessment approaches.

Clinical implications of this work.

Cervical EIS assessment during pregnancy provides a novel preterm birth risk assessment modality which can be adopted into clinical practice. Further studies may enable its incorporation into risk assessment algorithms and decision support tools to drive interventions and enhance personalized care.

Abstract:

Objectives: Preterm birth (PTB) accounts for two-thirds of deaths of structurally normal babies and is associated with colossal lifetime health care costs. Prevention of PTB remains limited by prediction methods of modest accuracy– transvaginal ultrasound (TVUS) cervical length (CL) and quantitative cervicovaginal fetal fibronectin (FFN) estimation. We report the first substantive study detailing the predictive performance of a cervical probe device based on Electrical Impedance Spectroscopy (EIS) for PTB – the EleCtriCaL Impedance Prediction of Preterm birth by spectroscopy of the cervix (ECCLIPPx) studies. We aimed to compare the accuracy of cervical EIS-based prediction of spontaneous PTB in asymptomatic women in mid-trimester to TVUS CL and FFN.

Methods: We studied 449 pregnant women at 20-22 weeks (V1) and 26-28 weeks (V2). EIS was performed using the Sheffield Mark 5.0 device that makes measurements in the frequency range 76Hz to 625kHz using a small probe housing tetrapolar electrodes. TVUS CL and FFN were also measured. Associations between cervical EIS, TVUS CL, and FFN and spontaneous delivery < 37 weeks and < 32 weeks were determined by multivariate linear and non-linear logistic regression analyses. The areas under the Receiver Operator Characteristic (AuROC) curve plots of sensitivity against specificity were used to compare the predictive performance of all parameters, singly and in combination.

Results: Of the 365 asymptomatic women studied at 20-22 weeks who received

no treatments, there were 29 spontaneous PTBs, 14 indicated PTBs, and 322 term births. At the higher frequencies assessed, cervical EIS predicted spontaneous delivery < 37 weeks (AuROC 0.76, 95% CI 0.71-0.81) compared to TVUS CL (AuROC 0.72, 95% CI 0.66-0.76) and FFN (AuROC 0.62, 95%CI 0.56-0.67). Combining all three assessments improved prediction of spontaneous PTB <37 weeks (AuROC 0.80, 95% CI 0.74-0.83) compared to TVUS CL and FFN alone. Incorporating previous history of PTB into the cervical EIS prediction model improved accuracy of prediction of spontaneous PTB < 37 weeks (AuROC 0.83, 95% CI 0.78-0.87) and < 32 weeks (AuROC 0.86, 95% CI 0.82-0.90). **Conclusion**: Mid-trimester cervical EIS assessment predicts spontaneous PTB. Larger confirmatory studies investigating its potential clinical utility and to inform effective preventive interventions are required.

Introduction

Preterm birth (PTB) affects 15 million babies (6 - 18% of live births) annually. Early (< 28 weeks) and late preterm babies suffer neurocognitive deficits[1-3] and educational difficulties[4] respectively, some developing chronic diseases[5, 6]. Although survival rates following PTB have improved, its incidence has plateaued or risen[7, 8]. Prevention of PTB remains limited by a lack of accurate predictive tests and therapies.

The current mainstay of PTB risk assessment is transvaginal ultrasound (TVUS) measurement of cervical length (CL)[9-11] and determination of cervicovaginal fluid fetal fibronectin (FFN) (12-14). Whilst both techniques have modest predictive values in high-risk asymptomatic women, their sensitivities are low in low-risk populations[15, 16, 17]. Data is variable but most reports suggest that majority of women with a short cervix (<15mm) will not deliver before 32 weeks[11, 18], limiting its utility for spontaneous PTB prevention (SPTB)[19, 20], particularly among nulliparous women with singleton pregnancies [21, 22]. Their cost-effective utility is also unclear [23].

The effectiveness of interventions based on CL-determined risk of PTB remains controversial [18]: progesterone treatment for women with a previous history of PTB or a short cervix on ultrasound has established but limited efficacy for prolonging gestation[24-26] and, based on some studies, for preventing adverse neurodevelopmental and other health outcomes[27, 28]. Given that the syndrome of PTB has multiple aetiologies, novel risk assessment modalities may improve

stratification of patients and enhance effectiveness of existing and new preventive interventions[29].

Assessing the cervical matricellular remodelling changes that precede birth is a logical target for preterm birth screening. Electrical impedance spectroscopy (EIS) non-invasively quantifies cervical tissue impedance to the injection of a small electrical current. Tissue impedance is influenced by several factors including cell layering, the intra- and extracellular spaces[30], cell membrane capacitance[31, 32], and tissue hydration[33, 34]. EIS is finding increasing application to screen for cervical[35, 36], oral[37] and breast[38] cancers. Having demonstrated that EIS can detect pre-labour cervical remodelling changes[39-42], and observing in a limited pilot study that midtrimester cervical impedance in women at risk of PTB seemed to predict delivery < 37 weeks with accuracies comparable to ultrasound-derived CL[43], we aimed to determine whether cervical EIS can predict spontaneous premature delivery, compared to TVUS CL and/or FFN.

Methods

The setting. This prospective observational cohort study was carried out at the Jessop Wing (JW) Maternity Unit of the Royal Hallamshire Hospital, Sheffield UK, a tertiary preterm birth referral centre with approximately 7,500 births annually, between January 2014 and August 2016. The study was approved by the Yorkshire & Humber (Sheffield) Committee of the UK National Research Ethics Service (REC Number 13/YH/0167). All study participants gave written informed consent prior to each study.

