OBSTETRICS

Development and validation of a multivariable prediction model of spontaneous preterm delivery and microbial invasion of the amniotic cavity in women with preterm labor

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BACKGROUND: Early spontaneous preterm delivery is often associated with microbial invasion of the amniotic cavity and/or intraamniotic inflammation.

OBJECTIVE: The objective of the study was to develop and validate clinically feasible multivariable prediction models of spontaneous delivery within 7 days and microbial invasion of the amniotic cavity in women admitted with diagnose of preterm labor and intact membranes below 34 weeks.

STUDY DESIGN: We used data from a cohort of women admitted from 2012 to 2018 with diagnosis of preterm labor below 34 weeks who had undergone amniocentesis to rule out microbial invasion of the amniotic cavity. The main outcome was spontaneous delivery within 7 days from admission. The secondary outcome was microbial invasion of the amniotic cavity, defined by a positive culture and/or 16S ribosomal RNA gene in the amniotic fluid. The sample (n = 358) was divided into derivation (2012−2016) and validation cohorts (2017−2018). Logistic regression models using a stepwise selection of variables were developed for the outcomes evaluated. We explored as predictive variables ultrasound cervical length measurement at admission, maternal C-reactive protein, gestational age, amniotic fluid glucose, and interleukin-6 (expressed as log units). Models were developed in the derivation cohort and applied to the validation cohort and diagnostic performance was calculated.

RESULTS: The derivation cohort included 263 women and the validation cohort 95 women. One hundred five of the women (39%, 105 of 268) spontaneously delivered in the following 7 days and 68 (19%, 68 of 358) had microbial invasion of the amniotic cavity. For spontaneous delivery within 7 days after admission, 4 predictors were identified: cervical length at admission, gestational age, amniotic fluid glucose, and interleukin-6. The diagnostic performance of the model was assessed in the validation cohort using the receiver operating characteristic curve and showed an area under curve of 0.86 (95% confidence interval, 0.77−0.95) with a detection rate of spontaneous delivery within 7 days of 87%, a false-positive rate of 33%, a negative predictive value of 80%, and a negative likelihood ratio of 0.1908. For microbial invasion of the amniotic cavity, 2 independent predictors of the amniotic cavity were identified: amniotic fluid glucose and maternal C-reactive protein. The receiver operating characteristic curve and an area under curve in the validation cohort was 0.83 (95% confidence interval, 0.70−0.96) with a detection rate of 76%, a false-positive rate of 8%, a negative predictive value of 93%, and a negative likelihood ratio of 0.2591.

CONCLUSION: In women with preterm labor, we propose 2 clinically feasible prediction models to classify as low vs high risk of spontaneous delivery within 7 days and of microbial invasion of the amniotic cavity. The models showed a high diagnostic performance and could be of value to optimize clinical management.

Key words: amniocentesis, cervical length, interleukin-6, intraamniotic infection, intraamniotic inflammation, microbial invasion of the amniotic cavity, multivariable prediction models, preterm birth, preterm labor, spontaneous preterm delivery

One third of women who are admitted to hospitals with a diagnosis of preterm labor and intact membranes (PTL) below 34 weeks will deliver within the following 7 days.[1,2] These cases are more often associated with microbial invasion of the amniotic cavity (MIAC) and/or intraamniotic inflammation (IAI).[2−4]

Identification of women with PTL at high risk of delivery within 7 days and/or of MIAC remains an unsolved clinical challenge. Several ultrasound (US)[5] and biochemical markers[6] have been proposed as predictors of spontaneous preterm delivery within 7 days.[7] Similarly, amniotic fluid glucose[8−12] or interleukin (IL)-6 concentrations[13−15] have been strongly related to MIAC and/or IAI. However, none of these markers have shown enough accuracy to be used as stand-alone predictors in clinical practice.

There is growing evidence suggesting that multivariable prediction models can improve the diagnostic performance of different adverse outcomes such as preeclampsia[16] or fetal growth restriction.[6] Concerning spontaneous preterm delivery, different prediction models have been proposed. In women with PTL, Carter et al[17] developed and validated a multivariable prediction model (QUiPP app) that integrates maternal risk factors (symptoms, previous cervical surgery, previous preterm birth <37.0 weeks, previous preterm prelabor rupture of membranes, number of fetuses), US (transvaginal ultrasound assessment of cervical length), and biochemical markers (cervicovaginal fluid quantitative fetal fibronectin test results) to

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AJOG at a Glance

Why was the study conducted?

To develop feasible multivariable prediction models of spontaneous preterm delivery within 7 days and microbial invasion of the amniotic cavity in women with preterm labor

Key findings

The model to predict spontaneous preterm delivery within 7 days included gestational age at admission, ultrasound cervical length measurement, amniotic fluid glucose, and inteleukin-6 and showed an area under the curve of 0.86 (95% confidence interval, 0.77−0.95) with a detection rate of spontaneous delivery within 7 days of 87% and a false-positive rate of 33%. The model to predict microbial invasion of the amniotic cavity included maternal C-reactive protein and amniotic fluid glucose and showed an area under the curve of 0.83 (95% confidence interval, 0.70−0.96) with a detection rate of 76% and a false-positive rate of 8%.

What does this add to what is known?

