European Journal of Obstetrics & Gynecology and Reproductive Biology 208 (2017) 103–108

Contents lists available at ScienceDirect

European Journal of Obstetrics & Gynecology and Reproductive Biology

journal homepage: www.elsevier.com/locate/ejogrb

Full length article

The effect of blood staining on cervicovaginal quantitative fetal fibronectin concentration and prediction of spontaneous preterm birth☆

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ARTICLE INFO

Article history:
Received 13 September 2016
Received in revised form 23 November 2016
Accepted 27 November 2016
Available online xxx

Keywords:
Blood staining
Fetal fibronectin
Preterm birth

ABSTRACT

Objective: Spontaneous preterm birth is the leading cause of neonatal morbidity and mortality. Cervicovaginal fetal fibronectin (fFN) has enhanced prediction of preterm birth and, more recently, quantified results have become available so that management can planned more effectively and targeted to individual women. Manufacture guidelines stipulate that fetal fibronectin (fFN) samples should be discarded in the presence of moderate to heavy vaginal bleeding but there hasn't yet been any formal investigation into the effect of blood staining on fetal fibronectin concentration and subsequent preterm birth prediction. The objective for this study was to determine the impact of blood stained swabs on quantitative fetal fibronectin (qfFN) concentration and prediction of spontaneous preterm birth (sPTB) in asymptomatic high-risk women.

Study design: Predefined blinded sub-analysis of a larger prospective study of qfFN in asymptomatic women at high-risk of preterm labour. Women with and without blood stained swabs were matched for gestational age at testing and delivery, risk factors and cervical length measurement.

Results: Median fFN concentration in blood stained swabs (n = 58) was 66 ng/ml vs. 7.5 ng/ml in the controls (n = 58) ($p < 0.0001$). At ≥50 ng/ml threshold the false positive ratio (FPR) in blood stained was 25/33 (75.8%) vs. 8/15 (53%) in controls, (risk difference 22.4; −6.8 to 51.6, p = 0.18). At ≥50 ng/ml threshold the false-negative ratio (FNR) in blood stained was 2/25 (8.0%) vs. 1/43 (2.3%) in controls (risk difference −5.7; −17.2 to 5.9, p = 0.55).

At each threshold 10, 50 and 200 ng/ml blood stained swabs had higher sensitivity but lower specificity for predicting preterm birth. Receiver Operating Characteristic (ROC) curve, the strongest global measure of test performance, for prediction of delivery at <34 weeks gestation was similar in blood stained vs. control groups. (0.78 vs. 0.84) in blood stained vs. control groups respectively.

Conclusion: Blood stained swabs have elevated qfFN concentrations but may still have predictive value, and clinical utility. Very low fFN values (<10 ng/ml) are especially reassuring and indicate lower risk of delivery than non-blood stained swabs. The higher false positive rate must be noted and explained to the patient.

Introduction

Spontaneous preterm birth (sPTB), birth before 37 completed weeks' of gestation), is the leading cause of neonatal morbidity and mortality [1]. Prediction of sPTB in symptomatic and asymptomatic high-risk women has been enhanced in recent years by the use of cervicovaginal fluid (CVF) fetal fibronectin (fFN) testing, now widely used in clinical practice. fFN is a glycoprotein found at the interface between chorion and decidua [2] which is usually present in low levels in CVF from 18 weeks of gestation; high levels after

☆ Natasha L. Hezelgrave is funded by a National Institute for Health Research (NIHR) Doctoral Research Fellowship (DRF-2013-06-171). The views expressed are those of the authors and not necessarily those of Tommy's, the NHS, the NIHR or the Department of Health.

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http://dx.doi.org/10.1016/j.ejogrb.2016.11.027
0301-2115/

this time may indicate choriodecidual disruption preceding preterm labour.

fFN has repeatedly been shown to have a high negative predictive value; an excellent 'rule out' test for spontaneous delivery between 23 and 34 weeks gestation. In contrast, the positive predictive value is sub-optimal (<20%) [3]. Traditionally a qualitative test (positive/negative at a threshold concentration of 50 ng/ml), we have now demonstrated improved accuracy in symptomatic [4] and asymptomatic [5] women using a novel bedside analyser (Hologic, Marlborough MA, USA) allowing rapid quantification of fFN concentration; quantitative fetal fibronectin (qfFN), with alternative concentration thresholds of 10 ng/ml and 200 ng/ml more accurately defining those at low and high risk respectively. This has enabled more accurate risk prediction amongst women who would have all traditionally been classified as 'positive', enhancing the positive predictive value of the test (up to 50%), whilst maintaining strong negative prediction.

Manufacture guidelines for both qualitative and quantitative tests stipulate that they should not be used with 'moderate or heavy vaginal bleeding' as plasma fFN can interfere with the CVF fibronectin assay giving potential false positive tests [6]. This is undesirable for any diagnostic test, especially one with modest positive prediction. Similarly, it could be hypothesised that blood-staining of the swab, which independently of fFN can indicate preterm birth risk, could give rise inappropriately to a false negative fFN test. However, incidental macroscopic blood-staining on a cervicovaginal swab is not uncommon, often attributed to the disruption of friable cervical tissue or due to a cervical ectropion. Yet we have not been able to locate any published studies describing the effect of blood-staining on fetal fibronectin results; it is not known whether blood increases false positive rates randomly due to assay cross-over, or whether a test taken from those who had visible blood-staining may have value, but at a different threshold than those currently used. The introduction of the quantified test may allow this to have clinical utility in practice.