The participants. We studied women deemed to be at high risk of PTB (one or more previous PTB or mid-trimester pregnancy loss [44, 45]) at 20-22 weeks and again at 26-28 weeks' gestation. We also studied women with no previous history of PTB at 20-22 weeks' gestation. Recruitment details, inclusion and exclusion characteristics and clinical outcomes are in Figure 1. Women with a history of an abnormal cervical smear in the previous three years, cone biopsy or loop excision of the cervix were excluded, as were women with recent/current cervical infection or vaginal bleeding. Multiple pregnancy, pregnancy with fetal anomaly, cervical cerclage or ongoing progesterone treatment were also excluded. Women who were recruited but were subsequently treated with vaginal progesterone or cervical cerclage were also excluded from analysis.

The EIS device. We employed the Sheffield Mark 5.0 EIS device (Figure 2), which is an update on previous probes that we employed in earlier work[40, 43, 46-49]. The Mark 5.0 device incorporates an accelerometer and a pressure sensor for

taking measurements at a constant application pressure of 2 Newton (determined from preliminary experiments to be the optimum force for cervical EIS measurements). The probe tip comprises of eight electrodes (two tetrapolar configurations) mounted in two rings of diameters 3 mm and 5.5 mm. The electrodes are fabricated out of 37.5% gold and are 0.6 mm and 1.5 mm diameter for the inner and outer rings respectively. The overall tip diameter is 11 mm and by using a combination of a load cell and a tri-axial accelerometer the device measures and displays the applied force to the clinician. The measurement process was gated to ensure that the applied force was 2 ±0.2 N which corresponds to a pressure of 21 kPa (157 mmHg).

The device measures transfer impedance by applying current at 14 frequencies ranging from 76.3 Hz to 625 kHz in octave increments via an adjacent pair of injecting electrodes, and voltage is measured between the remaining pair of sensing electrodes. Each frequency sweep takes 200 ms and, as a quality control measure, these are repeated until the standard error of eight subsequent measurements is below a threshold. This yields a minimum time required to record the full frequency spectrum of 1.6 s and 3.2 s for both electrode rings. The transfer impedance spectral measurements together with their variance is transmitted to the bespoke controlling PC application via Bluetooth technology and stored on a custom central database (ArQ, by Scientific Computing Sheffield Teaching Hospitals, Sheffield).

Cervical EIS measurements are highly repeatable as demonstrated by a mean (SE) coefficient of variation of impedance measurement of 7.3 (1.1) % [47] in the frequency range 39.5-312.5 kHz determined by our preliminary pilot study [43] to be potentially predictive of premature birth. In this electrical frequency range, the mean Intra-class Correlation Coefficient (ICC) of variation of the Mark 5.0 device was predetermined to be 0.91 (95% CI 0.74-0.98).

Given that pregnancy is associated with changes in cervical epithelium that includes ectropion formation and physiological high-grade metaplasia[50], we matched the EIS spectra to previously generated templates corresponding to normal squamous and columnar epithelia using a minimum least squares method[51]. As previously described[51], these templates generated from three-dimensional finite element models of epithelial tissue types[52, 53] are shown to improve discrimination for clinical study outcomes in the cervix[51] and the tongue[37]. Measured EIS spectra were used to construct probability distributions for each tissue type, enabling the relative probability that the measurement corresponded to columnar or squamous epithelium, to be determined.

The experimental procedure: All measurements were taken by a single operator after structured training on capturing EIS measurements and transvaginal ultrasound measurement of cervical length, employing a standardised experimental protocol. On attendance, each woman had triple high vaginal swabs (sterile Dacron swabs - Deltalab Eurotubo 300263, Fisher

Scientific, UK) taken to obtain cervicovaginal fluid, after the passage of a sterile Cusco's vaginal speculum - one swab sample used to quantify fetal fibronectin using the 10Q Rapid FFN analyser (Hologic, MA, USA) and another sent for bacteriological assessment. The sterile electrical impedance probe was then introduced and gently touched on the anterior lip of the cervix and a button was pressed to capture data automatically once a steady state application pressure of 2 N was attained[48]. Three consecutive measurements were taken in quick succession over about 2 minutes from each subject. Following removal of the device and the speculum, a transvaginal scan was then performed to measure cervical length – the shortest of three measurements was recorded. All data obtained were captured automatically into the ArQ database software which also subsequently captured participant details from clinical records. The operator was blind to the EIS data at the time of capture. Clinicians provided standard clinical care for the participants without knowledge of the EIS results. Ultrasound-indicated cerclage, vaginal progesterone and expectant care were offered for short cervix <25mm taking into account previous pregnancy history, the current gestational age and the woman's preferences.

Study outcomes:

The primary prediction outcome of the study was spontaneous delivery before 37 weeks' gestation. The secondary outcomes were delivery before 32 weeks, birth weight < 2500gm and composite adverse perinatal outcome (admission to the

neonatal intensive care unit, perinatal death, respiratory distress syndrome, intraventricular haemorrhage, necrotizing enterocolitis, and sepsis).

Sample size estimations:

These were based on data from our pilot study[43] and Jessop Wing birth statistics. We estimated that in order for EIS to be clinically useful, it should demonstrate reasonable sensitivity and specificity of over 80%. We reasoned that if the Mark 5.0 EIS device predicted preterm delivery with a sensitivity of within ±10% (i.e. 95% CI from 75 to 95%) we would require that 49 mothers who deliver early be included in our study population to have a sufficient subset of spontaneous preterm birth cases. Assuming that about 25% of women at very high risk of PTB have their babies before 37 weeks, as reported in literature and confirmed in our pilot study, we would need 4 x 49 (i.e. around 200) high-risk women (previous history of PTB) to be studied. This number would be sufficient for estimating specificity with a reasonable degree of precision since we would have up to 150 high-risk women who did not deliver prematurely. The precision for the specificity estimate would therefore be within ± 0.06 points, i.e. 95% CI 79% to 91%. To assess EIS performance in women with no previous history of preterm birth, we also recruited 250 women with no risk factors for PTB, a population for which current predictive tests have limited value, yet they account for >50% of PTB[54].