The good diagnostic performance observed in these models might encourage clinicians to integrate the use of the amniocentesis, particularly in women with early preterm labor, to efficiently target the high-risk group of spontaneous delivery within 7 days and microbial invasion of the amniotic cavity, avoiding unnecessary overtreatment if the risk is low.

predict the occurrence of spontaneous preterm delivery.[17] However, the model was not designed to predict IAI and/or MIAC.

In this regard, other authors have proposed different combinations of proteomic biomarkers in the amniotic fluid and in the cervicovaginal fluid[18−20] with a good accuracy to predict MIAC and/or IAI. These models have not been properly validated[20] or are not feasible to be used as a tool for a rapid diagnosis in the clinical setting.[18,19]

In this scenario, we aimed to develop and validate clinically feasible multivariable prediction models of both spontaneous delivery within 7 days after admission and MIAC in women with PTL below 34 weeks of gestation that can be used in clinical decision making.

Material and Methods

Patient population

This retrospective, observational study included consecutive women recruited within a common research line for the prediction of adverse outcomes in PTL from 2012 to 2018 at the Hospital Clinic and Hospital Sant Joan de Déu, Barcelona. As part of institutional clinical protocols, women with singleton pregnancies admitted with diagnosis of preterm labor and intact membranes below 34 weeks were offered an amniocentesis.

Women with the following conditions were excluded: clinical signs of chorioamnionitis[21] at admission, cervical length measurement at admission greater than the fifth centile,[2] maternal age <18 years, and no consent to perform amniocentesis for this indication. Maternal characteristics of women who declined amniocentesis were similar to our study population.

Patient selection and sampling procedures of both studies were performed in accordance with the Declaration of Helsinki and applicable local regulatory requirements after approval from the Institutional Review Boards (HCB/2010/5811, HCB/2015/0367, PIC-82-15). Written informed consent was obtained for sample collection from all subjects.

The study group was divided into a derivation cohort and a validation cohort.

Clinical management

Standard management of women with a diagnosis of PTL included US transvaginal cervical length measurement and maternal blood analysis for evaluation of maternal C-reactive protein (CRP) and white blood cell count at admission. Transvaginal cervical length was measured by experienced staff following Fetal Medicine Foundation guidelines (https://www.fetalmedicine.com). Briefly, the vaginal probe was placed approximately 3 cm from the cervix to avoid pressure resulting in distortion of the position and shape of the cervix. A sagittal view of the full length was measured by placing the calipers at the farthest points at which the cervical walls were juxtaposed. Ultrasound cervical length was measured at least 3 times and the shortest measurement was recorded.

A complete course of antenatal steroids, betamethasone 12 mg intramuscular injection with 2 doses given 24 hours apart, was administered until 34+6 weeks for fetal lung maturation. If there was no clinical contraindication, tocolysis (nifedipine or atosiban) was administered to prolong pregnancy during steroid administration (a course of 48 hours).

Broad-spectrum antibiotics (endovenous ampicillin 1 g every 6 hours and gentamycin 80 mg every 8 hours and 1 dose of oral azithromycin 1 g) were administered in women with amniotic fluid glucose concentrations <5 mg/dL and/or with microorganisms identified by amniotic fluid Gram staining and/or positive amniotic fluid cultures.

In women with advanced cervical dilatation (Bishop >6), we also started prophylactic broad-spectrum antibiotics that were discontinued if amniotic fluid cultures were negative. In the case of the onset of uterine contractions after these 48 hours of steroid administration, tocolysis was reintroduced only if MIAC or clinical chorioamnionitis were excluded.

Cultures for genital mycoplasma (Mycoplasma IST 2, bioMérieux for *Ureaplasma* spp or *Mycoplasma hominis*), aerobic (chocolate agar), and anaerobic (Schaedler agar for anaerobes and thioglycollate broth) bacteria as well as amniotic fluid glucose concentrations and Gram stains were performed

immediately after amniocentesis and clinical management was made according to the results.

Amniotic fluid samples were also analyzed by specific polymerase chain reaction (PCR) amplification of the 16S ribosomal RNA gene using the primers: 5-AGA GTT TGA TCC TGG CTC AG-3and 5-GGA CTA CCA GGG TAT CTA AT-3 followed by Sanger sequencing in the Department of Microbiology. Sequences were identified using the Blast algorithm in the National Center for Biotechnology Information database, with a minimum 98% sequence identity.

Amniotic fluid IL-6 concentrations were measured by enzyme-linked immunoassay (Biosource; Invitrogen, Carlsbad, CA) in amniotic fluid samples previously centrifuged at 4000 rpm for 10 minutes at 4°C and stored at −80°C. The minimum detectable level of IL-6 was 0.2 ng/mL.

Information of 16s ribosomal RNA gene sequencing and IL-6 was not available for clinical decision making.

Classification of outcomes

The primary outcome was spontaneous delivery within 7 days after admission. Women who delivered because of maternal or fetal indications were consequently censored. The secondary outcome was the occurrence of MIAC, defined by the presence of a positive amniotic fluid culture for bacteria, fungi, and *Ureaplasma* spp or *Mycoplasma hominis* and/or by specific PCR amplification of the 16S ribosomal RNA gene. Gestational age was established according to crown-rump length at the first-trimester US scan.[22]

Predictors

Predictors used to develop multivariable prediction model were those that showed to be independent predictors in the univariate logistic regression analysis and included the following continuous variables: US cervical length measurement (millimeters) at admission, maternal CRP concentrations (milligrams per liter), gestational age (weeks), amniotic fluid glucose concentrations (milligrams per deciliter), and amniotic fluid IL-6 (nanograms per milliliter) (expressed in a log scale).

