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ORIGINAL RESEARCH

Assessment of novel sonographic and biochemical tools for spontaneous preterm birth prediction in asymptomatic twin pregnancies

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Abstract

Introduction: Prematurity is a major global health issue. Twin pregnancies are a group at especially high risk of preterm birth. Sonographic mid-trimester cervical length has limited accuracy in predicting preterm birth. This study aimed to evaluate the association between mid-trimester sonographic markers of early cervical remodeling and cervical inflammatory biomarkers and fetal fibronectin, alone or in combination, as predictors of preterm birth before 34+0 weeks in asymptomatic twin pregnancies.

Material and Methods: Prospective cohort study, including uncomplicated dichorionic or monochorionic-diamniotic twin pregnancies, recruited and assessed between 18+0 and 24+6 weeks, from a single tertiary referral center between 2020 and 2023. At inclusion, transvaginal ultrasound was performed to assess the following sonographic markers (cervical length, uterocervical angle, cervical consistency index, cervical texture) and an endocervical sample was obtained prior to ultrasound to quantify the following cervical inflammatory biomarkers (tumor necrosis factor alpha, interleukins 1b, 6, 8, 18, matrix metalloproteinase-8 and 9) and fetal fibronectin. The diagnostic performance of those sonographic and biochemical markers independently associated with spontaneous preterm birth before 34 weeks was analyzed by receiver operating characteristic curves and assessed through sensitivity and specificity analysis for several cutoffs.

Results: Of the 172 women included, cervical length was shorter (36 mm vs. 40 mm; $p = 0.025$) and uterocervical angle was wider (137° vs. 120°; $p = 0.004$) in the preterm group. Cervical consistency index, cervical texture score, cervical inflammatory

Abbreviations: AP, anteroposterior; AUC, area under the ROC curve; CI, confidence interval; IL, interleukin; MMP, matrix metalloproteinase; PPROM, preterm prelabor rupture of membranes; ROC, receiver operating characteristics; sPTB, spontaneous preterm birth; TNF-α, tumor necrosis factor alpha.

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biomarkers, and fetal fibronectin were similar among the study groups. The area under the curve to predict spontaneous preterm birth before 34+0 weeks was 0.722 (95% CI 0.577 to 0.866) for cervical length, 0.789 (95% CI 0.683 to 0.895) for uterocervical angle, and 0.852 (95% CI 0.752 to 0.952) for a combination of both. Based on the receiver operating characteristics curve cutoff, sensitivity and specificity for cervical length ≤37 mm was 55.6% and 66.3%, for an uterocervical angle ≥135° was 77.8% and 76.1%, and for both criteria present 44.4% and 93.3%, respectively.

Conclusions: This finding of this study suggests that the combination of cervical length and uterocervical angle in mid-trimester sonographic assessment may improve the prediction of preterm birth before 34 weeks in asymptomatic and uncomplicated twin pregnancies.

KEYWORDS

cervical inflammatory biomarkers, cervical length, early cervical remodeling, multiple pregnancy, preterm birth prediction, spontaneous preterm birth, twin pregnancy, uterocervical angle

1 | INTRODUCTION

Prematurity is the leading cause of neonatal mortality and long-term neurodevelopmental impairment worldwide.[1,2] Multiple pregnancies have a substantially higher rate of preterm birth,[3–5] and their incidence has increased over the last decades.[6–8] Medically indicated delivery accounts for a considerable proportion of preterm births in twins; nevertheless, half of them are due to spontaneous labor or preterm prelabor rupture of membranes (PPROM).[4,6]

Predicting and preventing spontaneous preterm birth (sPTB) in twin pregnancies remains a challenge for maternal-fetal specialists.[4,9] The pathophysiological mechanisms involved in sPTB in twins are likely to differ from those in singletons.[6,10] It seems plausible that uterine overdistention plays an important role. Excessive uterine stretching could prematurely activate an inflammatory cytokine-mediated cascade and the release of labor mediators, leading to early myometrial activation and cervical ripening.[4,6,8,11–13] Moreover, there are differences in the effectiveness of interventions to prevent sPTB between twins and singletons.[4,5,10] These evidences highlight the need to investigate predictors of sPTB in well-designed prospective studies in twins.

