

Unlocking insights: Validating a comprehensive model for predicting spontaneous preterm delivery and microbial invasion in women facing preterm labor

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Abstract

Objective: Preterm labor is a multifactorial syndrome with diverse etiologies such as infection, inflammation, uteroplacental dysfunction, stress, and immune dysregulation. Identifying women at high risk remains a challenge, and multivariate prediction models have been developed to enhance risk stratification. The primary objective of this study was to externally validate Cobo's prediction models for spontaneous delivery within 7 days and microbial invasion of the amniotic cavity (MIAC) in a new population.

Methods: This retrospective observational study aimed to externally validate Cobo's prediction models for spontaneous delivery within 7 days and MIAC in an independent cohort. A total of 130 pregnant women with threatened preterm labor or suspected intra-amniotic infection were included at Vall d'Hebron University Hospital between October 2016 and September 2019. Amniocentesis was performed, and both amniotic fluid and maternal blood samples were analyzed, including measurement of interleukin-6 levels in amniotic fluid. Model performance was evaluated using discrimination (area under the receiver operating characteristic curve) and calibration.

Results: Among the 130 women included in the study, chorioamnionitis was diagnosed in 43 (33.1%) cases. Positive amniotic fluid and endocervical cultures were found in 15 and 47 cases, respectively. Spontaneous delivery within 7 days occurred in 47 (36.2%) women, and MIAC was confirmed in 15 of 128 (11.7%) cases. The area under the receiver operating characteristic curve was 0.805 (95% confidence interval [CI]: 0.715–0.895) for predicting delivery within 7 days and 0.897 (95% CI: 0.800–0.995) for MIAC. Calibration was poor for delivery within 7 days (intercept 0.32; slope 3.65) but more accurate for MIAC (intercept –0.02; slope 0.91).

Conclusion: Cobo's models demonstrated strong discriminatory capacity but suboptimal calibration for delivery within 7 days. Calibration for MIAC was more accurate, supporting their clinical utility with room for refinement.

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KEYWORDS

amniocentesis, cervical length, chorioamnionitis, interleukin-6, intra-amniotic inflammation, microbial invasion of amniotic cavity, multivariable prediction model, preterm labor, proinflammatory factors

1 | INTRODUCTION

Preterm labor is thought to be a syndrome initiated by multiple mechanisms, including infection or inflammation, uteroplacental ischaemia or hemorrhage, uterine overdistension, stress, and other immunologically mediated processes.¹⁻³

Many maternal or fetal characteristics have been associated with preterm birth, including maternal demographic characteristics, nutritional status, pregnancy history, current pregnancy characteristics, psychological characteristics, adverse behaviors, infection, uterine contractions and cervical length, and biological and genetic markers.

Intra-amniotic infection and inflammation are well-documented causes of preterm labor with intact fetal membranes, especially at early gestational ages.⁴

Several recent studies have shown that intra-amniotic inflammation is associated with adverse perinatal outcomes and lower delivery latency, regardless of whether microbial invasion of the amniotic cavity (MIAC) is present.⁵

Identifying pregnant women at high risk of spontaneous preterm delivery within 7 days remains a persistent challenge. Several groups have tried identifying ultrasound, clinical, and laboratory parameters predicting preterm delivery. However, none of these efforts have successfully shown the ability of a single parameter to predict preterm birth. Consequently, multivariate prediction models have been developed to refine risk calculations and provide a more accurate assessment of the likelihood of preterm delivery.

In a recent study, Cobo et al. described two multivariate models to predict spontaneous delivery within 7 days and MIAC.⁶ The model included variables such as gestational age at admission, ultrasound-measured cervical length, the amniotic fluid concentration of glucose and amniotic fluid concentration of interleukin-6 (IL-6) for the prediction of spontaneous delivery within 7 days, and maternal blood C-reactive protein (CRP) and glucose levels in amniotic fluid for the prediction of MIAC.

The primary objective of this study was to externally validate Cobo's prediction models for spontaneous delivery within 7 days and MIAC in a new population. Specifically, we aimed to assess the models' discriminatory ability and calibration performance in predicting these two adverse outcomes. By evaluating the area under the receiver operating characteristic (AUROC) curve and calibration plots, we sought to determine whether these models maintain their predictive accuracy and reliability when applied to an external cohort and to identify any areas where further model refinement might be necessary for clinical application.

2 | MATERIALS AND METHODS

2.1 | Study design and setting

This observational retrospective study was carried out at Vall d'Hebron University Hospital, Barcelona (Spain), between October 2016 and September 2019.

2.2 | Study population

The study population were pregnant women attending our center with a diagnosis of preterm labor below 34 weeks of gestation or suspected intra-amniotic infection regardless of gestational age. Inclusion criteria were singleton pregnancies, intact membranes, and acceptance of an amniocentesis. Singleton pregnancies with a gestational age up to 40+ 0 weeks were included. Gestational age was determined based on the first-trimester ultrasound crown-rump length measurement.

The exclusion criteria were maternal age <18 years; multiple pregnancies; major fetal structural malformations; performing amniocentesis was not feasible; and no consent to perform amniocentesis for this indication. Women with clinical signs of chorioamnionitis at admission were excluded.