Sample size

The sample size[23] to develop the multivariable prediction model was established assuming an initial inclusion of 5 potential predictors selected a priori based on consensus among the investigators (T.C., M.P., F.F., E.G.) and for an outcome (delivery within 7 days after admission) with a prevalence of 30%,[1,2] resulting in a sample size of 167. To account for a proportion of censored cases in which delivery is indicated electively (30%) and 10% of cases with missing information for any of the predictors, a conservative number of 240 cases were estimated.

For the validation cohort, all consecutive cases attended within 2 years (2017−2018) after the model was developed were included.

Statistical analysis

Statistical analysis was performed using SPSS 20.0 for MAC OS (IBM Corporation, Armonk, NY). We compared maternal characteristics and perinatal outcomes between the derivation and the validation cohorts; continuous variables were compared using a nonparametric Mann-Whitney *U* test presented as median with interquartile range (25th, 75th percentile). Categorical variables were compared using the χ^2 or Fisher exact test. Differences were considered statistically significant with a value of $P < .05$ with 2-sided alternative hypotheses.

Multivariable analysis by stepwise logistic regression was used to identify independent factors associated with the outcomes (prediction model development). The models that could best predict spontaneous delivery within 7 days and MIAC were constructed based on the final regression model and the direction of effects. Goodness-of-fit models were assessed by calculating Nagelkerke's R^2.

Diagnostic performance was calculated (area under the receiver-operating characteristic (AUROC) curve, sensitivity, specificity, positive predictive value, negative predictive value, positive likelihood ratio, and negative likelihood ratio for spontaneous delivery within 7 days and for MIAC in the derivation and the validation cohorts.

A posterior probabilities for a given outcome were calculated using Fagan's plots (R statistics [R project; package ggplot2]), in which a priori risk was based on the prevalence of the outcome among the sample population multiplied by the positive and negative likelihoods ratios of the diagnostic test.

Finally, to assess the likelihood of patients to have significantly different results if receiving tocolysis based on their clinical characteristics, we performed a nearest-neighbor propensity score matching by logit regression, in which the outcome variable was spontaneous delivery within 7 days after admission. Predictor variables were gestational age at admission, cervical length, amniotic fluid glucose, and IL-6, and the treatment variable was tocolysis. Test balance for adequate matching was performed using variance ratios.

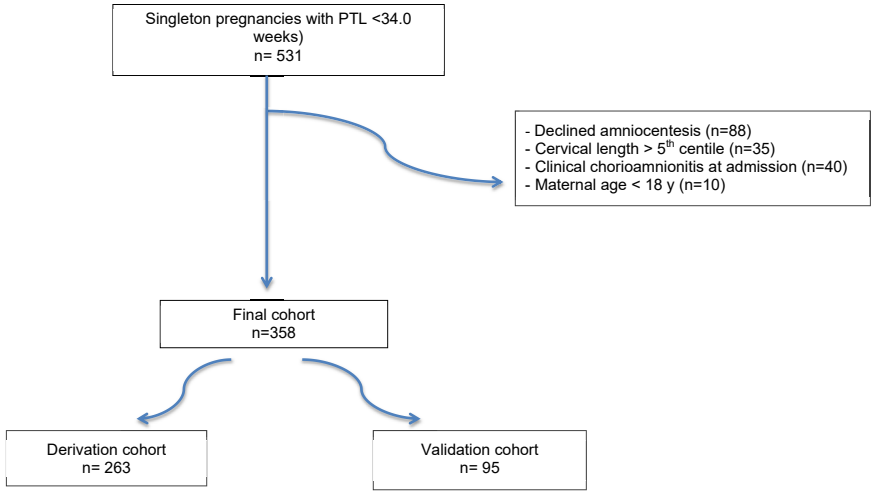
Results

During the study period 531 women were eligible for the study and 358 were finally included. Figure 1 shows flow chart of the entire study population.

For the overall population (n = 358), 105 (39%, 105 of 268) of women spontaneously delivered in the following 7 days and 68 (19%, 68 of 358) had microbial invasion of the amniotic cavity. Microorganisms isolated in the amniotic fluid with their amniotic fluid IL-6 concentrations are presented in a supplemental table (Supplemental Table 1).

The majority of women with MIAC (60 of 67, 89.6%) had high levels of amniotic fluid IL-6 (≥2.6 ng/mL). Women with MIAC had an earlier gestational age at admission (median [25th, 75th percentiles], 26.4 [24.2, 30.5] weeks) than women without (28.6 [25.6, 30.9] weeks). Gestational age at delivery was significantly earlier in women with MIAC (27 [25.1, 31.1] weeks) than in women without (35.3 [30.3, 38.5] weeks), and latency to delivery was significantly shorter in women with MIAC (1 [0, 3] day vs 35 [6, 64.5] days, respectively).

FIGURE 1
Distribution of the entire study group (2012–2018)



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Among women admitted below 28 weeks of gestation, 48% (59 of 123) spontaneously delivered in the following 7 days, and 24.6% (41 of 167) had MIAC. Below 32 weeks of admission, the prevalence of spontaneous delivery within 7 days and MIAC was 38.7% (86 of 222) and 19.1% (58 of 304), respectively.