Data Analysis:

This was carried out employing SPSS 24 (IBM Corp., NY, USA) and MedCalc 14.8.1 (Medcalc Software, bvba, BE). Being a study of diagnostic accuracy, the data is reported and presented according to the revised STAndards for the Reporting of Diagnostic accuracy studies (STARD) Statement[55]. Descriptive statistics was employed to summarise all quantitative data. Normality of data was assessed by the Kolmogorov-Smirnov test. Categorical outcomes such as spontaneous PTB < 32 or < 37 weeks' gestation were compared using a Chi-squared test (or Chi-squared test for trend in the case of ordinal outcomes). Continuous variables were compared using parametric (Student t tests) and nonparametric (Mann-Whitney U) tests as appropriate.

Multi-variate analyses, including multiple linear and multiple logistic regressions (LR), were employed to exclude subject and test variables that could influence and confound observations on EIS predictive performance for preterm delivery. Following analysis of the transfer impedance at the 14 frequencies studied, the top 5 frequencies that showed reduced transfer impedance magnitude in the spontaneous PTB group of women in this study (19.5 – 312.5 kHz), were used for further analysis using LR. In addition to these inputs, the full spectra were also matched to templates for normal squamous and columnar tissues as previously described[51, 56] to give two additional model inputs. Given that a model performs optimally on the dataset employed to generate it, validation on another dataset from a similar population provides a better reflection of the usefulness of the model. We therefore employed these inputs, taken from a random subset (30%

of all cases) of the spectra measured from both preterm-delivered and term-delivered women, as a training set (n = 110) to produce a final set of model parameters - the EIS index (consisting of tissue transfer impedance values in the frequency range 19.5 – 312.5 kHz and template-matched probability estimates for normal squamous and columnar tissues) – which was then used prospectively on the remaining measured spectra (n = 255), to test the performance of the model in separating preterm-delivered from term-delivered women. The output probability of spontaneous PTB was used to produce graphical receiver operator characteristics (ROC) curves of percentage sensitivity against one hundred minus percentage specificity, to determine predictive accuracies of EIS for delivery before 37 weeks, quantified as sensitivity, specificity, positive and negative predictive values, positive and negative likelihood ratios, and areas under the ROC curve. Similar test accuracy ROC curves were generated for TVUS CL and FFN. In further analysis the probabilities of spontaneous PTB based upon the analysis of the EIS spectra were combined with probabilities based upon CL and FFN, measured in the same women, to derive a ROC curve of the combined predictive probability of spontaneous PTB of the three modalities. Kaplan-Meier survival curves of time to delivery were produced to depict predictive performance of EIS for preterm birth based on an optimal cut-off value of the EIS index. Comparisons of test accuracies were also made for different sub-cohorts of pregnant women.

Results

Of the 449 pregnant women recruited, comprising a group deemed at high risk of preterm birth on account of a previous history and another group with no previous history of preterm birth, 365 women were included in the studies. 84 were excluded from analysis because of a previous history of colposcopic cervical treatment, progesterone therapy during the index pregnancy, or indicated cervical cerclage for presumed cervical insufficiency. Of the 365 untreated women included 159 were deemed at high risk of PTB on account of previous history whilst 206 women were deemed at low risk of PTB. Of this untreated cohort there were 29 spontaneous PTBs, 14 indicated PTBs, and 322 term births (Figure 1). Participant demographic and clinical outcome characteristics are detailed in Table 1. Smoking prevalence and maternal BMI were significantly higher in women who spontaneously delivered < 37 weeks' than those who delivered at term.

Cervical EIS measurements.

Consistent with data obtained during an earlier feasibility study[43] readings obtained from the inner 3mm tetra polar electrode ring arrangement showed better separation of findings between preterm- and term-delivered women and are reported here. Cervical transfer impedance was lower in women who delivered preterm compared to those who delivered at term in the frequency range 39.1 - 312.5 kHz, for the total cohort of women studied (Table 2). For the women at high risk of preterm birth recurrence who were studied at 26-28 weeks,

cervical transfer impedance was also lower in the subset who delivered preterm in the frequency range 19.5 – 312.5 kHz (Table 3).

Model validation data for EIS.

The predictive performance of the developed model was similar between the training set and the validation/test as summarized in (Table 4).

Predictive accuracy of cervical EIS measured at 20-22 weeks and at 26-28 weeks for PTD < 37 weeks.

The combined total cohort of women was used to determine the overall predictive performance of cervical EIS in comparison with TVUS CL and FFN (Figures 3a and 3b). EIS, TVUS CL and FFN measured at 20-22 weeks' gestation were independently predictive of preterm delivery before 37 weeks. Combining all three modalities improved prediction of PTB < 37 weeks than TVU CL ($p < 0.05$) or FFN ($p < 0.05$) alone (Figure 3a). Furthermore, cervical EIS showed higher predictive accuracy for preterm birth than TVUS CL for shorter CL categories (AuROC 0.83 vs 0.75 for CL < 15mm n = 16, and AuROC 0.84 vs 0.53 for CL 15 - 25mm, n = 51)

For the subset of women studied again at 26-28 weeks' gestation (n = 121), EIS, TVUS CL and FFN were also predictive of delivery before 37 weeks (Figure 3b). Combining all three modalities improved prediction of PTB <37 weeks compared to TVU CL ($p < 0.05$) and FFN ($p < 0.05$) alone (Figure 3b).