To date, sonographic mid-trimester cervical length measurement is the standard screening tool used for assessing the risk of sPTB in both singletons and twins. However, its accuracy is limited by low sensitivity as an isolated screening test.[10,14,15] In recent years, there has been increasing interest in the development of novel sonographic markers for evaluating the risk of sPTB by identifying biophysical changes in the early stages of cervical remodeling. Among these markers, there is increasing evidence to consider the mechanical flattening between the uterus and cervix represented by a wider uterocervical angle as a good predictor of sPTB in both singletons and twins.[16,17] The cervical consistency index, which evaluates the grade of cervical softening[18–20] and quantitative analysis of cervical texture, used to detect changes in cervical tissue microstructure, has been related to sPTB in singleton pregnancies.[21] On the contrary, intra-amniotic inflammation has been studied as a relevant contributor to preterm birth.[22–24] Hence, inflammatory biomarkers, such as cytokines and matrix metalloproteinases (MMPs), have been evaluated for identifying pregnancies at high risk of imminent preterm birth in complicated singleton pregnancies.[25,26] However, there is scarce and mostly retrospective evidence on the diagnostic accuracy of these novel sonographic and biochemical markers in the prediction of sPTB in twin pregnancies. Moreover, their potential contribution to sPTB prediction when combined with cervical length has not been explored.

The aim of this study was to evaluate the value of mid-trimester sonographic markers of early cervical remodeling and cervical inflammatory biomarkers and fetal fibronectin, alone or in combination, as predictors of sPTB <34+0 weeks in asymptomatic twin pregnancies.

Key message

Multiple pregnancy substantially contributes to preterm birth rate worldwide. Sonographic markers of preterm birth, such as cervical length measurement, have limited prediction accuracy. Combining sonographic mid-trimester cervical length with uterocervical angle improves the prediction of spontaneous preterm birth in asymptomatic and uncomplicated twin pregnancies.

2 | MATERIAL AND METHODS

2.1 | Study population

This was a prospective cohort study, including dichorionic and monochorionic-diamniotic twin pregnancies from a single tertiary center between 2020 and 2023. Exclusion criteria were as follows: monoamniotic twins, triplets or higher-order pregnancies, cases complicated by twin-to-twin transfusion syndrome or mid-trimester selective fetal growth restriction, major structural or chromosomal anomalies, or intrauterine fetal demise of one of the twins before recruitment. Women under treatment to prevent sPTB before recruitment were also excluded.

Women fulfilling the inclusion criteria were provided with information regarding the study and, after acceptance, a written informed consent was obtained. During the second trimester scan visit, between 18+0 and 24+6 weeks, a cervical mucus sample was acquired, and an ultrasound examination of the cervix was performed afterward to assess both sonographic and biochemical markers.

2.2 | Study outcomes

Gestational age at delivery and perinatal outcomes were prospectively collected. The primary outcome was defined as sPTB <34+0 weeks because of the clinical relevance on preterm birth management (e.g., use of antenatal corticosteroids for fetal lung maturation, use of tocolytics to arrest uterine contractions, and transfer to tertiary referral center with adequate neonatal intensive care unit). Women undergoing medically indicated preterm birth for maternal or fetal indication not related to sPTB <34 weeks were excluded from the final analysis.

2.3 | Data collection and clinical management

Demographic and obstetric data collected included maternal age, ethnicity, body mass index, nulliparity, use of assisted reproduction technology, chorionicity, and risk factors for preterm birth. Gestational age was based on the crown-rump length of the larger twin at the first trimester scan. Chorionicity was determined by the presence of the "T" or "lambda" sign before 14 weeks.

After assessing both sonographic and biochemical markers between 18+0 and 24+6 weeks, women were routinely followed according to the center protocol. Vaginal progesterone (400 mg/day) was administered if cervical length was found to be ≤25 mm before 24+0 weeks or below the 5th centile afterward, according to our normograms.[27] Cervical cerclage was placed if cervical length was ≤15 mm and progressive cervical shortening was observed or in cases of asymptomatic cervical dilatation before 24+6 weeks. Threatened preterm labor was defined as the presence of symptomatic and regular uterine contractions and cervical length shortening before term, and PPROM diagnosis was based on visualization of amniotic fluid leaking through the external cervical os and confirmed by detection of insulin-like growth factor-binding protein-1 using an immunochromatographic test (Amnioquick, Biosynex SA, Illkirch-Graffenstaden, France). According to the protocol of the center, elective delivery in uncomplicated dichorionic twins was proposed between 37+0 and 38+0 weeks and in monochorionic-diamniotic twins between 36+0 and 37+0 weeks.

2.4 | Sonographic cervical images acquisition and measurements

All cervical images in the study were obtained by a single trained sonographer (JP) using either a Voluson E6, Voluson E8, or Voluson E10 (GE Medical Systems, Zipf, Austria) equipped with a 2- to 10-MHz vaginal transducer, with the patient in the lithotomy position and an empty bladder. The intra- and inter-observer variability of significant sonographic markers associated with sPTB was assessed in 20 images by the principal examiner (JP) and a second examiner (JB).