Maternal history and pregnancy data were recorded. Maternal and pregnancy factors included age, body mass index, parity, ethnicity, smoking status and method of conception. Delivery variables included gestational age at delivery, birthweight, type of birth (vaginal or cesarean section), perinatal complications, and newborn gender.

2.3 | Clinical management

Patients with a diagnosis of threatened preterm labor or suspected intra-amniotic infection underwent a blood test for the evaluation of maternal white blood cells and maternal CRP. In addition, a determination of uterine contractions was performed by cardiotocography. A transvaginal ultrasound was also performed to measure cervical length according to the guidelines of the Fetal Medicine Foundation.⁷

Threatened preterm labor was diagnosed based on the presence of regular uterine contractions associated with cervical changes before 37 weeks of gestation. For patients included in the study, a cervical length of <25 mm was considered indicative of a short cervix before 30 weeks of gestation, and a cervical length of <15 mm after 30 weeks of gestation was also used as a threshold for inclusion.

Moreover, amniocentesis was offered to women to rule in or rule out intra-amniotic infection. Amniocentesis was performed with the patient in the supine position. Skin asepsis of the abdominal wall was performed using chlorhexidine or povidone-iodine. The puncture site was selected by ultrasound based on placental location, fetal position and movements, and the area with the largest pool of amniotic fluid. Amniotic fluid was collected using a 22-gauge needle. When possible, the transplacental puncture was avoided.

Amniotic fluid cultures were performed using standard microbiological techniques, including aerobic, anaerobic, and genital mycoplasma cultures. Pathogen identification was carried out using conventional biochemical methods. Additionally, 16S ribosomal RNA (rRNA) gene sequencing was used to detect bacterial DNA.

Interleukin-6 levels were measured in the amniotic fluid using a fluorescence immunoassay with microfluidic technology (ELLA ProteinSimple, Bio-Techne). Samples were run in triplicate with a 1:5 dilution using the buffer provided in the kit. Samples were stored at -20°C until they were processed. Glucose, leukocytes, and Gram stain were also analyzed in the amniotic fluid. CRP levels were measured in maternal blood, obtained by peripheral venipuncture at the time of admission, prior to the performance of amniocentesis.

A regimen of antenatal steroids, consisting of betamethasone 12mg administered intramuscularly in two doses 24h apart, was given between 24+0 and 34+6 weeks for fetal lung maturation. In cases of suspected imminent delivery, fetal neuroprotection with magnesium sulfate was initiated.

In cases of threatened preterm labor, tocolytic treatment with atosiban was started for 48h while awaiting the results of the amniotic fluid analysis. If MIAC was confirmed, tocolysis was discontinued. Antibiotics were not routinely administered at admission. Broad-spectrum antibiotic therapy was initiated in women with suspected intra-amniotic infection, particularly in cases showing progression of labor and amniotic fluid findings suggestive of infection. The first-line regimen consisted of intravenous ampicillin (1g every 6h) and gentamicin (80mg every 8h).

Following the resolution of preterm labor, management strategies such as vaginal progesterone, cervical pessary, or cerclage were implemented when clinically indicated. In our institution, cervical pessary is the preferred first-line preventive strategy in women with persistent cervical shortening. Vaginal progesterone is generally reserved for selected cases in which cervical pessary is contraindicated or considered less appropriate. In some patients who were already receiving vaginal progesterone before admission, a cervical pessary was subsequently added because of persistent cervical shortening. Cerclage was reserved for selected cases presenting with an extremely short cervix at diagnosis or in women who experienced progressive cervical shortening despite previous conservative treatment. All interventions were performed in accordance with local protocols and current clinical guidelines.

After delivery, a histopathological study of the placenta was requested. Based on this study, chorioamnionitis was diagnosed.

2.4 | Statistical analyses

For descriptive analysis, continuous variables were described as median and interquartile range (IQR) and categorical variables as absolute and relative (percentage) frequencies.

Continuous variables were compared using the Mann-Whitney test, and categorical variables were compared using the χ^2 -test or Fisher's test, as appropriate.

We assessed the predictive performance of Cobo's model in two key aspects: first, its discriminatory ability between the adverse event (delivery within 7 days or MIAC) and non-adverse event groups, quantified by the AUROC, where a value of 1 represents perfect discrimination and 0.5 indicates no discrimination beyond chance; and second, its calibration, which evaluates the concordance between predicted risk and observed outcomes.

The performance of the model was compared with the performance of the individual predictive variables using the DeLong test.

The risk calibration was evaluated visually using a graph that compared the observed incidence of the event (delivery within 7 days or MIAC) with the predicted incidence based on the risk calculations. The data was divided into bins according to risk levels, and for each group, the observed incidence was plotted against the model's predicted incidence. Ideally, a perfectly calibrated model would show a slope of 1.0 and an intercept of 0, indicating that the observed incidence matches the predicted delivery risk within 7 days or MIAC.

