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Abstract

Background: Preterm birth continues to be a major cause of infant morbidity and mortality worldwide, but advances have recently been made in its prediction and prevention. A short cervix ($<25\text{mm}$) in the second trimester on transvaginal ultrasound scan and fetal fibronectin are important predictive tests. For over ten years, the Preterm Labour Clinic at the Royal Women's Hospital, Melbourne, Australia has provided care for women at high risk of preterm birth, including those with a previous preterm birth, previous cervical surgery, uterine malformation or incidental finding of short cervix at routine ultrasound. The purpose of this study was to review this clinic's outcomes for the first decade.

Methods: This was a retrospective cohort study of all referrals to the Preterm Labour Clinic during the period 2004 to 2013 inclusive. 756 cases met the study inclusion criteria of appropriate risk factor, singleton pregnancy, surveillance undertaken and outcome data available.

Results: The preterm birth rate (<37 weeks) was 21.4%. The rate of preterm birth by year decreased significantly when adjusted for risk ($p=0.002$). A short cervix was diagnosed in 32% of the sample, and positively correlated with lower gestational age at delivery. Fetal fibronectin and serum alkaline phosphatase were independent predictors of preterm birth <34 weeks and <37 weeks.

Conclusion: The adjusted preterm birth rate at the Royal Women's Hospital's Preterm Labour Clinic has decreased significantly over the decade studied. Positive fetal fibronectin at 26 weeks and elevated serum alkaline phosphatase are independent predictors of preterm birth.

Introduction

Prematurity is a key contributor to the global burden of disease:¹ it is the leading cause of neonatal mortality worldwide, and has potentially lifelong consequences for survivors.. The most serious morbidity and highest mortality are typically associated with earlier gestational age at delivery. Preterm birth, by definition, occurs prior to 37 completed weeks' gestation, and may be further categorised into extreme prematurity

(less than 28 weeks' gestation), severe prematurity (28 – 31 weeks), moderate prematurity (32 – 33 weeks) and near term (34 – 36 weeks).² Most preterm births occur spontaneously following either preterm prelabour rupture of membranes (PPROM) or spontaneous labour, while a smaller proportion are indicated preterm births for maternal and/or fetal concerns such as pre-eclampsia.

The early identification of women at risk of preterm birth allows targeted administration of potentially beneficial therapies, including antenatal corticosteroids for fetal lung maturation and magnesium sulphate for neuroprotection.^{3, 4} Both vaginal progesterone and cervical cerclage have been shown to reduce the incidence of premature birth and improve neonatal outcomes,⁵ however cerclage in particular carries significant risks, including PPRM, preterm labour and maternal sepsis.⁶

The advent of transvaginal ultrasound (TVUS) measurement of the cervix gave rise to observations of a strong association between a second trimester cervical length of less than 25mm and preterm birth.⁷ Additional ultrasound findings that have an association with preterm birth include funnelling of the cervix (opening of the internal os, with or without prolapsing of the amniotic membrane into the endocervical canal)⁸ and the presence of intra-amniotic “sludge” (an echogenic collection of inflammatory cells and bacteria).⁹ Recent research suggests that the shortening cervix is likely, in most cases, to be an accompanying sign of an abnormal process leading to preterm birth, rather than its aetiology. Preterm labour itself appears to be a common endpoint on which numerous pathological processes converge.¹⁰

The significance of a short cervix, and the emergence of other predictive tests shown to be useful in asymptomatic women (most notably fetal fibronectin),¹¹ have seen many tertiary hospitals establish specialist preterm labour clinics (PTLC). The Royal Women's Hospital (RWH), Melbourne, Australia, has held such a clinic for over 10 years for women at high risk of preterm birth.

The aim of this study was to detail the clinical outcomes of patients managed through a specialist preterm labour clinic, and examine the performance of predictive tests.