2.4.1 | Cervical length measurement

Cervical length was measured from the internal to the external cervical os using a sagittal plane to visualize the entire cervical canal and the internal and external os, without placing any pressure on the cervix (Figure S1).

2.4.2 | Uterocervical angle measurement

Uterocervical angle was measured at the delimitation of two intersecting lines at the internal os. The first was formed by a straight line from the internal to the external os and the second was drawn parallel to the lower anterior uterine segment crossing through the internal cervical os (Figure S2).

2.4.3 | Cervical consistency index measurement

First, a sagittal view of the cervix was obtained without performing any pressure with the transducer. Then, to acquire a second image at maximal compression, pressure was gradually applied on the cervix until no further compression of the anteroposterior (AP) diameter could be observed. Afterward, a perpendicular line crossing through the AP diameter of the cervix before and at maximal compression (AP′) was drawn. Cervical consistency index was then calculated as the ratio between AP′ and AP and expressed as percentage (Figure S3).[16]

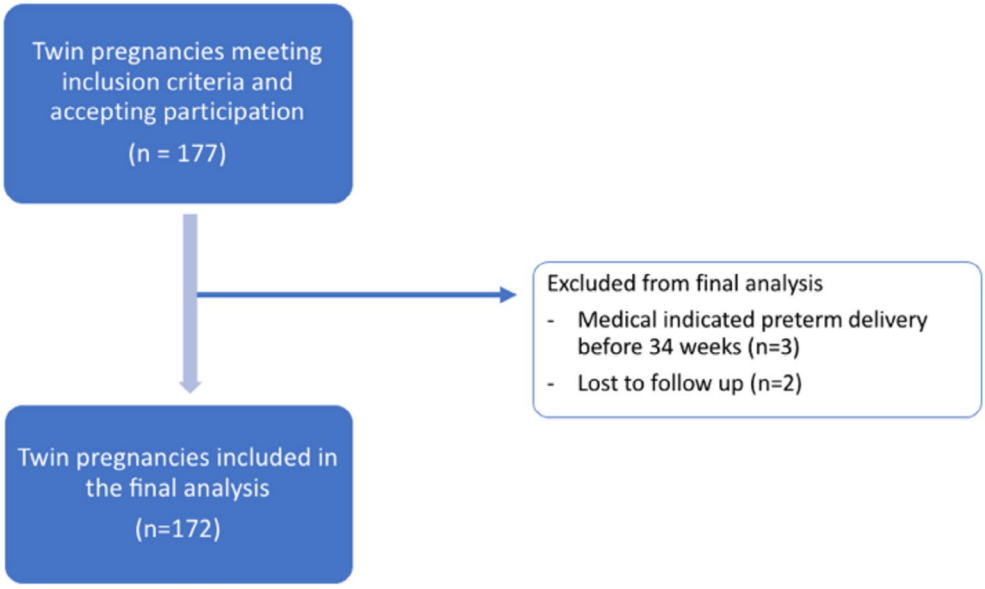

FIGURE 1 Flowchart of population recruited and analyzed.

2.4.4 | Cervical texture measurement and analysis

A sagittal view of the cervix without applying any pressure was obtained (Figure S4). Online measures and post-processing functions were turned off. Images were stored in the original Digital Imaging and Communication in Medicine format for offline analysis. To perform the quantitative analysis, the quantusPrematurity online tool (www.quantusprematurity.org) was used, in which a delineation of a region of interest of the entire cervix was automatically drawn by the tool.[28] To obtain the probability of risk of sPTB <34 weeks, the tool extracts texture features from the region of interest and then uses a machine learning classifier to obtain a confidence score.

All sonographic measures, except for the cervical texture analysis, were obtained at recruitment.

2.5 | Cervical samples collection and biochemical markers analysis

A cervical mucus sample was collected from the external cervical os and posteriorly kept at 4°C. Cervical fluid was centrifuged between 4000 and 6000 g for 10 min. Supernatants and pellets were frozen separately at −80°C. Determination of protein concentrations was performed using multiplex immunoassays to investigate the independence of inflammatory biomarkers (tumor necrosis factor alpha [TNF-α], interleukin [IL]-1b, IL-6, IL-8, IL-18, MMP-8 and MMP-9) and fetal fibronectin to predict sPTB <34 weeks. The total protein concentration was evaluated using the BCA test (Thermo Scientific, Rockford, IL). The samples were analyzed in duplicate and diluted as follows: an equal volume of cervical mucus homogenates (25 μL/well) or standards (25 μL/well) was incubated with pre-mixed bead sets in pre-wetted 96-well microtiter plates at 4°C overnight. The fluorescent detection antibody mixture was added and incubated for 1 h, followed by incubation with streptavidin-phycoerythrin for an additional 30 min at room temperature. The concentration of each analyte was determined using a Luminex 200 instrument (Luminex, Austin, TX), and data were reported as mean fluorescent intensities normalized by the amount of protein (mg).