The aim of this study was to compare qfFN concentration in a group of asymptomatic high-risk women, with visibly blood-stained swabs, taken between 18^{+0} and 27^{+6} weeks of gestation ('cases'), to a matched group of high-risk asymptomatic women with normal swabs ('controls'). Predictive statistics for sPTB <34 weeks of gestation were calculated and compared.

Materials and methods

A sub-analysis of a larger prospective blinded observational study (**E**valuation of **Q**uantitative Fetal Fibronectin **i**n **P**rediction of **P**reterm Birth, EQUIPP) evaluating the prediction of sPTB using qfFN in high-risk asymptomatic women [5]. The study took place between October 2012–September 2013 at five teaching hospitals in the United Kingdom and was approved by the South East London Research Ethics Committee (REC no: 10/H0806/68 London, UK). Written informed consent was obtained from all participants. Gestational age (GA) was confirmed by early obstetric ultrasound (11–14 weeks' gestation). Participant baseline demographics, obstetric history and risk factors were entered onto an online secure study specific database (www.medscinet.net/ptbstudies). Women were considered high risk if they were 18 + 0–27 + 6 weeks' gestation (the clinically recognized gestational window for fFN testing) [5,7] with one or more of: previous sPTB, previous premature preterm rupture of membranes (PPROM), previous late miscarriage ($16–23^{+6}$), previous cervical surgery (LLETZ, cone biopsy), uterine abnormality or a cervical length <25 mm in this pregnancy. Women presenting with moderate or heavy vaginal bleeding were not included.

Participants with 'macroscopically blood stained' qfFN swabs were matched (1:1) with women from the same database with normal swabs, according to gestational age at testing and delivery (±7 days) and risk factors for PTB (previous sPTB, previous late miscarriage, previous cervical surgery, uterine abnormality or cervical length <25 mm in the current pregnancy). Women with no suitable matched control were excluded.

The qfFN samples were collected as per manufacturer's instructions (Hologic). At speculum examination, Dacron swabs were rotated in the posterior fornix of the vagina for approximately 10 s. Swabs were placed in a test buffer (200 μl aliquots) which were then analysed simultaneously by the qualitative Rapid fFN TLI_{IQ} analyser (Hologic) and quantitative Rapid fFN 10Q analyser (Hologic). Clinicians were blinded to the quantitative result (a result code was generated by the analyzer) but the qualitative result was made available. The 10Q analyser has a range between 0 and 500 ng/ml (upper limit). The reliability of the Rapid 10Q analyser has previously been published [8]. Test thresholds (cut offs) of 10, 50 and 200 ng/ml were pre-defined prior to study data analysis based on the literature [9]. Pregnancy outcome details were obtained from handheld note review by trained research midwives and data entered onto the study database. Data entry was checked for inaccuracies contemporaneously by senior research midwives. Women were considered to have the outcome of interest (sPTB) if they had spontaneous onset of labour, or experienced PPROM, with subsequent premature delivery. Women with iatrogenic delivery <34 weeks' were excluded. Samples from women reporting prior sexual intercourse (within 48 h) were excluded from analysis due to known interference with the assay [10], as were results from women with PPROM, multiple pregnancy or cervical dilation ≥3 cm. A 'true positive' result was defined as spontaneous onset of labour (or PPROM) <34 weeks' with qfFN >50 ng/ml. A 'false positive' result was CVF qfFN ≥50 ng/ml at testing, and delivery $>33^{+6}$ weeks' gestation. Predictive statistics using alternative thresholds (10 ng/ml and 200 ng/ml) were also explored.

Statistical analysis was conducted using the Stata software (version 11.2; StatCorp LP, College Station, TX). Standard distributional checks were carried out, asymmetric qfFN values logged and checks repeated. Geometric means were generated after transformation of log-normal distributions. Quantitative fFN values were compared between groups using Student's *t*-tests on log transformed values and (nonparametric) area under the Receiver Operating Characteristic (ROC) curves. Medians were compared using the Wilcoxon rank sum test. Results are reported as ratios of geometric means. To check for a difference in performance between the blood stained and normal swabs, interaction between swab status and test result was compared using logistic regression with a correction to the standard errors for matching [10].

Results

A total of 63 asymptomatic high-risk participants with singleton pregnancies and blood stained swabs between 18^{+0} and 27^{+6} weeks' were identified. Of these, 2 participants who underwent iatrogenic deliveries (both pre-labour induction for pre-eclampsia) were excluded, and 1 was excluded due to an 'invalid' qfFN result (the bedside analyser was unable to provide a result). Two more were excluded due to lack of appropriate matched control, leaving 58 participants fulfilling criteria for analysis. These were matched with 58 controls according to gestational age at testing, gestational age at delivery, and risk factors for premature birth. Demographic, background, and obstetric characteristics for study participants are described in Table 1 and were comparable for cases and controls. Mean gestational age at testing for both groups was 23^{+1} weeks, and mean gestational age at delivery was 37^{+1} weeks. sPTB rate <34 weeks and <37 weeks gestation in the blood stained cases was 10/