Methods

Ethics

This project was granted exemption from formal ethics review by the local institutional Human Research Ethics Committee, as it was deemed to meet the criteria established for quality assurance activities outlined in the NHMRC guideline *Ethical Considerations in Quality Assurance and Evaluation Activities 2014*.

Participants

Women between 14 and 26 weeks' gestation who meet one or more of the following criteria are eligible for PTLC:

- ≥ 1 previous preterm birth or midtrimester loss
- ≥ 1 previous cold knife cone biopsy OR ≥ 2 large loop excisions of the transformation zone (LLETZ)
- ≥ 3 surgical terminations of pregnancy OR ≥ 4 dilatation and curettage procedures
- Cervical length $< 25\text{mm}$ (TVUS) noted incidentally on midtrimester fetal morphology scan
- Uterine malformation

Data pertaining to all women seen at the RWH PTLC from the first clinic of 2004 through to the end of 2013 were obtained. Pregnancies were excluded for the following reasons: no surveillance undertaken (single visit only or testing declined), multiple pregnancy, referral not meeting criteria, delivery data missing, or iatrogenic preterm birth. Where more than one cervical length measurement was available for a given week of gestation, the shortest was recorded for analysis. Ethnicity details were not collected due to limited relevance to risk of preterm birth in the Australian population (the best-described high-risk groups are African-American and Afro-Caribbean).¹²

Procedure

Women visit the clinic fortnightly, where they receive cervical surveillance via TVUS using a GE Logiq3 ultrasound machine (GE Healthcare, Fairfield, CT, USA). Women found to have a cervical length $< 25\text{mm}$ are offered treatment. Cervical swabs are taken

to identify the presence of abnormal flora at each visit, with appropriate antimicrobials offered as indicated. Screening for chlamydia trachomatis is performed at the first visit,¹³ and serum thyroid stimulating hormone (TSH)¹⁴ and alkaline phosphatase (ALP)¹⁵ levels are checked. At the final visit, a fetal fibronectin (fFN) test is performed (Hologic Inc., Marlborough, MA, USA), and women with negative results return to routine antenatal care thereafter.

This was a retrospective observational cohort study. Data were retrieved from the electronic pathology viewer and paper files, and entered into a Microsoft Excel spreadsheet (Microsoft Corporation, Redmond, WA, USA). Cervical length, presence of funnelling or intra-amniotic sludge, fFN test result, TSH (dichotomised according to >3.0 mIU/L or ≤ 3.0 mIU/L), serum ALP (dichotomised according to RWH laboratory cutoff >91 U/L or ≤ 91 U/L) were included as independent variables; the primary dependent variable was gestational age at delivery (dichotomised according to ≥ 37 weeks or <37 weeks). Analysis was also performed for a gestational age cutoff of 34 weeks.

Means, medians and standard deviations were calculated in Excel for demographic data and cohort outcomes. Proportions were compared using an online calculator (www.socscistatistics.com/tests/ztest/Default2.aspx). GraphPad Prism v5.01 was used for correlations, comparing medians and contingency tables (Spearman, Mann-Whitney U and chi-squared analyses respectively, with Fisher's exact test used for cell counts less than five). R version 3.1.2 was used for linear regression analysis, and Stata 13 for logistic regressions. Standard logistic regressions revealed separation or quasi-separation, so the Firth modification¹⁶ was applied to overcome this.

Results

A total of 939 cases were identified, with 756 included in the analysis once exclusion criteria had been applied. Demographic information and outcome data are summarised in Table 1. The main indications for surveillance are summarised in Figure 1; these were previous SPTB (53.79% of referrals), history of cervical surgery (24.20%); uterine malformation (11.35%) and incidental finding of short cervix (9.16%). Women frequently had two or more risk factors for SPTB, and in this situation the main or

underlying reason for referral guided allocation to groups. For example, a woman with a unicornuate uterus who had previously had a SPTB was considered to be referred due to her uterine malformation which was presumed to be the underlying cause of the SPTB. Proportions of referrals remained largely consistent over the study period.