2.6 | Statistical analyses

Continuous variables were presented as median with interquartile range (25th; 75th percentile). Categorical variables were presented as numbers with percentages (%). The Wilcoxon test was used to compare continuous variables, whereas the chi-squared or Fisher exact test was used to compare categorical variables. Multivariate logistic regression was performed to evaluate the variables independently associated with sPTB <34+0 weeks in the contingency tables.

The diagnostic performance of the sonographic and/or biochemical markers significantly associated with sPTB <34 weeks was reported by receiver operating characteristics (ROC) and expressed by their respective area under the ROC curve (AUC). The DeLong method was used to compare AUCs. Diagnostic accuracy was also assessed with the sensitivity, specificity, positive predictive value, negative predictive value, and positive and negative likelihood ratios (LHR+ and LHR−) for the significant markers. The 90th and the 5th-10th percentiles cutoffs in the cohort population, as well as the cutoffs established in the literature, were reported. Reproducibility of significant sPTB predictors was assessed using intraclass correlation coefficient by both the principal examiner (JP) and a second examiner (JB). Differences were considered statistically significant when $p < 0.05$. Statistical analyses were performed using Stata statistical package (version 15.1 software, StataCorp., College Station, TX).

TABLE 1 Demographic and perinatal outcomes between groups.

Variables	Delivery <34 weeks ($n=9$)	Delivery >34 weeks ($n=163$)	p-value
Maternal age	28.4 (24.6–40.7)	35.0 (31.3–37.8)	0.174
Ethnicity			0.703
White	6 (66.7)	117 (71.8)	
Maghreb	1 (11.1)	8 (4.9)	
African	0 (0)	2 (1.2)	
Hispanic	2 (22.2)	25 (15.4)	
Oriental Asia	0 (0)	2 (1.2)	
Meridional Asia	0 (0)	9 (5.5)	
Body mass index	27.9 (21.9–30.1)	23.8 (21.0–27.7)	0.501
Nulliparity	9 (100)	112 (68.7)	**0.045**
Assisted reproduction technology	3 (33.3)	71 (43.6)	0.904
Chorionicity			>0.99
Dichorionic	5 (55.6)	98 (60.1)	
Monochorionic diamniotic	4 (44.4)	65 (39.9)	
Risk factors sPTB[a]	0 (0)	17 (10.4)	0.307
Progesterone	1 (11.1)	16 (9.8)	>0.99
Cerclage	0 (0)	3 (1.8)	>0.99
Gestational age at delivery	32.9 (31.1–33.4)	36.9 (36.3–37.3)	**<0.001**
Preterm labor	7 (77.8)	11 (6.8)	**<0.001**
PPROM	2 (22.2)	23 (14.1)	0.620
Birthweight (g)	1640 (1530–1874)	2410 (2178–2643)	**<0.001**
Composite neonatal morbidity[b]	3/18 (16.7)	6/326 (1.8)	**0.008**

Note: Continuous variables were compared using non-parametric Wilcoxon test and presented as median (25th; 75th percentile). Categorical variables were compared using chi-squared or Fisher exact tests and presented as numbers (percentages).

Abbreviations: PPROM, preterm prelabor rupture of membranes; sPTB, spontaneous preterm birth.

[a]Risk factors of sPTB: smoking, previous sPTB <34+0 weeks or second trimester miscarriage between 17+0–22+6 weeks, uterine malformation, and cervical conization.

[b]Composite neonatal morbidity is defined as the presence of any of the following variables: Neonatal mortality, bronchopulmonary dysplasia, early sepsis, III–IV stage intraventricular hemorrhage, necrotizing enterocolitis.

3 | RESULTS

A total of 177 women were eligible for inclusion and consented to participate. Two women were lost to follow-up. The overall sPTB rate was 36/175 (20.6%) <37+0 weeks, 9/175 (5.1%) <34+0 weeks, 3/175 (1.7%) <32+0 weeks, and 1/175 (0.6%) <28+0 weeks. Three women underwent elective delivery for medical reasons not related to sPTB <34 weeks and were, therefore, excluded from the final analysis (Figure 1; Table S1).

Basal characteristics were similar between groups, except for a more significant proportion of nulliparous women in the sPTB group. Moreover, there were no significant differences in either the use of vaginal progesterone or the placement of cervical cerclage between groups (Table 1).

