**Non-invasive prediction models of intraamniotic infection in women with preterm labor**

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**Condensation:** We constructed non-invasive prediction models, using high-dimensional biology and machine learning, to screen the high-risk preterm labor group of intra-amniotic infection and/or early delivery.

**Short title:** Non-invasive prediction models of intra-amniotic infection and/or early delivery in preterm labor.

**AJOG at a Glance:**

**A. Why was the study conducted?** In preterm labor, to screen the high-risk group of the composite outcome intra-amniotic infection and/or spontaneous delivery within 7 days, using non-invasive methods suitable in the clinical setting

**B. What are the key findings?** Different models were developed and validated for the composite outcome including transvaginal cervical length, maternal CRP, vaginal pH, inteleukin-6, lactic acid concentrations or *Lactobacillus* genus. The diagnostic performance showed areas under the curve ranging from 82.2% (+-3.1% 95% confidence interval (CI)) to 85.2%(+-3.1% 95% CI) and sensitivities ranging from 76.1 to 85.9%.

**C. What does this study add to what is already known?** The good diagnostic performance observed might encourage clinicians to integrate the use of the amniocentesis in the management of preterm labor avoiding unnecessary amniocentesis if the risk is low.

**Abstract**

**Background:** Among women with preterm labor, those with intra-amniotic infection present the highest risk of early delivery and the most adverse outcomes. Identification of intra-amniotic infection requires amniocentesis, perceived as too invasive by women and physicians. Non-invasive methods for identifying intra-amniotic infection and/or early delivery are critical to focus early efforts on high-risk while avoiding unnecessary interventions in low-risk preterm labor women.

**Objective:** We modeled the best performing models integrating biochemical data with clinical and ultrasound information to predict a composite outcome of intra-amniotic infection and/or spontaneous delivery within 7 days.

**Study design**: From 2015-2020, w~~W~~e used data from a cohort of women admitted with diagnosis of preterm labor below 34 weeks at Hospital Clinic and Hospital Sant Joan de Déu, Barcelona, who had undergone amniocentesis to rule in/out intra-amniotic infection or inflammation. Transv~~V~~aginal ultrasound, maternal blood and vaginal samples were prospectively performed at admission. Using high-dimensional biology, we explored vaginal proteins (by multiplex immunoassay), amino acids (by high-performance liquid chromatography) and bacteria (by 16S rRNA gene amplicon sequencing) to predict the composite outcome. We selected ultrasound, maternal blood and vaginal predictors that could be tested with rapid diagnostic techniques and developed prediction models employing Machine Learning that were applied in a validation cohort.

**Results**: We studied a cohort of 288 women with PTL below 34 weeks, of which 103 (35%) had a composite outcome of IAI and/or spontaneous delivery within 7 days. The sample was divided into derivation (n=116) and validation cohorts (n=172). Four prediction models were proposed, including ultrasound transvaginal cervical length, maternal C-reactive protein, vaginal IL-6 (using automated immunoanalyzer), vaginal pH (using pH meter), vaginal lactic acid (using reflectometer) and vaginal *Lactobacillus* genus (using quantitative-PCR), with areas under the curve ranging from 82.2% (+-3.1% 95% confidence interval) to 85.2%(+-3.1% 95% confidence interval), sensitivities ranging from 76.1 to 85.9% and specificities of 75.2 to 85.1%.

**Conclusions**: These results provide proof-of-principle of how non-invasive methods suitable for point-of-care systems can select high-risk cases among women with preterm labor and might substantially aid in clinical management and outcomes, while improving use of resources and patient experience.