Employing the optimal predictive EIS index at 20-22 weeks (0.118) in a binary classification to assess pregnancy continuation rates to 42 weeks, the high predictive performance of EIS is depicted by the Kaplan-Meier survival analysis of time-to-delivery curves shown in Figure 4 (Chi-squared 37.4922, DF 1, $p < 0.0001$).

Influence of previous obstetric history on cervical EIS prediction of PTB.

Taking into account a previous history of PTB, the accuracy of prediction of spontaneous delivery before 37 weeks and before 32 weeks was significantly better for EIS than CL ($p < 0.01$) and FFN ($p < 0.05$), when taken at 20-22 weeks, as shown in Table 5. Furthermore, predictive accuracy of EIS for PTB did not improve further by the incorporation of TVUS CL and FFN.

Prediction of delivery < 37 weeks in low-risk women with no history of PTD and in nulliparous women.

In women with no previous history of PTB (n=206), cervical EIS demonstrated a non-significant trend towards improved predictive performance for PTB < 37 weeks (n=4 AuROC 0.79, 95% CI 0.73-0.85) than ultrasound cervical length (AuROC 0.69, 95% CI 0.62-0.75).

Cervical tissue transfer impedance at 19.5 – 312.5 kHz was significantly lower [mean (SEM) 3.0 (0.16) Ohm.m vs 3.5 (0.07) Ohm.m, $p < 0.05$] in nulliparous women that delivered at <37 weeks (n = 6) than the term-delivered counterparts

(n=132) whilst mean cervical length did not significantly differ [mean (SEM) 36.3 (4.1) mm vs 39.6 (0.6) mm respectively, p = 0.22].

Discussion

We detail, for the first time, cervical Electrical Impedance Spectroscopy (EIS) measurements in the mid-trimester of pregnancy to predict spontaneous PTB (delivery <37 weeks). In our cohort of asymptomatic pregnant women, we compared EIS to conventional clinical tests – TVUS CL[10, 20] and vaginal fetal fibronectin quantitation[12]. We show that cervical EIS has strong predictive potential for subsequent PTB in this untreated cohort, with predictive accuracies comparable to, and in some clinical situations superior to, TVUS CL and FFN estimation[21]. Larger studies are required to determine whether EIS may prove of clinical utility to predict preterm delivery, either as a standalone test or in conjunction with current clinical tests, more so when combined with maternal demographics and previous history of preterm birth.

Our findings agree with our limited feasibility study of women at high risk of preterm birth[43] that also showed lower cervical tissue transfer impedance values in mid-trimester in those destined to deliver preterm. How cervical remodeling changes influence this observation remains unclear. EIS assesses the dielectric properties tissue which are influenced by cell volume, intra- and extracellular conductivities, cell plasma membrane capacitance[31, 57], and the functional state of cellular gap junction proteins[58]. Tissue transfer impedance also decreases with increasing hydration and oedema[42, 59, 60]. Cervical EIS assesses these properties in the epithelium[51, 52], as well as the subepithelial stroma[49]. Determining the relative contribution of these tissue characteristics to measured impedance is hampered by limited information regarding cervical histologic and microscopic changes during pregnancy. However, it can be

speculated that cervical remodeling changes several weeks/months before labour account for the lower transfer impedance values in women destined to deliver preterm. Recent studies by us and others have also demonstrated vaginal dysbiosis (characterized by community state types deficient in lactobacillus species) in women destined to deliver preterm birth[61-63], which may modulate cervical epithelial and stromal remodeling leading to preterm birth[61].

We have demonstrated that mid-trimester cervical EIS had predictive accuracies for spontaneous PTB that matched or exceeded TVUS CL or FFN. Incorporating the past history of a preterm birth into the risk assessment model improved the prediction of PTB by all assessed modalities with highest accuracies being achieved by cervical EIS alone or when combined with TVUS CL and FFN. We have also shown that cervical EIS demonstrated higher predictive accuracy for PTB than TVUS CL for shorter CL categories in out limited cohorts. Larger studies will determine whether EIS will improve PTB risk assessment in pregnant women with a short cervix < 15mm given that less than 1 in 25 of them will deliver before 32 weeks' gestation[11, 18]. Such studies will also clarify the PTB predictive performance of EIS in nulliparous and low risk women. In this initial report we detail the predictive performance of cervical EIS solely in pregnant women receiving no treatment intervention for preterm birth in order to reduce their potential confounding influence on study observations. A larger study will be required to determine the value of EIS in directing or assessing such interventions and in women have had cervical surgery, who have congenital uterine

malformations[64], and in multiple pregnancies given the dearth of adequate PTB risk assessment approaches for these groups [65-68].

The predictive performance of TVUS CL and FFN for PTB before 32 or 37 weeks' gestation in asymptomatic women studied in mid-trimester in our series is in agreement with other reports for unselected pregnant women as well as for those with a previous history of PTB[15, 16, 21, 22, 69]. Furthermore, our limited data also highlights that combining EIS with TVUS CL and FFN for risk assessing asymptomatic nulliparous women may improve PTB prediction and warrants further study in these women who currently have limited options for PTB risk assessment. If these studies are coupled with effective interventions and demonstrate benefit, they may enable the generation of better predictive algorithms and decision support tools for preterm birth management[70, 71].