Regarding sonographic sPTB prediction parameters, cervical length was shorter (36 mm vs. 40 mm; $p=0.025$) and uterocervical angle was wider (137° vs. 120°; $p=0.004$) in the sPTB group. However, at the time of recruitment, only 1/9 (11.1%) women in the sPTB group had a cervical length <25 mm versus 6/163 (3.7%) in the other group ($p=0.318$). Cervical consistency index and cervical texture score were similar among the study groups. With respect to cervical inflammatory biomarkers and fetal fibronectin, there were no differences between groups (Table 2). After adjusting for potential confounders, both cervical length and uterocervical angle remained independently associated with sPTB <34 weeks (Table 3). Results for sonographic and biochemical predictors in patients delivering >37 weeks were similar to those delivering between 34 and 36 weeks (Table S2).

The ROC curves for cervical length and uterocervical angle are shown in Figure 2. The AUCs for cervical length and uterocervical angle to predict sPTB <34+0 weeks were 0.722 (95% confidence interval [CI] 0.577 to 0.866) and 0.789 (95% CI 0.683 to 0.895) ($p=0.496$), respectively. On including both cervical length and uterocervical angle in a logistic regression model for the prediction

TABLE 2 Sonographic and biochemical predictors of the two groups.

Variables	Delivery <34 weeks ($n=9$)	Delivery >34 weeks ($n=163$)	p-value
Gestational age at recruitment	22 (21.4–22.1)	21.1 (20.3–22.0)	0.058
Cervical length (mm)	36 (33–39)	40 (36–45)	**0.025**
Uterocervical angle (°)	137 (135–148)	120 (106–134)	**0.004**
Cervical consistency index (%)	62.5 (53.8–65.6)	62.1 (55.2–69.2)	0.747
Cervical texture-based score	−9.8 (−10.3–9.2)	−8.4 (−10.1–6.6)	0.209
Fibronectin (pg/mL)	20855 (12819–126220)	42902 (16149–80145)	0.735
TNF-α (pg/mL)	14.6 (7.5–26.6)	15.4 (7.2–29.2)	0.170
IL1b (pg/mL)	345 (275–795)	331 (118–1012)	0.753
IL6 (pg/mL)	42.4 (24.6–125.4)	59.9 (22.6–114.1)	0.182
IL8 (pg/mL)	715 (511–1154)	736 (432–1248)	0.236
IL18 (pg/mL)	119 (70–138)	102 (61–171)	0.142
MMP8 (pg/mL)	34059 (15557–41324)	25585 (16633–40873)	0.117
MMP9 (pg/mL)	15416 (7671–16879)	10988 (5425–22063)	0.294

Note: Continuous variables were compared using non-parametric Wilcoxon test and presented as median (25th; 75th percentile). Categorical variables were compared using chi-squared or Fisher exact tests and presented as numbers (percentages). All cervical inflammatory biomarkers and fibronectin values are adjusted for gestational age at sampling.

Abbreviations: IL, interleukin; MMP, matrix metalloproteinase; TNF-α, tumor necrosis factor alpha.

TABLE 3 Multivariate logistic regression for sonographic predictors significantly associated with spontaneous preterm birth <34 weeks.

Variables	OR (95% CI)	p-value	OR (95% CI)	p-value[a]
Cervical length	0.92 (0.86 to 0.98)	**0.023**	0.89 (0.79 to 0.99)	**0.039**
Uterocervical angle	1.05 (1.01 to 1.09)	**0.012**	1.05 (1.01 to 1.10)	**0.016**

Abbreviations: CI, confidence interval; OR, odds ratio.

[a]Adjusted for: Maternal age, ethnicity, risk factor for preterm birth, chorionicity, assisted reproductive technology, use of progesterone/cerclage placement.

of sPTB <34 weeks, the AUC significantly improved compared with the use of uterocervical angle alone (AUC 0.852 [95% CI 0.752 to 0.952]; $p=0.024$). The diagnostic accuracy based on the ROC-curve optimal cutoff point for both cervical length and uterocervical angle alone and in combination is shown in Table 4. Moreover, cutoffs based on the 5th-10th centiles for cervical length and the 90th centile for uterocervical angle, as well as those used in the literature, are reported.[29–31] Regarding the reproducibility of uterocervical angle, the intraobserver and inter-observer intraclass correlation coefficient (95% CI) value were 0.885 (0.820 to 0.928) and 0.931 (0.800 to 0.977), respectively.

4 | DISCUSSION

This study suggests that the addition of mid-trimester uterocervical angle to cervical length may improve the prediction of sPTB <34 weeks in asymptomatic and uncomplicated twin pregnancies. The study found a significant and independent association between uterocervical angle and sPTB <34 weeks, with an acceptable diagnostic accuracy based on an optimal ROC-curve cutoff point of 135° and an excellent reproducibility.