Maternal characteristics and perinatal outcome comparisons between women with and without MIAC and with and without sPTD within 7 days are presented in supplemental tables (Supplemental Tables 2 and 3, respectively).

The derivation cohort included 263 women and the validation cohort 95. Differences in the maternal characteristics and perinatal outcomes between women from the derivation and the validation cohorts are shown in Table 1. In the validation cohort, IL-6 concentrations were significantly higher, gestational age at delivery was significantly earlier and latency to delivery significantly was shorter than in the derivation cohort.

Multivariable analysis indicated that gestational age at admission, cervical length, amniotic fluid glucose, and IL-6 were independent predictors for spontaneous delivery within 7 days (Table 2). Maternal CRP and amniotic fluid glucose were independent predictors of MIAC ($P < .05$).

The regression formula for spontaneous delivery within 7 days was: −7.588 + 0.132 * gestational age at admission − 0.051 * US cervical length − 0.055 * amniotic fluid glucose + 1.438 * amniotic fluid log (IL-6). $R^2 = 51.5\%$.

The regression formula for MIAC was = 1.034 + 0.169 * maternal CRP −0.158 * amniotic fluid glucose. $R^2 = 65.6\%$.

According to the selected predictors and the direction of effects, we proposed 2 models: one for spontaneous delivery within 7 days and one for MIAC in which maternal CRP = milligrams per liter; amniotic fluid glucose = milligrams per deciliter; gestational age at admission = weeks; US cervical length = millimeters; and amniotic fluid IL-6 (nanograms per milliliter) were expressed in a log scale.

The diagnostic performance of the model for predicting spontaneous delivery within 7 days and MIAC was assessed using ROC curves. The AUROC of the model to predict the risk of spontaneous delivery within 7 days was 0.88 (95% confidence interval [CI], 0.83–0.93) in the derivation cohort and 0.86 (95% CI, 0.77–0.95) in the validation cohort. In the validation cohort, the detection rate of spontaneous delivery within 7 days was of 87% and the false-positive rate was 33%. We selected a cutoff with a high detection rate to efficiently target the high-risk group of women who will inevitably deliver in the following days.

The AUROC of the model for predicting MIAC was 0.94 (95% CI, 0.89–0.98) in the derivation and 0.83 (95% CI, 0.70–0.96) in the validation cohort. In the validation cohort, the detection rate of MIAC was 76% of women, and the false-positive rate was of 8%. We decided to select a cutoff with a low false-positive rate to avoid unnecessary antibiotic treatment in women without MIAC. The diagnostic performance of the 2 models is shown in Table 3.

Figure 2 shows Fagan nomogram of the validation cohort. According to this, if we test the model for spontaneous delivery within 7 days in a woman with a priori risk of 57% this risk increases to 79% in the high-risk group (after applying the positive LR value) and decreases to 20% in the low-risk group (after applying the negative LR). Regarding the risk of MIAC, if the priory risk is 22%, it increases to 73% in the high-risk group and decreases to 7% in the low-risk group.

Our analysis (propensity score) to account for an indication bias suggests a lack of such effect because we did not find tocolysis to be associated with spontaneous delivery within 7 days (Supplemental Table 4).

Comment

Principal findings

In this study, we developed and validated clinically feasible multivariable prediction models in women admitted with a diagnosis of PTL below 34 weeks of gestation that could help clinicians to efficiently manage low- and high-risk populations of spontaneous delivery within 7 days and of MIAC.

The good diagnostic performance observed in these models might encourage clinicians to integrate the use of the amniocentesis, considered a safe procedure, even in a more challenging

TABLE 1
Maternal characteristics and perinatal outcomes of the women in the derivation and the validation cohorts

Variables	Derivation cohort (n = 263)	Validation cohort (n = 95)	*P* value
Maternal age, y	33.1 (28.1, 36.5)	34.2 (29.0, 37.9)	.069
Caucasian ethnicity	190 (72)	59 (62)	< .001
Smoking	30/203 (15)	13 (14)	.861
Nulliparity	123 (47)	58 (61)	0.023
Prior preterm birth	34 (13)	3 (3)	.006
Cervical length, mm	11 (5; 18)	9 (3; 15)	.149
CRP, mg/L	0.75 (0.38, 1.98)	1.06 (0.49, 2.48)	.087
WBC ($\times 10^9$/L)	12,280 (10,230, 14,940)	12,330 (10,710, 15,800)	.517
GA at admission, wks	28.6 (25.3, 31.0)	27.4 (24.4, 30.4)	.084
GA at amniocentesis, wks	28.6 (25.4, 31.0)	27.4 (24.6, 30.6)	.093
AF glucose, mg/dL	31 (18, 43)	26 (13, 39)	.140
AF IL-6, ng/mL	2.47 (1.02, 20.8)	16.95 (2.2, 121.4)	< .001
MIAC	47 (18)	21 (22)	.364
Antenatal steroids	232/247 (94)	85 (90)	.168
Antenatal antibiotics	149 (57)	62/94 (66)	.142
GA at delivery, wks	34.6 (29.1, 38.3)	31 (26.7, 36)	.001
GA <37.0 weeks	163 (62)	74 (78)	.005
Spontaneous onset of labor	201 (76)	67 (71)	.271
Spontaneous delivery within 48 h after admission	34/201 (17)	17/67 (25)	.158
Spontaneous delivery within 7 days after admission	67/201 (33)	38/67 (57)	.001
Latency to delivery, d	31 (3; 62)	7 (1; 50)	.004
Clinical chorioamnionitis at delivery	37/262 (14)	29/87 (33)	< .001

Continuous variables were compared using a nonparametric Mann-Whitney *U* test presented as medians (25th and 75th interquartile percentiles). Categorical variables were compared using χ^2 or Fisher exact tests and presented as number (percentage).