All statistical analyses were performed using the computing environment R (v 4.0.0).⁸

2.5 | Ethics statement

This study was approved by the Ethics Committee of Vall d'Hebron Research Institute (registration number PR(AMI)541-2016) on September 1, 2016. All participants provided their written informed consent.

3 | RESULTS

A total of 130 pregnant women agreed to participate in the study. The median gestational age at admission was 25.1 weeks (IQR, 22.8, 27.5 weeks), and the mean gestational age at delivery was 31.1 weeks (IQR 26.5, 37.5 weeks). In two women, we could not obtain data for MIAC, and the delivery date was missing in six patients.

Maternal characteristics, pregnancy outcomes, and neonatal outcomes are shown in Tables 1 and 2.

3.1 | Microbiological and amniotic fluid studies

The microbiological studies at admission showed a positive endocervical culture in 37 women (28.5%) and a positive urinary culture in

TABLE 1 Maternal characteristics.

Variables	Results
Maternal age, years	32 [27, 36]
Body mass index, kg/m ²	24.7 [20.5, 28.1]
Ethnicity (%)	
White	67 (51.5%)
Black	5 (3.8%)
Arab	12 (9.2%)
Indian	8 (6.2%)
Latin-American	38 (29.2%)
Smoking	15 (11.5%)
Assisted reproduction techniques	11 (8.5%)
Gestational age at inclusion, weeks	25.1 [22.8, 27.5]
Cervical length, mm	9 [0, 17]
C-reactive protein, mg/dL	1.1 (0.5, 2.7)
Amniotic fluid interleukin 6	25568.4 (142573.2)
Amniotic fluid glucose	39.40 (24.56)
Amniotic fluid leukocytes	8 (6.2%)
Amniotic fluid Gram	6 (4.7%)
Positive endocervical culture	37 (28.5%)
Positive urine culture	13/126 (10.2%)
Positive amniotic fluid culture	15 (11.5%)
Post-acute management after resolution of preterm labor (%)	
Cervical pessary	21 (16.2%)
Cerclage	24 (18.5%)
Vaginal progesterone	2 (1.5%)
Combined treatment (pessary and vaginal progesterone)	3 (2.3%)
Antibiotic treatment due to suspected chorioamnionitis	14 (11.8%)

Note: Continuous variables are shown as mean (standard deviation) or median [interquartile range]. Categorical variables are shown as absolute frequency (percentage).

13 out of 126 women (10.2%). The median CRP level was 1.1 mg/dL (IQR, 0.5, 2.7 mg/dL).

The amniotic fluid studies showed that the median IL-6 level was 1048 pg/mL (IQR, 493, 4992 pg/mL), the median glucose level was 35 mg/dL (IQR, 26, 49 mg/dL), leukocytes were detected in eight cases (6.2%), Gram stain was positive in six samples (4.7%), and 15 out of 128 women (11.7%) had a positive amniotic fluid culture. The microorganisms identified in the amniotic fluid are detailed in Table 3.

3.2 | Outcomes

Forty-seven women (36.2%) delivered within 7 days, and 15 out of 128 women (11.7%) had MIAC. The median gestational age at delivery was 31.1 (IQR, 26.5, 37.5 weeks).

TABLE 2 Pregnancy and neonatal outcomes.

Variables	Results
Gestational age at delivery, weeks	31.1 [26.5, 37.5]
Antenatal steroids	94/129 (72.9%)
Spontaneous delivery within 7 days after admission	47 (36.2%)
Microbial invasion of the amniotic cavity	15 (11.5%)
Signs of chorioamnionitis in the histopathological study of the placenta	43/126 (34.1%)
Neonatal death	8/109 (7.3%)
Infant respiratory distress syndrome	42/107 (39.3%)
Intraventricular hemorrhage	
Grade I–II	96/107 (89.7%)
Grade III–IV	11/107 (10.3%)
Neonatal sepsis	19/107 (17.8%)
Necrotizing enterocolitis	2/107 (1.9%)
Retinopathy of prematurity	8/107 (7.5%)

Note: Continuous variables are shown as median [interquartile range]. Categorical variables are shown as absolute frequency (percentage).

TABLE 3 Information about microorganisms isolated in the amniotic fluid culture.

Variables	Microorganisms in the amniotic fluid
1	<i>Peptoniphilus asaccharolyticus</i> , <i>Ureaplasma parvum</i>
2	<i>Citrobacter kasseri</i>
3	<i>Fusobacterium nucleatum</i>
4	<i>Polymicrobial anaerobic flora</i>
5	<i>Ureaplasma urealyticum</i>
6	<i>Lactobacillus jensenii</i>
7	<i>Staphylococcus aureus</i>
8	<i>Listeria monocytogenes</i>
9	<i>Ureaplasma parvum</i>
10	<i>Ureaplasma parvum</i>
11	<i>Candida albicans</i>
12	<i>Fusobacterium nucleatum</i>
13	<i>Ureaplasma parvum</i>
14	<i>Ureaplasma urealyticum</i>
15	<i>Bacteroides fragilis</i>

In 126 cases, a histopathological study of the placenta was performed. A total of 43 (34.1%) showed signs of chorioamnionitis, and 83 (65.9%) showed no signs of inflammation.