**Keywords –** spontaneous preterm delivery, intra-amniotic infection, preterm labor, multivariable prediction models, amniocentesis

## Introduction

Current management of women admitted with preterm labor (PTL) is highly inefficient. A majority of women diagnosed with PTL are at low-risk and will eventually deliver at term[1]. On the other hand, identification of women with high-risk PTL (those effectively delivering within 7 days of admission) is still poor. It is well known that the worst outcomes in PTL[2], and also in preterm prelabour rupture of membranes[3], occur in women with intra-amniotic infection (IAI) and/or inflammation. The earliest spontaneous preterm delivery (sPTD) is most likely related to IAI[3,4]. Women with IAI are the group who might really benefit from antenatal strategies that have shown to improve perinatal outcomes (e.g., antenatal steroids[5], magnesium sulfate[6], and probably antibiotics[7]). On the contrary, most women without IAI deliver near term and do not require close follow-up and interventions.

Despite the fact that women with and without IAI have a completely different perinatal prognosis, current antenatal management for both groups is similar. The diagnosis of IAI requires the performance of an amniocentesis although it is not universally practiced clinically, being ~~being~~ met by substantial resistance from women and even physicians. However, evidence supports that, once diagnosed, IAI might be eradicated with broad-spectrum antibiotics[7] and this adds to the above-mentioned reasons to stress the impact of targeting this group for early intervention, while avoiding unnecessary interventions in the remaining low-risk PTL women.

In the field of sPTD, efforts have focused on the development of multiparameter prediction models for high-risk PTL women[8,9]. However, they were either not designed to predict IAI[9] or require amniocentesis[8]. Altogether, there is a critical need for clinically feasible non-invasive methods capable of selecting PTL women at high risk of IAI. In this regard, using a multiplex immunoassay, several proteins in the maternal serum or in the cervico-vaginal samples have been described to predict IAI or imminent delivery[10-12]. Similarly, the vaginal microbiome of IAI[12] and the vaginal metabolome expression of IAI have been characterized[14,15]. Unfortunately, none of technologies used are feasible for clinical application and, to date, no methods suitable for application on a point-of-care basis have been evaluated.

We aimed to conduct an exploratory study using high-dimensional biology to investigate and select vaginal proteins, amino acids and bacteria that could be suitable for integration in rapid diagnostic systems. We modeled the best performing models integrating biochemical data with clinical and ultrasound information to predict a composite outcome of IAI and/or spontaneous delivery within 7 days.

## Material and methods

### Study design

This is a prospective observational study including singleton pregnancies admitted with a diagnosis of PTL below 34 weeks at Hospital Clinic and Hospital Sant Joan de Déu, Barcelona (2015-2020). As part of the institutional clinical protocols, these women were offered amniocentesis to rule in/out IAI. We included singleton pregnancies admitted with a diagnosis of preterm labor and intact membranes between 23.0 and 33.6 weeks, not in arrested labor at admission, and who do not meet exclusion criteria.

We excluded maternal age < 18 years, multiple gestations, clinical chorioamnionitis, defined by the presence of fever ≥ 38ºC, fetal tachycardia (> 160 heart beat per minute > 10 minutes) and maternal white blood cells > 15000/$mm^3$ (not justified by the administration of antenatal corticosteroids)[16], cervical dilatation > 3 cm, major structural malformations of fetal complications, transvaginal cervical length measurement at admission >> $5^{th}$ centile (defined as a cervical length greater than 25 mm in women with PTL below 28.0 weeks; greater than 20 mm between 28.0-31.6 weeks and greater than 15 mm above 32.0 weeks of gestation)[17], not feasible to perform amniocentesis and no consent to perform amniocentesis for this indication.

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

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**Classification of the main outcomes**

We selected a composite outcome defined by the presence of IAI and/or the occurrence of spontaneous delivery within 7 days.

IAI was defined as the presence of a positive amniotic fluid culture for aerobic (chocolate agar), anaerobic (Schaedler agar), yeasts (thioglycollate broth), genital mycoplasma (Mycoplasma IST 2, bioMérieux for *Ureaplasma* spp. or *Mycoplasma hominis)*, and/or by specific PCR amplification of the 16S ribosomal RNA gene[8].