Evidence on mid-trimester uterocervical angle as a predictor of sPTB in twins is scarce, mostly retrospective, and has been conducted with variable cutoff values.[30–34] In the largest study on 424 twin pregnancies, Benito-Vielba et al. reported that an angle >120° was associated with an increased risk of sPTB <34 weeks (OR 2.66 [1.24 to 5.69]; $p=0.01$), with an AUC of 0.676.[30] In this study, uterocervical angle was measured retrospectively in stored images originally performed to measure the cervical length. A smaller prospective study using 130° as a cutoff reported a similar AUC (0.72).[31] The present study represents the largest prospective cohort, with a similar AUC to those previously reported, and further reports good inter- and intraobserver agreement scores of uterocervical angle measurements, which had not been assessed in previous studies.

Regarding the evaluation of the combination of cervical length and uterocervical angle together to predict sPTB, evidence is limited

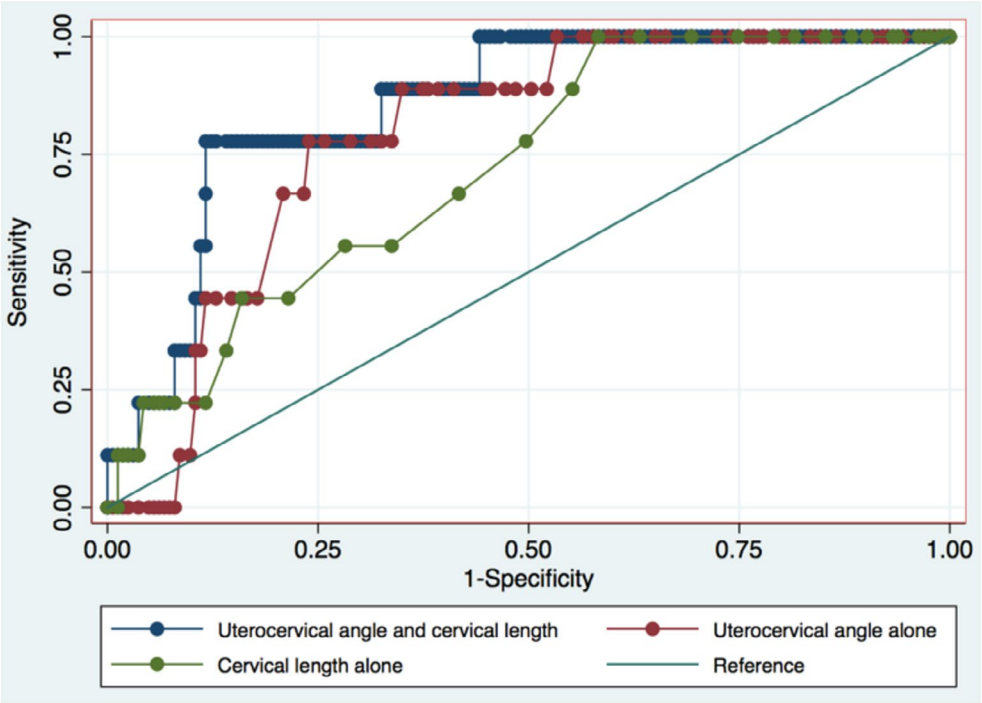

FIGURE 2 Receiver operating characteristics curves of cervical length and uterocervical angle for spontaneous preterm birth <34+0 weeks prediction.

and derived mainly from singleton series. To our knowledge, only one previous study assessed cervical length and uterocervical angle together in twins and did not observe an improvement for sPTB with respect to the use of cervical length alone; differences that could be related to the smaller and retrospective origin of their cohort and also because a different gestational age cutoff for sPTB was proposed (37 weeks vs. 34 weeks).[32] The main reason for using 37 mm as the cutoff point for cervical length, in its combination with uterocervical angle, instead of using shorter cutoff points (20–27 mm), which have demonstrated a stronger association to sPTB prediction in previous studies,[29] is because of the low number of patients having a cervical length less than 27 mm in the sPTB group ($n = 2$), decreasing significantly the sensitivity of this marker.