3.3 | Performance of the prediction models: Discrimination

Amniotic fluid IL-6 levels were significantly higher if spontaneous delivery occurred within the first 7 days (3919 [IQR, 1031, 41261]

TABLE 4 Performance of multivariate prediction model for MIAC and spontaneous delivery within 7 days.

	Specificity	Sensitivity	PPV	NPV	LR (+)	LR (-)
AUROC spontaneous delivery ≤ 7 days						
0.805 (95% CI 0.715–0.895)	0.95	0.37	0.81	0.73	7.4	0.66
	0.90	0.60	0.77	0.80	6.0	0.44
	0.85	0.67	0.72	0.82	4.47	0.39
AUROC MIAC						
0.897 (95% CI 0.800–0.995)	0.95	0.73	0.66	0.96	14.60	0.28
	0.90	0.73	0.50	0.96	7.30	0.30
	0.85	0.87	0.44	0.98	5.80	0.15

Abbreviations: AUROC, area under the receiver operating characteristic curve; CI, confidence interval; LR, likelihood ratio; MIAC, microbial invasion of the amniotic cavity; NPV, negative predictive value; PPV, positive predictive value.

pg/mL) than if delivery occurred after 7 days (736 [IQR, 392, 1725] pg/mL), $P < 0.001$. They were also significantly higher in the MIAC group (65 897 [IQR, 41261, 66 300] pg/mL) than in the non-MIAC group (980 [IQR, 429, 2834] pg/mL), $P < 0.001$.

Applying the regression formula of Cobo's model to our population, we achieved an AUROC of 0.805 (95% CI, 0.715–0.895) for predicting spontaneous delivery within 7 days and an AUROC of 0.897 (95% CI, 0.800–0.995) for predicting MIAC. The performance of both prediction models are shown in Table 4.

Tables 5 and 6 and Figures 1 and 2 display AUROC curves for various parameters predicting delivery within 7 days and MIAC.

3.4 | Performance of prediction models: Calibration

Calibration plots for prediction of delivery within 7 days and MIAC are shown in Figures 3 and 4. For predicting delivery within 7 days, the calibration intercept was 0.32 (95% CI –2.22 to 2.86), and the slope was 3.65 (–17.86 to 25.17). For predicting MIAC, the calibration intercept was –0.02 (–0.25 to 0.20), with a slope of 0.91 (0.37 to 157).

4 | DISCUSSION

4.1 | Principal findings

The principal findings of this study demonstrate that Cobo's prediction models for spontaneous delivery within 7 days and MIAC exhibit good discriminatory performance in an external population. Specifically, the AUROC of 0.805 for predicting delivery within 7 days indicates a good discriminatory ability to distinguish between cases that will and will not deliver within this timeframe. Similarly, the AUROC of 0.897 for predicting MIAC reflects excellent discriminatory power for this adverse outcome.

The diagnostic accuracy of the evaluated parameters for predicting spontaneous delivery within 7 days was assessed using the AUROC. The Cobo model demonstrated the highest predictive

TABLE 5 Prediction of spontaneous delivery within 7 days: Performance of the Cobo model was compared with the performance of each variable using the DeLong test.

Spontaneous delivery within 7 days	AUROC	95% CI	P-value
Cobo model	0.80	0.71–0.89	
Interleukin-6 in amniotic fluid (pg/mL)	0.75	0.65–0.84	0.4541
Glucose in amniotic fluid (mg/dL)	0.72	0.61–0.83	0.0028
Cervical length at admission (mm)	0.59	0.48–0.70	0.0011
Gestational age at admission (weeks)	0.68	0.58–0.78	0.1148
C-reactive protein (mg/dL)	0.62	0.52–0.72	0.0017

Abbreviations: AUROC, area under the receiver-operating characteristic; CI, confidence interval.

performance, with an AUROC of 0.80 (95% CI: 0.71–0.89), indicating good discrimination. Among the individual biomarkers, IL-6 in amniotic fluid showed an AUROC of 0.75 (95% CI: 0.65–0.84), suggesting moderate predictive accuracy. Glucose in amniotic fluid followed with an AUROC of 0.72 (95% CI: 0.61–0.83). Conversely, cervical length at admission had limited predictive value (AUROC: 0.59, 95% CI: 0.48–0.70), as did gestational age at admission (AUROC: 0.68, 95% CI: 0.58–0.78) and CRP (AUROC: 0.62, 95% CI: 0.52–0.72). These results underscore the superior predictive performance of the Cobo model compared to individual markers.

The Cobo model demonstrated the highest predictive performance for predicting MIAC with an AUROC of 0.90 (95% CI: 0.80–0.99), reflecting excellent discrimination. Among individual biomarkers, IL-6 in amniotic fluid showed a comparable AUROC of 0.91 (95% CI: 0.80–1), although the difference was not statistically significant ($P = 0.6051$). Glucose in amniotic fluid also exhibited a strong predictive value with an AUROC of 0.90 (95% CI: 0.81–1) but similarly showed no significant difference from the Cobo model ($P = 0.4439$). In contrast, cervical length at admission had moderate predictive accuracy with an AUROC of 0.59 (95% CI: 0.42–0.77), and gestational age at admission showed

the lowest predictive value (AUROC: 0.51, 95% CI: 0.37–0.66). CRP had a moderate AUROC of 0.78 (95% CI: 0.67–0.89). These findings emphasize the superior predictive ability of the Cobo model compared to individual biomarkers, especially in predicting MIAC.