Amniocentesis procedure was previously reported[18]. Briefly, the area of needle insertion should be planned. The selected largest vertical pocket should be located in a transverse view of the abdomen, avoiding peripheral pools near the uterine fundus, and ones near the lateral uterine walls. The image on the US screen should include the maternal abdominal skin. Whenever possible, a transplacental insertion should be avoided. Transplacental puncture is contraindicated in cases of alloimmunization or viral maternal infection by human immunodeficiency virus (HIV), hepatitis B virus (HBV) or hepatitis C virus (HCV). In obese patients, it is important to take into account the distance that the needle must travel towards the amniotic cavity, which may be estimated by ultrasound measurement previous to the puncture. An appropriate needle length (20–22 G) should be chosen based on this distance; 12-, 15- and 20-cm needles are available commercially, although operators must be aware that longer needles are prone to bending.

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Gestational age was established according to crown-rump length at the first-trimester US scan. Women who delivered because of maternal or fetal indications were censored.

**Vaginal fluid collection**

Vaginal fluid was collected using swabs submerged in 5.0 mL of sodium chloride (NaCl) and kept at 4ºC until processing. Vaginal fluid was centrifuged, ranging between 2,000-3,000 x g at 4ºC for 10 minutes. Supernatants and pellets were stored separately at -80ºC.

**Exploratory study using high-dimensional biology**

**Determination of amino acid concentrations using high-performance liquid chromatography (HPLC)**

Amino acid concentrations were analyzed with an ion exchange chromatography process using a Biochrom 30 amino acid analyzer (Pharmacia Biochrom Ltd, Cambridge, UK) as was previously described[15]. Data was normalized by total vaginal fluid protein concentration (measured by PierceTM BCA Protein Assay Kit, Thermo Scientific™, ref: 23225).

**DNA extraction, 16s ribosomal RNA gene amplification and sequencing**

DNA extraction from the vaginal swabs was performed using the Purelink Microbiome DNA Purification Kit (Invitrogen) according to the manufacturer's instructions. DNA concentrations were measured using a Qubit® 2.0 Fluorometer (Life Technology, Carlsbad, CA, USA).

The 16S rRNA amplicon sequencing was performed as described previously[13]. The DADA2 pipeline[19~~8~~] was used to achieve quality filtering, sequence joining and chimera removal. Then, taxonomic assignment, including species level classification, was performed using the Silva v132 database[20~~19~~]. Samples with less than 1,000 sequence reads were removed. Singletons and amplicon sequence variant level with a relative frequency <0.01% were also removed. The resulting taxonomical tables were used as Total Sum Scaling normalization at genus for further analysis and combination with the other measurements to build the mathematic models.

**Determination of protein concentrations using multiplex immunoassays (Luminex® Technology)**

We decided to investigate the independence[2][10-2][32] of metalloproteinase-8 (MMP-8), Interleukin (IL)- 1β, IL-6 and IL-8 to predict IAI and/or spontaneous delivery within 7 days. All samples were thawed and immediately centrifuged at 16,000-x g for 4 minutes. The total protein concentration was evaluated using PierceTM BCA Protein Assay Kit (Thermo Scientific™, ref: 23225). The samples were analyzed in duplicate and diluted as follows: 1/20 and 1/40 for MMP-8; 1/2 and 1/4 for IL-1β and IL-6; 1/10 and 1/20 for IL-8. The human MMP-8 Magnetic Luminex Performance Assay (LMPM908) was used for protein MMP-8 detection and the Magnetic Luminex® performance assay Human cytokine premixed kit A (FXTM 03-03) was used for cytokine IL-1β, IL-6 and IL-8 detection, both of which are manufactured by R&D systems™. Seven standards with a 1/3-dilution factor were used to perform the calibration curve from a stock solution of 2,100 pg/mL for IL-1β, 4,200 pg/mL for IL-6, 3,200 pg/mL for IL-8 and 65,000 pg/mL for MMP8. All procedures were performed following the manufacturer's recommendations. Total time to perform a Luminex assay following manufacturer protocol ranged between 4 and 5 hours.