AF, amniotic fluid; *CRP*, C-reactive protein; *GA*, gestational age; *MIAC*, microbial invasion of the amniotic cavity; *WBC*, white blood cells.

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condition such as preterm prelabor rupture of membranes,[24] as part of the management of PTL, particularly in women with early PTL (eg, before 32 weeks).

Results

Prediction models of spontaneous delivery within 7 days after admission have previously been proposed in symptomatic[25] women. Thus, Carter et al[17] developed and validated a multivariable prediction model (QUiPP app) in women with symptoms of PTL showing similar diagnostic performance to our prediction model with the strength to be a minimally invasive tool. However, what is of note in Carter et al[17] paper is the low prevalence of spontaneous delivery within 7 days reported in their validation cohort (4.9% vs 57% observed in our cohort).

Based on these differences, we might hypothesize that QUiPP app was used by the authors as a screening tool to increase confidence in the admission decision of women attending with symptoms. In our admission decision, we are already performing a QUiPP-like screening because we take into consideration gestational age and cervical length. This explains the high rate of spontaneous delivery within 7 days observed in our validation cohort. What differentiates our model from QUiPP app is that our model was developed not to be a screening tool but to be a diagnostic tool of spontaneous delivery within 7 days.

Our model integrates not only maternal (gestational age) and US factors (cervical length measurement), as QUiPP app does, but also information related to IAI (amniotic fluid glucose and IL-6). To include information related to IAI in a prediction model of spontaneous delivery within 7 days leads to a better prognosis and more efficient clinical management. Up to 40% of women with early diagnosis of PTL (< 28 weeks) have IAI (with or without MIAC[2–4]). In addition, early spontaneous preterm delivery[2] is considered to most likely be related to this

TABLE 2
Multivariable prediction models of MIAC and spontaneous delivery within 7 days after admission using stepwise logistic regression analysis

	Spontaneous delivery within 7 days				MIAC		
Variables	OR	95% CI	*P* value	Variables	OR	95% CI	*P* value
GA at amniocentesis, wks	1.141	1.007–1.292	.038	CRP (mg/L)	1.184	1.017–1.380	.030
Cervical length, mm	0.951	0.913–0.990	.014	AF glucose (mg/dL)	0.854	0.816–0.893	< .001
AF glucose, mg/dL	0.947	0.921–0.973	< .001				
AF IL-6 (log)	4.211	2.316–7.657	< .001				

AF, amniotic fluid; *CI*, confidence interval; *CRP*, C-reactive protein; *IL-6*, interleukin-6; *MIAC*, microbial invasion of the amniotic cavity; *OR*, odds ratio.
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inflammatory condition. Finally, there has been widely reported the association of IAI with a worse perinatal outcome.[3,4,26]

There have been other authors who proposed a prediction model of spontaneous preterm delivery including IAI information. Thus, Holst et al[27] proposed a good model to predict spontaneous delivery within 7 days including a combination of amniotic fluid and cervical fluid inflammatory proteins using multiplexed immunoassay technology. However, these results were not validated in an independent cohort, thus limiting the reproducibility of their findings. Similarly, Hitti et al[19] and Combs et al[18] developed and validated 2 different predictive models of MIAC in women with PTL including different proteins measured in the cervicovaginal fluid. The main limitation is that such protein measures are not readily available in clinical laboratories.

Conversely, we propose clinically feasible prediction models that can be used in clinical decision making. Indeed, all the variables included in the models reported here can be measured immediately after admission (such as gestational age, US cervical length) or within a few hours (eg, maternal CRP, amniotic fluid glucose). There are currently even IL-6 bedside tests[13–15] that provide rapid information and show a good correlation with the gold standard enzyme-linked immunoassay analysis.

Clinical implications

For a clinical perspective, the classification of the risk of spontaneous delivery within 7 days and/or MIAC into low and high risk might help clinicians to more efficiently manage women with diagnosis of PTL. In the high-risk group, expected early delivery should be planned with antenatal strategies that have shown to improve neonatal outcome, such as patient transfer to facilities with neonatal intensive care units, treatment with antenatal steroids[28] and

TABLE 3
Diagnostic performance of MIAC and spontaneous delivery within 7 days after admission in the derivation and the validation cohorts

	Spontaneous delivery within 7 days		MIAC	
Variables	Derivation cohort (n = 201)	Validation cohort (n = 67)	Derivation cohort (n = 263)	Validation cohort (n = 95)
AUC (95% CI)	0.88 (0.83–0.93)	0.86 (0.77–0.95)	0.94 (0.89–0.98)	0.83 (0.70–0.96)
Sensitivity n (%)	55/67 (82.09)	33/38 (86.84)	39/47 (82.98)	16/21 (76.19)
Specificity n (%)	114/134 (85.07)	20/29 (68.97)	207/216 (95.83)	68/74 (91.89)
Positive predictive value n (%)	55/75 (73.3)	33/42 (78.57)	39/48 (81.25)	16/22 (72.73)
Negative predictive value n (%)	114/126 (90.48)	20/25 (80)	207/215 (96.28)	68/73 (93.15)
Positive LR	4.53	2.80	19.91	9.40
Negative LR	0.1838	0.1908	0.1776	0.2591