Interleukin-6 and glucose in amniotic fluid demonstrated good predictive value for spontaneous preterm birth within 7 days and MIAC, with AUROC values comparable to those of the Cobo model. This supports their potential utility as standalone biomarkers. However, while a parsimonious approach is often preferred in clinical practice, predictive models that incorporate multiple parameters

TABLE 6 Prediction of microbial invasion of the amniotic cavity (MIAC): Performance of the Cobo model was compared with the performance each variable by the DeLong test.

	AUROC	95% CI	P-value
Cobo model	0.90	0.80–0.99	
IL-6 (pg/mL)	0.91	0.80–1	0.6051
Glucose in amniotic fluid (mg/dL)	0.90	0.81–1	0.4439
Cervical length at admission (mm)	0.59	0.42–0.77	0.0030
Gestational age at admission (weeks)	0.51	0.37–0.66	0.0002
C-reactive protein (mg/dL)	0.78	0.67–0.89	0.0303

Abbreviations: AUROC, area under the receiver-operating characteristic; CI, confidence interval.

might provide added clinical value by improving robustness across different patient populations and clinical contexts. The inclusion of multiple markers might enhance predictive accuracy in certain subgroups and facilitate broader applicability. The choice between using an individual biomarker or a predictive model should ultimately be guided by clinical judgment and the specific clinical scenario.

In the comparative analysis of predictive models, the number of patients included in each calculation might influence the statistical results. In our dataset of 129 patients, two had missing glucose values and four had missing IL-6 values. The AUROC for glucose was initially calculated using 127 patients, while the AUROC for the Cobo model was based on 123 patients, as it excluded those with missing IL-6 or glucose values. To ensure consistency in DeLong's test, the glucose model was applied to the subset of 123 patients, where its AUROC was slightly lower than in the full dataset. This methodological adjustment explains the statistically significant difference observed ($P=0.0028$) despite the overlap in confidence intervals.

However, while the model's ability to discriminate between outcomes was strong, the calibration results were mixed. The calibration for MIAC was satisfactory, with the predicted risks closely aligning with the observed incidence. In contrast, the model for delivery within 7 days showed poorer calibration, suggesting that while the overall risk ranking was adequate, the predicted probabilities for individual patients were less accurate.

These findings underscore the clinical potential of Cobo's models in predicting key adverse outcomes in high-risk pregnancies.

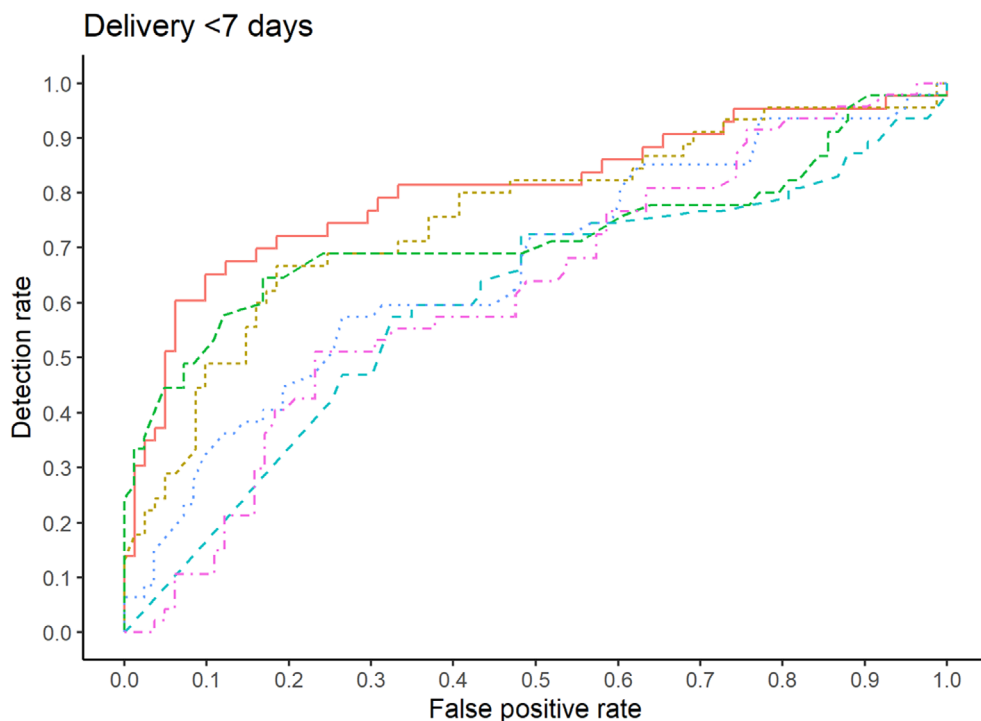


FIGURE 1 AUROC for prediction of delivery within 7 days. The receiver operating characteristic (ROC) curve shows the area under the curve (AUROC=0.805) for the model's ability to predict spontaneous delivery within 7 days. The red solid line represents the Cobo model. The ochre dashed line indicates interleukin-6 (IL-6) levels (pg/mL). The green dashed line shows glucose levels (mg/dL). The blue dashed line represents cervical length (mm). The blue dotted line shows gestational age at inclusion (weeks). The pink dash-dotted line indicates C-reactive protein (CRP) levels (mg/dL).