If our observations are confirmed in larger studies, clinical adoption of cervical EIS as a preterm birth risk assessment tool may confer several advantages. It would provide a hand-held point-of care test that would require limited additional training of front-line care practitioners in the maternity health care space. Regardless of the varied aetiologies of the preterm birth syndrome, the device assesses cervical remodelling which is the final common path to the onset of preterm labour. It could potentially be employed for risk assessment for the majority of pregnant women. It can be employed in obese women subject to possible technical difficulties of visualizing the cervix during a pelvic examination. Minimal training is required, and technical proficiency may be

achieved after about 10 measurements by a medically trained person able to perform a pelvic examination. An operating manual and/or training video would suffice to deliver such training. Being distinct from existing risk assessment approaches, EIS could prove complimentary to them.

This study has several limitations. The sample size is limited and has precluded assessment of women who have had cervical loop excisional treatment, cervical cerclage or progesterone, as well as those with multiple pregnancy. The study population was mainly Caucasian and further studies of women of other ethnicities and races will be required to demonstrate generalizability of findings. Although a single operator obtaining all study measurements has ensured better reliability of the data, inter-observer performance of the measurement has not been assessed. However, we have previously reported high inter- and intra-observer repeatability and reproducibility of cervical EIS [47]. The limited sample size also precluded assessing EIS prediction of secondary outcomes such as neonatal morbidity. Larger, multi-centre studies will be required to determine the potential value of cervical EIS for routine unselected screening during pregnancy, the effects of treatments on EIS measurements, and the potential incorporation of EIS into assessment and treatment algorithms, as well as health economic and value of information analyses.

Conclusion: Subject to confirmation in larger studies our observations suggest potential clinical utility for cervical EIS for preterm birth risk assessment, either as a standalone test or in conjunction with existing modalities.

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Figure Legends

Figure 1

Flowchart showing participant recruitment details, inclusion and exclusion characteristics, cohorts and preterm birth outcomes.

Figure 2.

The Sheffield Mark 5.0 Electrical Impedance Spectroscopy device comprising the device base housing the electronics, accelerometer and pressure sensor and the device probe piece (A), showing the tetrapolar probe configuration (B), and the complete coupled device ready for use to take measurements.

Figures 3a

Receiver operator characteristic curves depicting predictive accuracy of EIS (Electrical Impedance Spectroscopy), TVUS CL (Transvaginal ultrasound cervical length) and FFN (Fetal fibronectin) assessed at 20-22 weeks for spontaneous preterm delivery before 37 weeks' gestation. EIS AuROC 0.76, 95% CI 0.71-0.81, $p < 0.0001$; TVUS CL AuROC 0.72, 95% CI 0.66-0.76, $p < 0.001$; FFN AuROC 0.62, 95%CI 0.65-0.74, $p=0.05$. Combining all three modalities (EIS + CL + FFN) demonstrated significantly better prediction of spontaneous PTB < 37 weeks (AuROC 0.79 95% CI 0.74-0.83) than TVUS CL ($p < 0.05$) or FFN ($p < 0.05$) alone.

Figure 3b

Receiver operator characteristic curves depicting predictive accuracy of EIS (Electrical Impedance Spectroscopy), TVUS CL (Transvaginal ultrasound cervical length) and

FFN (Fetal fibronectin) assessed at 26-28 weeks for spontaneous preterm delivery before 37 weeks' gestation. EIS AuROC 0.80, 95% CI 0.71 - 0.88, $p < 0.0001$; TVUS CL AuROC 0.66, 95% CI 0.57 - 0.74, $p < 0.05$); FFN AuROC 0.66, 95%CI 0.57 - 0.75, $p = 0.37$). Combining all three modalities (EIS + CL + FFN) demonstrated significantly better prediction of spontaneous PTB < 37 weeks (AuROC 0.79 95% CI 0.74-0.83, $p < 0.0001$) than TVUS CL ($p < 0.05$) or FFN ($p < 0.05$) alone.

Figure 4

Kaplan-Meier survival analysis of time-to-delivery curves for all the women studied at 20-22 weeks' gestation employing a binary classification based on an optimal predictive cut-off EIS index value of 0.118 to assess pregnancy continuation rates to 42 weeks' gestation.

Table 1. Subject characteristics and birth outcomes.

	Preterm delivery (<37 weeks) N=43	Term delivery (≥ 37 weeks) N=322	P value
Race			
White (93%)	36	299	
Asian (2.7%)	2	8	
Black (3.3%)	5	9	
Arab (0.3%)	0	2	
Mixed (0.6%)	0	4	
Age, y, median (IQR)	31 (28 to 36.0)	30 (26-33)	0.081
Body mass index (BMI), kg/m2, median (IQR)	29.4 (25.8 to 32.5)	24.6 (22.4 to 28.3)	< 0.001
Parity			
0 (39.3%)	6	132	
≥1 (60.7%)	37	190	
Previous preterm delivery (24 - 37 weeks), N = 132	31/43 (72%)	101/322 (31.4%)	< 0.0001
Smoking	13/43 (30.2%)	51/322 (15.8%)	< 0.01

Previous mid-trimester miscarriages (14 - < 24 weeks), N = 48	8/43 (18.6%)	40/322 (12.4%)	0.1271
Cervical length, mm, mean (SD) at 20 - 22 weeks at 26 - 28 weeks	 33 (9.8) 31 (8) N = 14,	 40 (6.7) 36 (7), N = 107	 P < 0.001 P = 0.03
Fetal fibronectin, ng/mL, median (IQR) at 20 - 22 weeks at 26 - 28 weeks	 9 (4 - 52) 12.5 (4 - 67), N = 14	 6 (3 - 13) 4 (2.8 - 12.5), N = 107	 P = 0.010 P =0.038
Birth outcomes	Spontaneous preterm delivery (<37 weeks) N = 29	Term delivery (≥ 37 weeks) N = 322	P value
Gestation at delivery, weeks, median (IQR*)	36 (31 - 35)	39 (39 - 40)	< 0.001
Birthweight g, median (IQR)	2340.5 (1747.3 to 2592.5)	3435 (3100.0 to 3780.0)	P < 0.001

*IQR: interquartile range,

Table 2. Cervical transfer impedance values measured across 14 frequencies at 20-22 weeks' gestation, in term-delivered and spontaneous preterm-delivered women.