In relation to mid-trimester cervical consistency index, this study's findings are in line with those of Rosen et al., who analyzed a prospective cohort of 80 twin pregnancies and reported no significant association between this marker and sPTB <34 weeks, showing a poor AUC (0.538).[35] On the contrary, Van der Merwe et al. found cervical consistency index to be the strongest marker for the prediction of sPTB <34 weeks (AUC 0.82).[31] Nonetheless, some methodological differences should be underlined, such as their images being analyzed offline, while our measurement was calculated at the time of examination, and a higher proportion of sPTB <34 weeks in their cohort (16.3% vs. 5.1%). Regarding quantitative cervical texture analysis, no significant association between this parameter and sPTB in twins was found. This is similar to the results of Van der Merwe et al.[31] and contrary to what is described in singletons.[21]

Mid-trimester cervical inflammatory biomarkers were not associated with sPTB <34 weeks in this study. The development of non-invasive biomarkers to identify intra-amniotic inflammation avoiding the need for amniocentesis has raised increasing interest.[36,37] Our findings might suggest that mid-trimester intra-amniotic inflammation does not play a relevant role in sPTB in asymptomatic twin pregnancies, although this finding should be interpreted with caution as discussed later in this section. To our knowledge, only one previous study evaluated inflammatory ILs between 24 and 34 weeks in cervico-vaginal mucus. The authors reported that a higher IL-8 concentration at 28 weeks was related to preterm birth <37 weeks in twin pregnancies,[38] suggesting a potential contribution of intra-amniotic inflammation later in gestation.

Contrary to other studies,[39–41] fetal fibronectin did not show a significant association with sPTB in this cohort. It is worth noting that in most previous studies, this marker was evaluated in symptomatic twin pregnancies or at a higher gestational age (22–34 weeks) than in the present study.[42] This finding is supported by Kuhrt et al. who observed that fibronectin tests undertaken before 22 weeks had a limited predictive value for sPTB in twins.[43]

Mid-trimester cervical length has limited accuracy in sPTB prediction in twins, being 20–25 mm the best-established cutoff points with a reasonable balance between false negatives and positives in sPTB prediction.[29,44] This study supports that adding uterocervical angle to cervical length improves the prediction of sPTB as compared with the evaluation of each measure alone. Uterocervical angle can be easily measured in a very similar ultrasonographic section to that used for cervical length, and as shown in this study, it has high intra- and inter-observer reproducibility. From a pathophysiological point of view, these results further support the role of uterine overdistension in the genesis of sPTB in twins and may help

TABLE 4 Diagnostic performance of the measurement of cervical length and uterocervical angle for the prediction of spontaneous preterm birth <34+0 weeks.

Cutoff (*n*/*N*)	Sensitivity (% (*n*/*N*))	Specificity (% (*n*/*N*))	PPV (% (*n*/*N*))	NPV (% (*n*/*N*))	LHR+ (95% CI)	LHR− (95% CI)
CL						
≤20 mm (5/172)	11.1 (1/9)	97.5 (159/163)	20 (1/5)	95.2 (159/167)	4.53 (0.56 to 36.46)	0.91 (0.72 to 1.15)
≤25 mm (7/172)	11.1 (1/9)	96.3 (157/163)	14.3 (1/7)	95.2 (157/165)	3.02 (0.41 to 22.48)	0.92 (0.73 to 1.16)
≤27 mm (9/172)	22.2 (2/9)	95.7 (156/163)	22.2 (2/9)	95.7 (156/163)	5.17 (1.25 to 21.43)	0.81 (0.57 to 1.15)
≤32 mm (21/172)	22.2 (2/9)	88.3 (144/163)	9.5 (2/21)	95.4 (144/151)	1.91 (0.52 to 6.95)	0.88 (0.62 to 1.25)
≤37 mm (60/172)	55.6 (5/9)	66.3 (108/163)	8.3 (5/60)	96.4 (108/112)	1.64 (0.88 to 3.07)	0.67 (0.32 to 1.40)
UCA						
≥150° (17/172)	11.1 (1/9)	90.2 (147/163)	5.9 (1/17)	94.8 (147/155)	1.13 (0.17 to 7.61)	0.98 (0.78 to 1.25)
≥135° (46/172)	77.8 (7/9)	76.1 (124/163)	15.2 (7/46)	98.4 (124/126)	3.25 (2.09 to 5.07)	0.29 (0.09 to 0.99)
≥130° (62/172)	77.8 (7/9)	66.3 (108/163)	11.3 (7/62)	98.2 (108/110)	2.30 (1.53 to 3.47)	0.33 (0.10 to 1.14)
≥120° (90/172)	88.9 (8/9)	49.7 (81/163)	8.9 (8/90)	98.8 (81/82)	1.77 (1.34 to 2.33)	0.22 (0.04 to 1.43)
Combination CL and UCA						
≥135° or ≤37 mm (101/172)	88.9 (8/9)	42.9 (70/163)	7.9 (8/101)	98.6 (70/71)	1.56 (1.19 to 2.03)	0.26 (0.04 to 1.66)
≥135° and ≤37 mm (15/172)	44.4 (4/9)	93.3 (152/163)	26.7 (4/15)	96.8 (152/157)	6.59 (2.61 to 16.64)	0.60 (0.33 to 1.07)

Note: Cutoffs proposed for cervical length (in order): Conde-Agudelo[29] proposed cutoff, 5th centile cutoff, 10th centile cutoff, ROC-curve proposed cutoff. Cutoffs proposed for uterocervical angle (in order): 90th centile cutoff, ROC-curve proposed cutoff, Van der Merwe[31] proposed cutoff, Benito-Vielba[30] proposed cutoff.