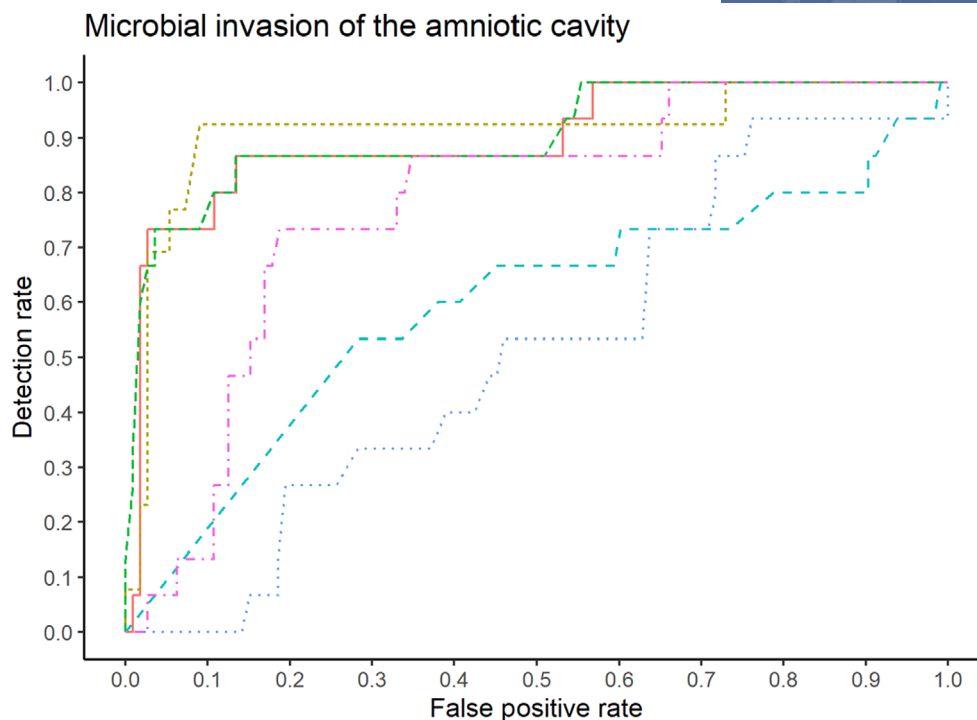


FIGURE 2 AUROC for prediction of microbial invasion of the amniotic cavity (MIAC). ROC curve demonstrating the model's predictive performance for MIAC, with an AUROC of 0.897. The red solid line represents the Cobo model. The ochre dashed line indicates interleukin-6 (IL-6) levels (pg/mL). The green dashed line shows glucose levels (mg/dL). The blue dashed line represents cervical length (mm). The blue dotted line shows gestational age at inclusion (weeks). The pink dash-dotted line indicates C-reactive protein (CRP) levels (mg/dL).

However, they also reveal opportunities for further refinement, particularly in enhancing the calibration for predicting delivery within 7 days, which could significantly improve the model's accuracy in estimating individual patient risks.

4.2 | Results in the context of what is known

Over the past 10 years, there has been an emphasis on studying risk factors for preterm birth, and risk prediction models have been developed to aid in its diagnosis. This is because it is known that most pregnant women admitted for threatened preterm labor will not experience spontaneous delivery in the following days; in other words, they are falsely thought to be in threatened preterm labor.

Various ultrasound and biochemical markers have been suggested as predictors of spontaneous preterm delivery within 7 days.^{9,10} Likewise, amniotic fluid glucose or gram stain concentrations have been closely associated with MIAC and/or intra-amniotic inflammation.¹¹⁻¹⁴

Further, it has been demonstrated that proinflammatory factors play a crucial role in preterm birth. These factors have been studied both at the vaginal and amniotic fluid levels.^{5,15-19} Among these factors, the most studied is IL-6.^{18,20} It is elevated in cases of MIAC and high-risk preterm birth. Our group has recently published the normal values of IL-6 in amniotic fluid, emphasizing that they are not affected by maternal parameters or gestational age.²¹

Cobo's model encompasses various factors associated with threatened preterm birth and MIAC, aiming to develop a predictive

model for preterm birth within the next 7 days and for MIAC. This model seeks to classify patients into high-risk and low-risk categories, allowing early and accurate identification of pregnant women who might be at a higher risk. This approach could be invaluable for implementing preventive interventions and more personalized clinical management based on the estimated risk.