AUC, area under curve; *CI*, confidence interval; *LR*, likelihood ratio; *MIAC*: microbial invasion of the amniotic cavity.
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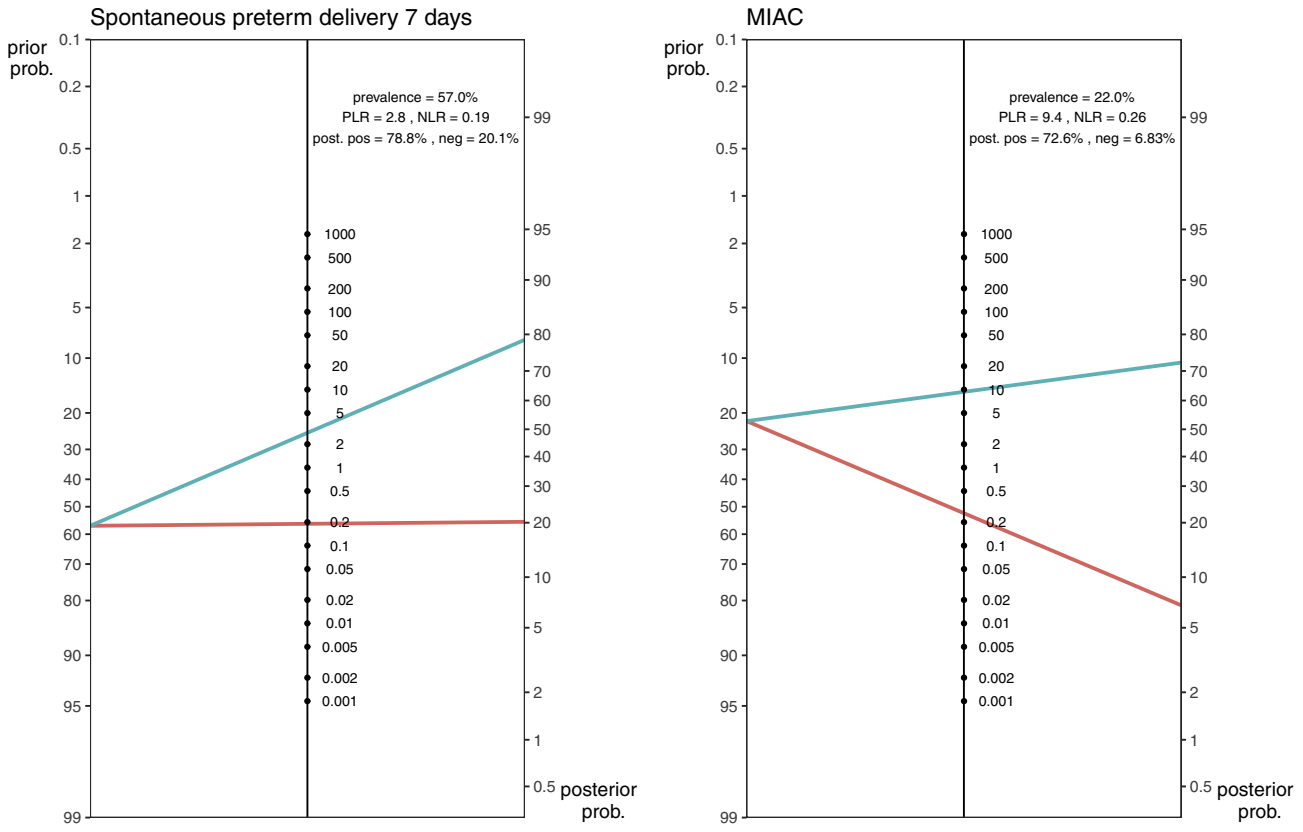
FIGURE 2
Fagan nomogram of the validation cohort



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magnesium sulfate for neuroprotection,[29] and, for those with MIAC, early initiation of broad-spectrum antibiotics.[30]

In this regard, although systematic antenatal antibiotic prophylaxis has been questioned in women with PTL,[31,32] emerging data suggest that eradication of MIAC and IAI is possible in a substantial number of women with PTL after early administration of broad-spectrum antibiotic treatment.[30]

With respect to the low-risk group, the management of these women could be potentially ameliorated with a lower-level intensity intervention.

Research implications

Future studies are required to prospectively evaluate the influence of these models on improving clinical management and the potential benefit of early antibiotic treatment in women with a high-predicted risk of MIAC. Studies evaluating the cost-effectiveness of our models (eg, hospital stay length, cost of treatment, or work leave) are also warranted.

Strengths and limitations

A main strength of this study is that the model was validated in an independent sample. We included a well-characterized large cohort with information of MIAC and with close perinatal follow-up. Moreover, the diagnosis of MIAC was based on microbial cultures as well as PCR targeting the 16S ribosomal RNA gene sequence. Moreover, all the predictors included in the 2 models are variables that can be obtained at admission or within 24 hours in most clinical settings.