The rate of preterm birth (< 37 weeks) in the overall cohort was 21.40%. The mean gestational age at delivery was 37.16 ± 4.47 weeks (range 15 – 42 weeks). No significant difference in mean gestational age or preterm birth rate was found when comparing outcomes for years 2004 – 2008 to years 2009 – 2013. However, with adjustment for the increased proportion of sonographically short cervixes amongst attendees over the decade (a marker of risk status), linear regression showed a significant decrease in the rate of preterm births ($p=0.007$) (cf. Figure 2).

Tables 2.1 and 2.2 summarise the univariate analysis of potential risk factors for SPTB; Tables 3.1 and 3.2 outline the properties of the predictive tests used at the clinic.

The incidence of a short cervix (closed endocervical length of less than 25 mm) amongst attendees of the PTLC was 32%. Cervical length was positively correlated with gestational age at delivery ($r=0.2758$, $p < 0.0001$); the “short cervix” group had a mean gestational age at delivery of 35.44 weeks (± 5.46), significantly earlier than the “long cervix” group (37.95 ± 3.66 weeks, $p < 0.0001$). A short cervix was a significant predictor of SPTB < 37 weeks in univariate analysis ($p < 0.0001$) but not in multivariate analysis. Despite this, a short cervix had limited positive predictive value in the cohort as a whole (36.25%, refer to Table 3). In subgroup analysis, its PPV was best amongst the uterine malformation subgroup (50%), as compared to previous preterm birth (39.47%) and cervical surgery groups (27.27%).

Funnelling of the cervix was present in 27.21% of women: 75.42% of women with a short cervix had funnelling compared to 4.84% of women with normal cervical length. Univariate analysis revealed funnelling to be a significant predictor of SPTB ($p < 0.0001$). Sludge was only reported from 2009 onwards and occurred in 10.80% of pregnancies during this period, also in association with a short cervix (28.77% vs 1.43%

of the long cervix group). It was also a significant predictor of SPTB on univariate analysis ($p=0.0079$).

Of the short cervix group, 80% ($n=196$) received one or more treatments (see Table 1). The “no treatment” subgroup was seen before 2008, in the period before vaginal progesterone was supported by evidence. A short cervix was noted for the first time at 24 weeks’ gestation or later in 32% of this subgroup (15/47), and most of these (9/15) women were advised to have a period of bed rest at home or in hospital.

Second trimester TSH and ALP were not performed over the entire study period, and therefore yielded small numbers of abnormal results. ALP and fFN were highly significant predictors of SPTB in univariate analysis, whereas TSH was not (see Table 3.1 and 3.2). Use of assisted reproductive technology (ART, excluding ovulation induction alone) was not predictive of SPTB.

All single variables that were moderately predictive of SPTB on univariate analysis ($p < 0.2$, see Table 2.1 and 2.2) were entered into a logistic regression; these included cervical length $< 25\text{mm}$, fFN result, ALP result, parity, funnelling, sludge, and history of cervical surgery. In this model, both fFN and ALP emerged as independently predictive of SPTB ($p=0.048$ and 0.018 respectively), but cervical length $< 25\text{mm}$ was not. When only ultrasound indicators (cervical length $< 25\text{mm}$, funnelling, sludge) were used in the regression, a short cervix was the only statistically significant predictor of SPTB ($p=0.001$). Results were comparable for preterm birth at < 34 weeks, and when cervical length $< 15\text{mm}$ was used as an alternative threshold.