Recent advances in omics technologies have identified novel biomarkers for the prediction of spontaneous preterm birth. In particular, Kindschuh et al. demonstrated that vaginal metabolomic signatures obtained during the second trimester could predict subsequent spontaneous preterm birth with good accuracy (AUROC 0.78), outperforming models based solely on clinical characteristics or vaginal microbiota composition.²² These findings suggest that metabolomics-based approaches might become valuable tools for risk stratification and early identification of women at increased risk of preterm birth. However, these techniques require specialized laboratory infrastructure, remain costly, and are not yet routinely available in most clinical settings. In contrast, the prediction models evaluated in the present study rely on clinical and laboratory variables that are readily accessible in tertiary obstetric units, facilitating their potential implementation in routine practice. Further, it should be noted that the objectives of both approaches differ substantially, as the metabolomic model was developed for early prediction of spontaneous preterm birth in asymptomatic women, whereas the present study evaluates prediction of imminent delivery and microbial invasion of the amniotic cavity in women presenting with preterm labor.

Delivery within 7 days

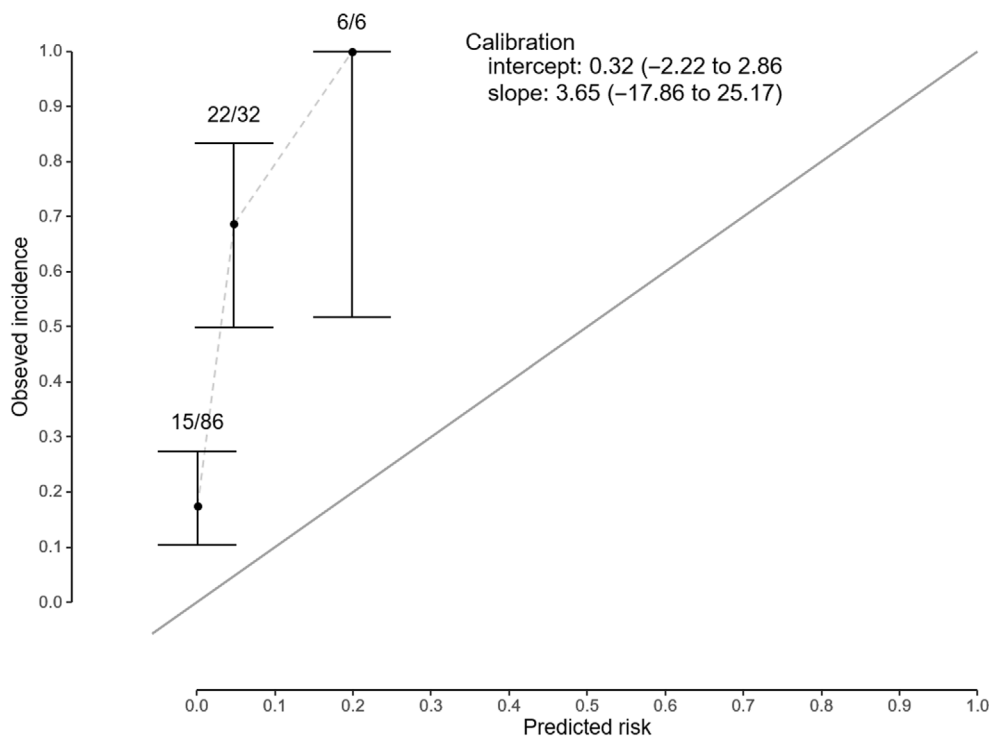


FIGURE 3 Calibration plot for prediction of delivery within 7 days. Calibration plot showing the relationship between predicted and observed probabilities for delivery within 7 days.

4.3 | Clinical implications

Only one-third of the patients admitted for preterm birth with intact membranes will experience spontaneous delivery within the first 7 days after admission. When this occurs, it is often associated with MIAC or intra-amniotic inflammation.¹ However, the clinical management of these patients is the same, and identifying these higher-risk patients remains a challenge for obstetricians.^{5,10,23,24}

Prediction models can assist in classifying patients based on the risk of spontaneous delivery and/or MIAC. Based on this risk, a change in clinical management could be considered, although further studies would be needed to confirm this hypothesis.

4.4 | Research implications

Future research must assess the impact of these models on enhancing clinical care and the potential advantages of aggressive treatment in women with a high predicted risk of MIAC.

4.5 | Strengths and limitations

One of this study's main strengths is that it is a replication and external validation of a previously conducted and validated study with

an independent internal sample, which lends more robustness to the proposal of Cobo et al. This study has several limitations. Although it is an external validation study, which strengthens the robustness of the findings, the retrospective design might still introduce selection bias. Unlike the study by Cobo et al., which only included pregnant women under 34 weeks of gestation, this study included women up to 40 weeks. This broader inclusion criterion reflects the inclusion of patients not only with threatened preterm labor under 34 weeks but also with clinical suspicion of intraamniotic infection, which can occur at later gestational ages. While this broader inclusion criterion might provide a better representation of the general population, it introduces heterogeneity in the study population. Additionally, some data related to delivery outcomes were unavailable for certain participants, as these women were discharged after the acute phase of threatened preterm labor and delivered at other centers.