Abbreviations: CL, cervical length; LHR, likelihood ratio; NPV, negative predictive value; PPV, positive predictive value; UCA, uterocervical angle.

to explain that certain preventive measures might work differently in singletons versus twins.

As far as we are aware, this study is the first to prospectively evaluate the combination of cervical length and uterocervical angle to predict sPTB <34 weeks in a cohort of asymptomatic and uncomplicated twin pregnancies. Likewise, it reports the largest prospective series assessing novel sonographic markers of early cervical remodeling and cervical inflammatory biomarkers as predictors of sPTB. All the sonographic measures used, except for cervical texture, were calculated at the time of the examination and not offline. This ensured that the examiner was blinded to the perinatal results when sonographic measures were obtained.

However, some limitations must be acknowledged. There was a small number of cases of sPTB delivering <34 weeks ($n=9$). As this may impact the diagnostic accuracy of the parameters evaluated, the predictive value of parameters other than uterocervical angle and cervical length may be underestimated. Furthermore, due to the low number of sPTB cases in our cohort, the results found on uterocervical angle and cervical length as sPTB predictors need to be interpreted with caution and must be confirmed in larger multicentric cohort studies in order to validate our findings. On the contrary, the role of inflammatory biomarkers and intra-amniotic inflammation in the pathogenesis of sPTB in twins must be interpreted with caution. An association between cervical inflammatory biomarkers and sPTB could not be demonstrated. However, intra-amniotic parameters were not evaluated. Thus, higher IL and MMP levels in amniotic fluid cannot be completely excluded. In a cohort of asymptomatic twins who underwent second trimester genetic amniocentesis with normal karyotype and absence of major structural abnormalities, Lee et al. reported significantly higher mid-trimester concentrations of IL-8 and MMP-9 in fetuses delivering preterm.[45] Since in our cohort amniocentesis was not performed, the role of these biomarkers in sPTB in twins has yet to be elucidated. Finally, our study population was composed of asymptomatic twin pregnancies, which hampers comparability with other cohorts that included complicated twin pregnancies.

Preterm birth remains a challenge. Although the risk of prematurity and its clinical and economic impact is even higher in multiples compared with singletons, the evidence on useful preventive strategies in this population is scarce, probably due to differences in its etiopathology, with uterine overdistension being a significant trigger factor. In this study, we have shown that uterocervical angle could be a novel and useful sonographic marker in preterm birth prediction in twin pregnancies. Patients presenting a wide uterocervical angle and short cervical length may have a higher risk of preterm delivery. Therefore, preventive strategies, such as the use of progesterone or cervical pessary, should be evaluated considering these parameters. Future investigations are needed in order to confirm the findings reported here and to evaluate targeted preventive strategies in those patients with an increased uterocervical angle.

5 | CONCLUSION

This study's findings suggest that the combination of cervical length and uterocervical angle in mid-trimester sonographic assessment may improve the prediction of preterm birth <34 weeks in asymptomatic and uncomplicated twin pregnancies. Mid-trimester cervical

consistency index, cervical texture score, cervical inflammatory biomarkers, and fetal fibronectin were not associated with preterm birth <34 weeks. Further prospective series are needed to confirm these findings.

AUTHOR CONTRIBUTIONS

Júlia Ponce: Conceptualization; methodology, participants' inclusion; data curation; formal analysis; writing—original draft; writing—review and editing. Teresa Cobo: Methodology; writing—review and editing. Clara Murillo, Anna Gonce, Francesca Crovetto, and Laura Guirado: Participants inclusion; data curation; writing—review and editing. Ana B. Sánchez-García, Ana P. Dantas, and David Coronado: Formal analysis. Judit Bruch: Participants inclusion; data curation. Eduard Gratacós: Writing—review and editing. Montse Palacio: Conceptualization; methodology; writing—original draft, writing—review and editing. Mar Bennasar: Conceptualization; methodology; participants inclusion; data curation, writing—original draft, writing—review and editing.

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CONFLICT OF INTEREST STATEMENT

David Coronado is employed, and Montse Palacio served as scientific adviser to Transmural Biotech S.L. The remaining authors report no conflict of interest.

ETHICS STATEMENT

The study protocol was approved by the Research Ethics Committee of Hospital Clinic de Barcelona (HCB/2020/0192) on July 3, 2020. A written informed consent was obtained from all women participating in the study.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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