Finally, we evaluated whether clinical management with tocolysis interfered with the outcome of spontaneous delivery within 7 days in some women (eg, discontinuation of tocolysis if amniotic fluid cultures were positive or glucose was <5 mg/dL) and found no association between them (Supplemental Table 1). This might be due to the fact that, regardless of our management, these women with MIAC will inevitably deliver in few days because of the inflammatory exposition.[3,4,26]

As limitations, IL-6 was measured in frozen samples, although there is strong evidence showing a good correlation between frozen and fresh samples.[13,28] Finally, this study was not designed to evaluate whether our prediction models improve maternal and neonatal outcomes.

Conclusion

In conclusion, we propose 2 clinically feasible multivariable prediction models

that may help clinicians to individualize the management of women admitted with a diagnose of PTL before 34 weeks of gestation: first, targeting the high-risk group of spontaneous delivery within 7 days after admission and/or of MIAC who require efficient planning of expected early delivery; and second, avoiding unnecessary overtreatment in the low-risk group. ■

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SUPPLEMENTAL TABLE 1

Information about microorganisms isolated in the amniotic fluid

Variables	Microorganisms in the amniotic fluid	AF IL-6, ng/mL	Latency to delivery, d
1	*Ureaplasma* spp	0.102	3.00
2	*Ureaplasma* spp	20.106	15.00
3	*Ureaplasma* spp	90.832	1.00
4	*Ureaplasma* spp	892.642	15.00
5	*Ureaplasma* spp	22.891	1.00
6	*Ureaplasma* spp	1.799	6.00
7	*Ureaplasma* spp	133.250	1.00
8	*Ureaplasma* spp	158.600	1.00
9	*Ureaplasma* spp	37.760	1.00
10	*Ureaplasma* spp	4.306	3.00
11	*Ureaplasma* spp	9.005	1.00
12	*Ureaplasma* spp	0.376	13.00
13	*Ureaplasma* spp	3.736	2.00
14	*Ureaplasma* sp., *Streptococcus anginosus*	161.051	1.00
15	*Ureaplasma* spp, Fusobacterium spp	24.522	0.00
16	*Ureaplasma* spp	236.856	1.00
17	*Ureaplasma* spp	80.7325	12.00
18	*Ureaplasma* spp	75.7254	1.00
19	*Ureaplasma* spp	838.025	4.00
20	*Ureaplasma* spp	148.620	0.00
21	*Ureaplasma* spp	151.632	3.00
22	*Ureaplasma* spp	125.220	1.00
23	*Ureaplasma* spp	26.7074	4.00
24	*Ureaplasma* spp	48.1035	2.00
25	*Ureaplasma* spp	139.401	3.00
26	*Ureaplasma* spp	152.103	3.00
27	*Ureaplasma* spp	1214.000	3.00
28	*Ureaplasma* spp, *Haemophilus influenzae*	207.199	.00
29	*Ureaplasma* spp., *Mycoplasma hominis*	340.687	1.00
30	*Ureaplasma* spp, *Fusobacterium* spp, *Candida albicans*	24.432	2.00
31	*Ureaplasma* spp	41.151	36.00
32	*Mycoplasma hominis*	598.0	0.00
33	*Mycoplasma hominis*, *Fusobacterium* spp	30.313	2.00
34	*Fusobacterium* spp	2.774	0.00
35	*Fusobacterium nucleatum*	1888.000	17.00
36	*Fusobacterium* spp	2.790	0.00
37	*Fusobacterium* spp	44.420	3.00
38	*Fusobacterium nucleatum*	591.869	1.00
39	*Fusobacterium nucleatum*	789.989	0.00

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SUPPLEMENTAL TABLE 1

Information about microorganisms isolated in the amniotic fluid *(continued)*

Variables	Microorganisms in the amniotic fluid	AF IL-6, ng/mL	Latency to delivery, d
40	*Fusobacterium nucleatum*	1595.000	2.00
41	*Fusobacterium* spp	498.415	0.00
42	*Fusobacterium nucleatum*	837.164	5.00
43	*Fusobacterium nucleatum*, *Streptococcus viridans*	2879.000	0.00
44	*Fusobacterium* spp, *Candida albicans*	1332.000	1.00
45	*Fusobacterium nucleatum*, *Candida albicans*	0.339	1.00
46	*Streptococcus viridans*	2.829	1.00
47	*Streptococcus agalactiae*, *Candida albicans*	2.460	2.00
48	*Streptococcus mitis*	1.176	53.00
49	*Streptococcus pyogenes*	0.2627	46.00
50	*Streptococcus agalactiae*	542.438	0.00
51	*Listeria monocytogenes*	242.202	3.00
52	*Listeria monocytogenes*	18.151	0.00
53	*Listeria monocytogenes*	127.575	0.00
54	*Lactobacillus*	28.060	11.00
55	*Lactobacillus*	118.842	1.00
56	*Escherichia coli*	281.509	0.00
57	*Escherichia coli*	2.8948	0.00
58	*Escherichia coli*	20.8493	0.00
59	*Capnocytophaga sputigena*	38.480	1.00
60	*Capnocytophaga sputigena*	123.386	0.00
61	*Candida albicans*	142.649	0.00
62	*Candida albicans*	6.020	2.00
63	*Candida albicans*	14.720	7.00
64	*Leptotrichia* spp	262.367	1.00
65	*Proteus mirabilis*	48.940	1.00
66	*Prevotella amnii*	413.000	1.00
67	*Peptostreptococcus*	36.499	0.00
68	*Bacteroides fragilis*	696.715	0.00