Interleukin-6 levels were measured in frozen samples, which show a strong correlation with fresh samples, although minimal variability might occur.

The prediction model includes clinical and analytical parameters that can be quickly obtained in the laboratory. Obtaining amniotic fluid requires performing an amniocentesis, and some obstetricians might be hesitant to perform it. It is well known that amniocentesis is a safe invasive technique, although the patient should be informed of the associated risks. In addition to its usefulness for applying the risk model, it is also valuable for conducting an amniotic fluid culture to study a

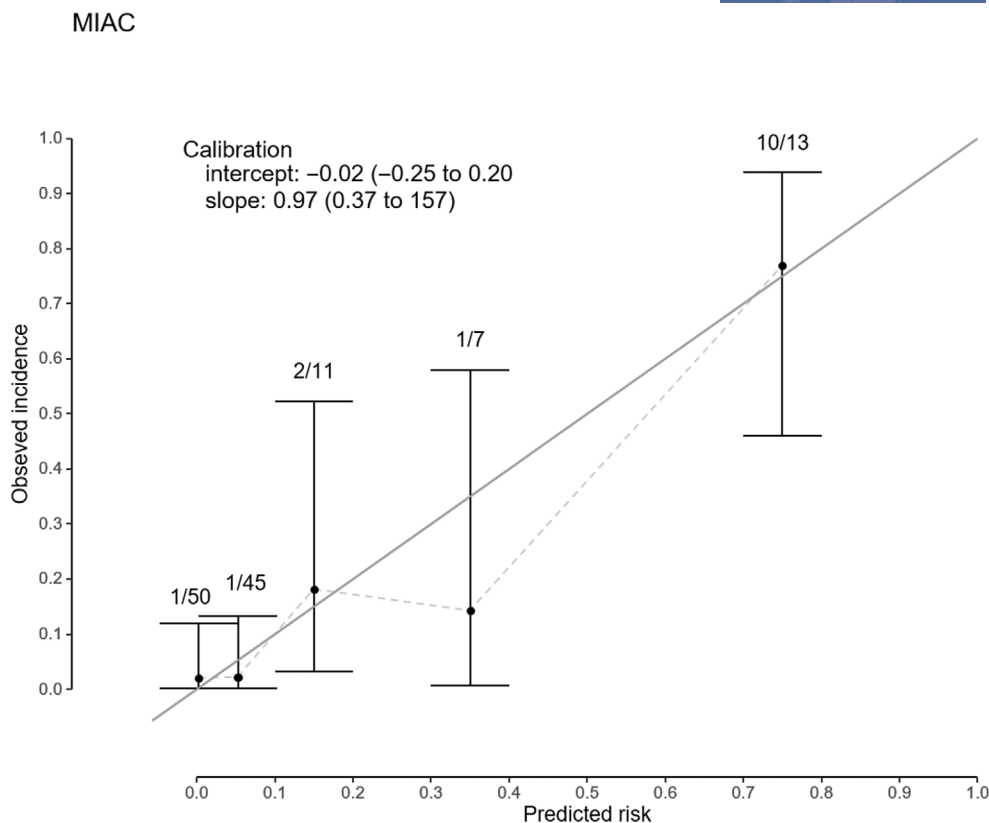


FIGURE 4 Calibration plot for prediction of microbial invasion of the amniotic cavity (MIAC). Calibration plot displaying the agreement between predicted and observed risks for MIAC.

potential MIAC. In addition, the reliance on invasive procedures and specific biomarkers in the model might restrict its applicability in clinical settings where such resources are not routinely available.

Further, a cost-effectiveness study of the risk prediction models should be conducted to assess whether there are any changes in perinatal outcomes.

5 | CONCLUSION

In conclusion, the external validation of Cobo's prediction models for spontaneous delivery within 7 days and MIAC demonstrated satisfactory predictive performance. The model showed good discrimination for both outcomes, with AUROC values of 0.805 and 0.897, respectively. However, calibration for the delivery prediction within 7 days was suboptimal, indicating less accuracy in individual risk estimates, while the calibration for MIAC was closer to the ideal, suggesting better agreement between predicted and observed outcomes. Notably, single amniotic fluid parameters provided predictive values comparable to those of the models for both delivery within 7 days and MIAC. These findings highlight the potential utility of Cobo's models in clinical practice but underscore the need for further refinement, particularly in improving calibration for predicting delivery within 7 days.

AUTHOR CONTRIBUTIONS

EB was the principal investigator at Hospital Vall d'Hebron, contributed to the study design, patient follow-up and data collection, and wrote the first draft of both the protocol and manuscript. EB is the guarantor for the manuscript. MG was the study coordinator, contributed to the study design, patient follow-up and data collection, and wrote the protocol and manuscript with EB. NM, MV, MM, and CF-J contributed to the study design and data collection. SA, MAS-D, CR, MD, and AC contributed to data collection. MH-G and AS contributed to the study design. All authors had complete access to the data at the end of the study and the decision to submit the manuscript for publication was taken at a team joint meeting where everyone reviewed and approved the final version of the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