Ninety six percent of women (763/791) attending PTLC had at least one cervical swab taken. Abnormal flora were cultured from one or more of these tests in 65% (493/791) of this group. The most prevalent microorganisms were ureaplasma species (urealyticum or parvum, 39.84%), candida albicans (20.18%) and mixed anaerobes (16.64%). No significant association between presence of abnormal flora in any swab, or ureaplasma colonisation, and preterm delivery was found, nor was there any association between antibiotic treatment and preterm birth. When persistent colonisation was considered, defined as two or more consecutive swabs yielding the same pathogen

(excluding Group B streptococcus), there was a moderate association with preterm birth before 34 weeks that was not seen on multivariate analysis.

Routine testing for chlamydia at the first PTLC visit began in 2008. In the entire cohort, 212 women were tested for chlamydia, with a 2% infection rate.

Discussion

The rate of preterm birth amongst this cohort is somewhat lower than other published results for a high-risk population.¹⁷⁻¹⁹ Its reduction over the study period, despite an increase in the risk profile of clinic attendees as determined by their cervical length, is likely due to the use of new preventive treatments, most notably vaginal progesterone, which became available in the latter half of the decade studied. The relatively low rate of SPTB also confirms the results of other studies that suggest that a history of SPTB alone – the basis of a majority of referrals to this clinic – is an inadequate predictor of subsequent SPTB.²⁰ Our multivariate analysis of this and other historical risk factors also verifies that these do not independently predict SPTB.

Both smoking during pregnancy and use of ART have been previously reported as risk factors for SPTB in epidemiological studies,¹² however neither of these emerged as significant in this study. Recording of these data was inconsistent and frequently incomplete, which may explain the difference in findings.

A short cervix on TVUS in the second trimester is widely reported as a key predictor of SPTB in asymptomatic, high-risk women,²¹ as it was in our study. The ability of a short cervix to predict SPTB in a given patient, however, is fairly limited. We found that the PPV of a short cervix varied somewhat by subgroup, according to the main pathology, with higher PPVs in women with uterine anomalies than other subgroups and the cohort overall. This may be consistent with different dominant aetiologies of SPTB in each group.

The finding of a poorer PPV for a short cervix in women with a past history of cervical surgery is particularly important in light of previous studies that have found an increased rate of midtrimester short cervix in this population.²² Although it has been reported that the volume of cervical tissue excised for the biopsy may elevate the risk of SPTB,²³ other studies conclude that the increased risk is independent of the presence of the mean shorter cervical length found in this group of women.²⁴

In keeping with other studies,^{11, 25} fFN was a significant predictor of preterm birth in both univariate and multivariate analyses, however its NPV is more clinically useful, especially given the small numbers of positive results. Bolt et al.²⁶ went so far as to suggest that fFN is a better predictor of preterm birth and that cervical length adds nothing to its predictive value. However, the effectiveness of interventions such as progesterone are closely linked to cervical length, and its use at earlier gestations than fFN increases its utility in clinical practice.

Thyroid disease has a known association with adverse pregnancy outcome, including SPTB,¹⁴ however midtrimester TSH was not found to be a significant predictor in our study. ALP is produced by the placenta, and a large epidemiological study has reported an association with SPTB.¹⁵ Only a small number of women underwent testing (n=49), however an ALP outside the RWH laboratory reference range proved a significant predictor of SPTB. While Goldenberg et al²⁷ found no association between a 19-week elevated serum ALP and SPTB (with an association present at 26 weeks), we found that any second trimester raised ALP (usually performed between 14 and 18 weeks) was a significant predictor of preterm birth in univariate and multivariate analysis. The cited studies used different reference ranges for ALP, which may explain the difference in results. Further investigation of the clinical use and significance of ALP as a predictor of SPTB is indicated.

The role of ureaplasma species in preterm birth remains unresolved. Whilst some studies identify both ureaplasma urealyticum and ureaplasma parvum as important pathological agents,²⁸ other studies suggest these can simply colonise the amniotic cavity without ill effect.²⁹ No significant association between the presence of

ureaplasma species (or any other abnormal flora) and preterm birth was found in this cohort. Ureaplasma urealyticum has also been implicated in key morbidities of premature infants, including early onset neonatal sepsis, bronchopulmonary dysplasia, intraventricular haemorrhage and necrotising enterocolitis.³⁰ Our ureaplasma-colonised subgroup had no significant increase in rates of neonatal morbidity or incidence of low Apgar scores, perhaps due to treatment with erythromycin.