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SUPPLEMENTAL TABLE 2

Maternal characteristics and perinatal outcomes according to the outcome of MIAC

Variables	MIAC (n = 68)	No MIAC (n = 290)	*P* value
Maternal age, y	33.3 (26.3, 36.5)	31.8 (27.9, 36.3)	.233
White ethnicity	47 (69)	202 (70)	.541
Smoking	8/59 (14)	35/239 (15)	1.000
Nulliparity	35 (52)	146 (50)	.893
Prior preterm birth	6 (9)	31 (11)	.825
Cervical length, mm	5 (0, 14)	11 (5;18)	.002
CRP, mg/L	3.4 (1.1, 7.3)	0.6 (0.3, 1.4)	< .001
WBC ($\times 10^9$/L)	14,110 (12,085, 1,7285)	12,090 (9890, 14,720)	< .001
GA at admission, wks	26.4 (24.8, 30.5)	28.6 (25.7, 31.0)	.017
GA at amniocentesis, wks	26.4 (24,8, 30.5)	28.6 (25.7, 31.0)	.018
AF glucose, mg/dL	4 (0, 6)	33 (24, 44)	< .001
AF IL-6, ng/mL	48.1 (10.4, 273.9)	2.2 (0.9, 10.6)	< .001
Antenatal steroids	57/59 (97)	260/283 (92)	.276
Antenatal antibiotics	66 (97)	145/289 (50)	< .001
GA at delivery, wks	26.9 (25.2, 31.1)	35.0 (29.7, 38.3)	< .001
Spontaneous onset of labor	49 (72)	219 (76)	.539
Latency to delivery, d	1 (0, 3)	31 (6, 62)	< .001
Clinical chorioamnionitis at delivery	39/66 (59)	27/283 (10)	< .001

Continuous variables were compared using a nonparametric Mann-Whitney *U* test presented as medians (25th and 75th interquartile percentiles). Categorical variables were compared using χ^2 or Fisher exact tests and presented as number (percentage).

AF, amniotic fluid; *CRP*, C-reactive protein; *GA*, gestational age; *MIAC*, microbial invasion of the amniotic cavity; *WBC*, white blood cells.

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SUPPLEMENTAL TABLE 3

Maternal characteristics and perinatal outcomes according to the outcome of sPTD within 7 days

Variables	sPTD, 7 d (n = 105)	No sPTD, 7 d (n = 163)	*P* value
Maternal age, y	33.0 (27.6, 36.8)	31.9 (27.6, 36.3)	.554
White ethnicity	70 (67)	121 (74)	.167
Smoking	21/98 (21)	10/126 (8)	.006
Nulliparity	58 (55)	80 (49)	.381
Prior preterm birth	12 (11)	18 (11)	1.000
Cervical length, mm	6 (0, 16)	11 (6, 18)	< .001
CRP, mg/L	1.8 (0.7, 4.4)	0.5 (0.3, 1.1)	< .001
WBC ($\times 10^9$/L	14040 (11875, 17340)	11540 (9700, 14080)	< .001
GA at admission, wks	27.4 (25.0, 31.0)	28.9 (26.0, 31.0)	.148
GA at amniocentesis, wks	27.4 (25.0, 31.0)	29.0 (26.0, 31.0)	.171
AF glucose, mg/dL	16 (3.5, 30)	36 (26, 46)	< .001
AF IL-6, ng/mL	32.0 (4.5, 127.6)	1.3 (0.8, 2.89)	< .001
Antenatal steroids	97/99 (98)	147/160 (92)	.054
Antenatal antibiotics	95 (91)	66/162 (41)	< .001
GA at delivery, wks	27.4 (25.1, 31.3)	37.3 (33.0, 38.9)	< .001
Spontaneous onset of labor	105 (100)	163 (100)	—
Latency to delivery, d	2 (1, 3)	49 (27, 72)	< .001
Clinical chorioamnionitis at delivery	37/99 (37)	10/162 (6)	< .001

Continuous variables were compared using a nonparametric Mann-Whitney *U* test presented as medians (25th and 75th interquartile percentiles). Categorical variables were compared using χ^2 or Fisher exact tests and presented as number (percentage).

AF, amniotic fluid; *CRP*, C-reactive protein; *GA*, gestational age; *MIAC*, microbial invasion of the amniotic cavity; *WBC*, white blood cells.

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SUPPLEMENTAL TABLE 4

Association between model variables for prediction of spontaneous preterm delivery at 7 days after nearest neighbor propensity score matching

Outcome	GA at admission	Cervical length	AF glucose	AF IL-6
sPTD within 7 days	Coefficient (95% CI)			
No tocolysis (n = 10)	Reference	Reference	Reference	Reference
Tocolysis (n = 84)	0.016 (−0.032 to 0.062)	−0.025 (−0.06 to 0.01)	−0.001 (0.17−0.02)	−0.49 (−0.13 to 0.31)
Estimated average treatment effect in population comparing tocolysis vs. no tocolysis				
Outcome	Coefficient	SE	95% CI	*P* value
sPTD within 7 days	0.22	0.2	−0.18 to 0.62	0.285

AF, amniotic fluid; *CI*, confidence interval; *GA*, gestational age; *IL*, interleukin; *sPTD*, spontaneous preterm delivery.

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