Cervical EIS measurement frequency (Hz)	Spontaneous preterm delivery <37 weeks' gestation (N = 29)		Term delivery ≥ 37 weeks' gestation (N = 322)		
	Mean cervical impedance (Ohm.meter)	SEM*	Mean cervical impedance (Ohm.meter)	SEM	P value
76	29.83	5.14	29.88	1.29	0.9916
153	28.25	4.85	28.23	1.20	0.9968
305	26.22	4.39	26.24	1.09	0.9947
610	23.23	3.70	23.42	0.94	0.9542
1221	19.16	2.78	19.78	0.75	0.8142
2441	14.66	1.87	15.56	0.57	0.6474
4883	10.46	1.20	11.61	0.41	0.4202
9766	7.19	0.75	8.34	0.30	0.2634

19531	4.92	0.42	5.90	0.22	0.1819
39063	3.50	0.22	4.14	0.08	0.0219
78125	2.66	0.12	3.09	0.04	0.0048
156250	2.17	0.08	2.44	0.03	0.0035
312500	1.79	0.06	1.97	0.02	0.0062
625000	1.47	0.05	1.55	0.01	0.0641

*SEM: standard error of the mean

Table 3. Cervical transfer impedance values measured across 14 frequencies at 26-28 weeks' gestation (high risk pregnancies that continued to this gestation), in term-delivered (n=107) and spontaneous preterm-delivered (n=14) women with a previous history of preterm birth.

Cervical measurement frequency (Hz)	Spontaneous preterm delivery <37 weeks' gestation (N = 14)		Term delivery ≥ 37 weeks' gestation (N = 107)		
	Mean cervical impedance (Ohm.meter)	SEM*	Mean cervical impedance (Ohm.meter)	SEM	P value
76	18.56	4.73	28.37	1.90	0.0781
153	17.64	4.51	26.77	1.77	0.0795
305	16.53	4.23	24.76	1.60	0.0817
610	14.97	3.81	21.86	1.36	0.0887
1221	12.87	3.21	18.22	1.08	0.0981
2441	10.50	2.55	14.22	0.79	0.1198
4883	8.13	1.85	10.67	0.55	0.1280

9766	6.02	1.21	7.84	0.36	0.0954
19531	4.36	0.71	5.71	0.22	0.0381
39063	3.21	0.39	4.23	0.12	0.0070
78125	2.51	0.21	3.25	0.08	0.0015
156250	2.06	0.12	2.57	0.05	0.0014
312500	1.70	0.07	2.03	0.04	0.0055
625000	1.41	0.05	1.56	0.03	0.0529

*SEM: standard error of the mean

Table 4. Model development: Accuracy of prediction of spontaneous delivery < 37 weeks, training set and testing/validation set)

	Training set (N = 110)	Test/Validation set (N = 255)
Births < 37 weeks', n (%)	10 (9%)	19 (9%)
AuROC (95%CI)	0.80 (95% CI 0.72 - 0.87)	0.77 (95% CI 0.69 - 0.86)
Sensitivity (%)	80.0 (95% CI 44 - 98)	73.0 (95% C1 67-80)
Specificity (%)	85.3 (95% CI 77 - 92)	84.0 (95% C1 77-92)

Table 5 The influence of previous history of preterm birth on the predictive accuracy of cervical EIS, TVUS CL and FFN assessed at 20-22 weeks' gestation (visit 1, n = 365) for spontaneous preterm birth (n = 29).

Prediction of spontaneous delivery before 37 weeks (n = 29)								
Parameter	**AuROC (95%CI)**	**Sensitivity**	**Specificity**	**PPV**	**NPV**	**+LR**	**-LR**	**P value**
Cervical EIS + previous history (20-22 weeks)	0.83 (0.78 - 0.87)	81.0 (60.6 – 93.4)	72.0 (66.2 – 76.8)	20.4 (16.5-25.0)	97.7 (95.0-98.9)	2.86 (2.2-3.7)	0.27 (0.1-0.6)	< 0.0001
CL + previous history (20-22 weeks)	0.71 (0.66 - 0.76)	62.1 (42.3-79.3)	74.8 (69.7 – 79.5)	18.2 (13.6-23.8)	95.6 (93.2-97.2)	2.47 (1.8-3.5)	0.51 (0.3-0.8)	< 0.001

FFN + previous history (20-22 weeks)	0.75 (0.70 - 0.80)	79.3 (60.3 – 92.0)	66.3 (60.8-71.4)	17.6 (14.3-21.3)	97.2 (94.5-98.6)	2.35 (1.8-3.0)	0.31 (0.2-0.6)	< 0.0001
Cervical EIS + CL + FFN + previous history	0.81 (0.77 - 0.85)	65.3 (44.3 - 82.8)	88.2 (83.9 - 91.7)	33.3 (24.7-43.3)	96.6 (94.3-98.0)	5.54 (3.6-8.4)	0.39 (0.2-0.7)	< 0.0001
Prediction of spontaneous delivery before 32 weeks (n = 13)								
Cervical EIS + previous history (20-22 weeks)	0.86 (0.82-0.90)	75 (34.9 – 96.8)	91 (87.1 – 93.9)	17.6 (11.2 - 26.8)	99.3 (97.7 - 99.8)	8.25 (4.8 - 14.1)	0.28 (0.08 - 0.9)	< 0.0001