Chlamydia trachomatis infection has been causally linked to preterm birth,³¹ although a low rate of infection made statistical analysis unhelpful in our cohort. Rours, Duijts and Moll et al reported a 3.9% infection rate, which was comparable to the 2% infection in our study population. The low absolute numbers of women with chlamydia infection meant it did not contribute significantly to the preterm birth rate in our study, however a previously reported strong association with moderate to extreme premature birth (aOR 4.3, 95% CI 1.3 – 15.2³¹) supports the value of ongoing screening.

Conclusion

Among women at high risk of preterm birth, this cohort study identified that a short cervix (<25 mm) on TVUS in the second trimester was the only ultrasound indicator predictive of SPTB <37 and <34 weeks, although its PPV is relatively low, at 36.25 and 24.17% respectively. Fetal fibronectin at 26 weeks' gestation was an independent predictor of SPTB, although its most useful clinical application is its NPV, at 83.67%. An elevated serum ALP (>91 IU/L) at any time in the second trimester was also an independent predictor of SPTB: further research into the pathophysiological basis for and potential clinical utility of this novel finding is warranted.

The observed decline in the rate of spontaneous preterm birth among preterm labour clinic attendees, when adjusted for cervical length, provides useful evidence for the value of the assessments and interventions provided by these specialist clinics. Although much remains to be understood regarding the pathogenesis and prevention of preterm labour, the apparent benefits of this targeted approach to prediction and prevention offers hope that we may finally be able to achieve a reduction in the hitherto relatively static rates of prematurity, and thus the suffering of infants and their families alike.

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Table 1. Summary of descriptive statistics: demographics, treatment, delivery data and perinatal mortality

Demographic variable	n or mean	% or range	Comments
Maternal age (years)	31.70	18 - 46	Standard deviation = 5.22
Gravidity	3	1 - 14	
Parity	1	0 - 8	
Smoking in last 12 months (n=786)	195	24.81%	
Treatment type			
Progesterone pessaries	63	26.25%	
Progesterone + transvaginal (TV) cerclage	62	25.83%	
TV cerclage alone	60	25.00%	9 elective, 51 ultrasound-indicated or rescue
Arabin pessary	7	2.917%	Fetal Medicine Foundation study participants
Transabdominal (TA) cerclage alone	4	1.667%	2 TA cerclage patients received progesterone
No treatment	47	19.58%	
Total:	243		2 patients from pessary trial received ≥ 1 other treatments, true total number of women with short cervix = 240/757 (32.0%)
Outcome			
Gestational age at delivery (weeks)	37.2	15 - 42	
Birth weight (g)	3001	111 - 4875	
Delivery type			
Normal vaginal delivery	377	49.80%	
Instrumental vaginal delivery	121	15.98%	

Elective Caesarean section	131	17.31%	
Emergency Caesarean section	125	16.51%	
Outcomes for infants delivered ≥ 20 weeks (n=747)			
Live births	727	97.32%	
Stillbirths	10	1.339%	
Neonatal deaths	11	1.473%	
"Take home" rate for infants delivered ≥ 24 weeks (n = 730)	725	99.32%	
PPROM	89	54.94%	% of preterm births
Documented histological chorioamnionitis (n=194)	86	44.3%	
Post-partum pyrexia ($T \geq 38^0$ C)	20	2.9%	

Figure 1.

Proportions of PTLC referrals by category (where 100% represents the total number of indications for referral; some women referred for multiple indications)

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Figure 2.

Rate of preterm birth per year, adjusted for diagnosis of short cervix in that year, with statistically significant downward trend ($p=0.007$).

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