CL + previous history (20-22 w)	0.78 (0.74-0.82)	54 (25.1 - 80.8)	90 (85.8 - 92.5)	15.9 (9.5-25.4)	98.1 (96.7-99.0)	5.12 (2.8-9.2)	0.52 (0.3-0.9)	< 0.0001
FFN + previous history (20-22 w)	0.81 (0.76-0.85)	69 (38.6 – 90.9)	89 (85.4 – 92.2)	19.1 (12.9-27.5)	98.7 (97.2-99.5)	6.38 (4.0-10.2)	0.35 (0.2-0.8)	< 0.001
Cervical EIS + CL + FFN + previous history	0.88 (0.84 – 0.92)	75 (34.9 – 96.8)	94 (90.9 – 96.5)	25.0 (15.5-37.8)	99.0 (97.7-99.8)	12.8 (7.0-23.3)	0.27 (0.08-0.90)	< 0.0001

FFN: Fetal fibronectin, previous history: previous history of preterm birth, CL: cervical length, PPV: positive predictive value, NPV: negative predictive value, +LR: likelihood ratio, -LR: negative likelihood ratio.

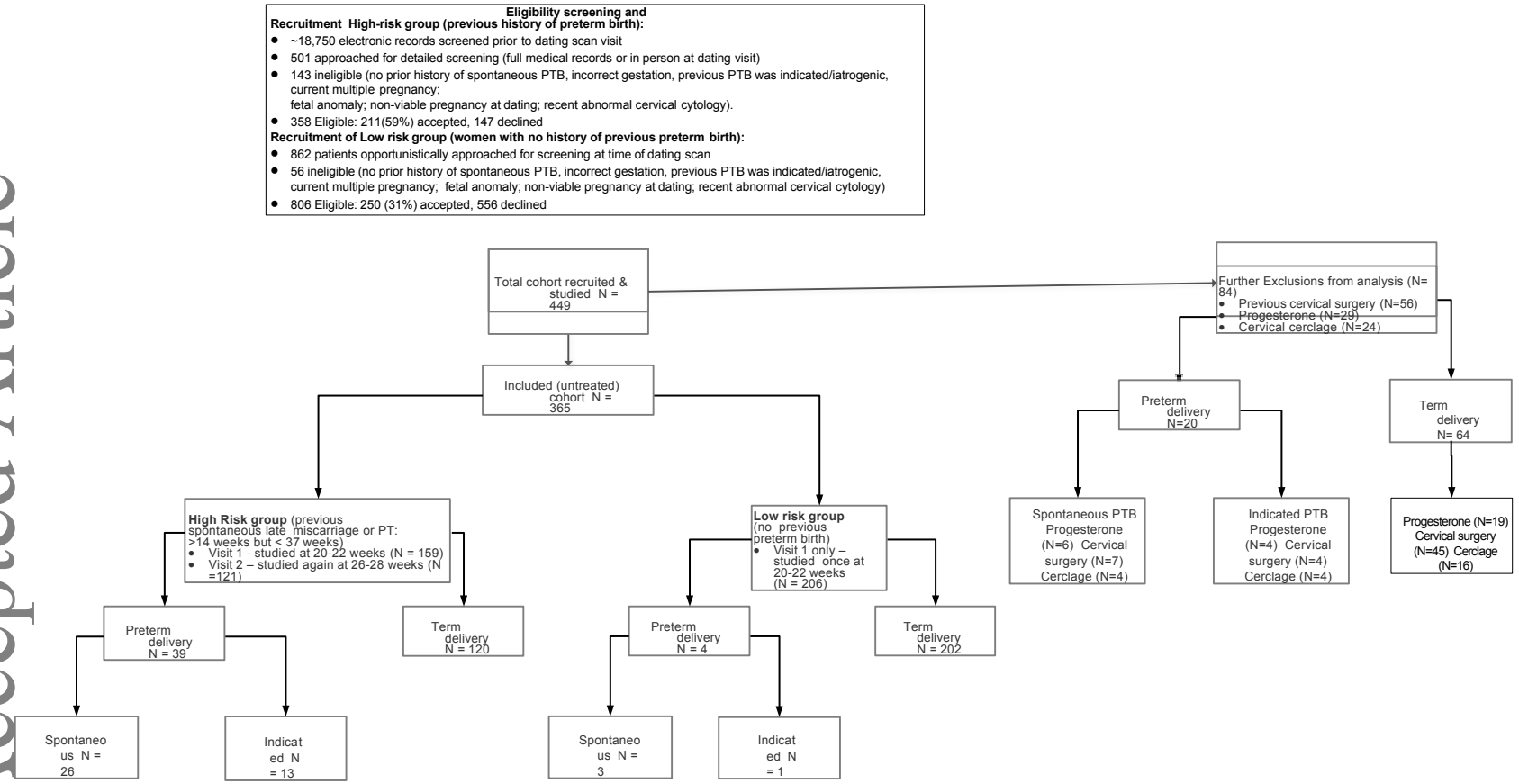

Figure 1

Figure 2

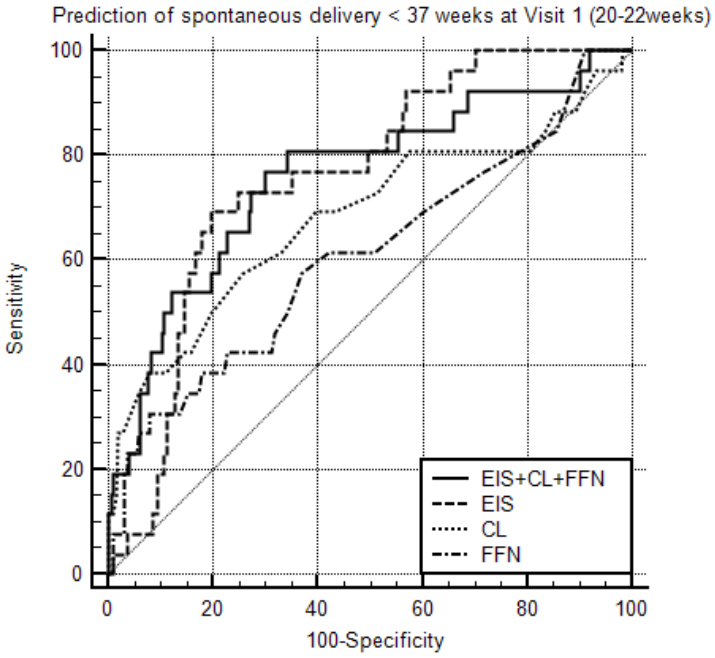

Figure 3a

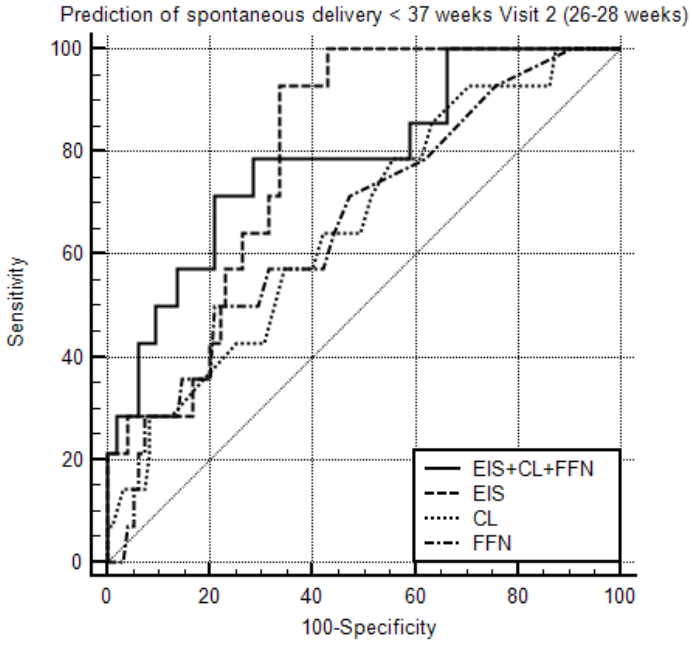

Figure 3b

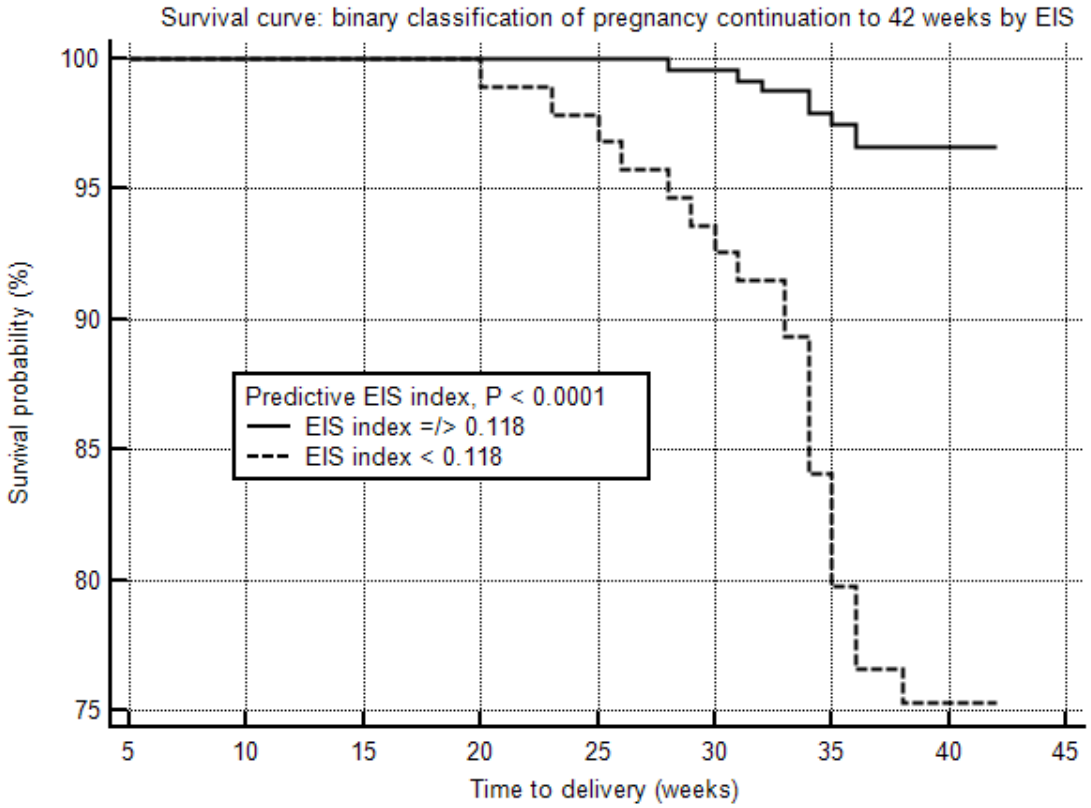

Figure 4