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**Statistical analysis**

To investigate the independence to predict the composite outcome, for each biomarker we used a machine learning supervised Bayesian classification method (Sparse Bayesian learning[2][43]) and classified the women according to the values of the biomarkers retaining only those that obtained a minimum sensitivity of 50% at 70% specificity when used alone. Sensitivity and Specificity was measured in a stratified k-fold cross-validation scenario with K=5 and 10 random repetitions to incorporate variance estimation and avoid pitfalls due to random data separation.

**Development and validation study**

For the development and validation of the prediction models we included the cohort used in the exploratory analysis and a new cohort of women.

Based on exploratory analysis findings, the investigators (TC, EG) decided to include independent predictors for the composite outcome that could be tested using feasible techniques with rapid diagnosis. This is why they decided not to include amino acids evaluated by HPLC but to include vaginal pH (tested by specific pH-meter) and vaginal lactic acid (measured by Reflectoquant® System Lactic acid test, Merck Millipore) in the prediction models instead. This was based on the knowledge of the influence of *Lactobacillus* genus on vaginal pH and lactic acid production[24,5].

Finally, based on previously reports[24,5,26,27], we explored the independence of clinical variables such as gestational age at admission (weeks), US transvaginal cervical length measurement (mm), and maternal CRP concentrations (mg/L) as predictors of IAI and/or spontaneous delivery within 7 days.

Transvaginal cervical length was measured by experienced staff following Fetal Medicine Foundation guidelines (htto://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 furthest points at which the cervical walls were juxtaposed. Ultrasound cervical length was measured at least three times and the shortest measurement was recorded.

**Bacterial load of *Lactobacillus* spp. by targeted quantitative PCR**

qPCR amplification and detection were performed with specific primers targeted to the 16S region for *Lactobacillus* genus in each vaginal sample as described elsewhere[13]. Each reaction mixture of 20 µl was composed of KAPA Sybr Fast qPCR Kit (KAPA Biosystems), 0.4 µl of each primer (10 µM concentration) and 1 µl of template DNA in a LightCycler 480 Real-Time PCR System (Roche Technologies). All amplifications were performed in duplicate. The bacterial concentration in each sample was calculated by comparison with the Ct values obtained from a

standard curve and also, a negative control was included in each reaction plate. These were generated using serial 10-fold dilutions of genes. Data was normalized for total DNA concentrations (ng/µL) and presented in a logarithmic scale (log number copies gene/ng total DNA).

**Vaginal fluid IL-6 analysis using an automated Cobas e602 electrochemiluminescence immunoanalyzer**

Vaginal and amniotic fluid IL-6 concentrations were measured using an automated Cobas 801 electrochemiluminescence immunoanalyzer (Roche Diagnostics, Mannheim, Germany)[28,7].

**Determination of vaginal pH and lactic acid**

The RQflex 10 Reflectoquant® reflectometer (Merck Millipore, Burlington, Massachusetts, USA) was used for the lactate measurements. The test strips (Reflectoquant, Merck Millipore) for lactate have a range of 3–60 mg/L and undiluted samples were directly added to the strips and incubated according to the manufacturer's instructions.

pH was determined using a pH meter Basic 20 + (CRISON, Italy) with resolution of 0.01 pH and a micro-pH sensor (100ul, Hach).

**Sample size**

The overall cohort (N) was divided into derivation (Nder) and validation (Nval) cohorts. To establish Nval, we used sample size computation for single group mean[28] with a confidence level >95% ($p<0.05$) and two-sided margin of error <8% (beta>0.82). The last Nval women (according to their admission date) were used for validation and the (Nder = N - Nval) remaining women were left for derivation.

**Statistical analysis**

Statistical analysis was performed using Python (Python foundation, USA). The Shapiro Wilk test was initially used to assess continuous data for normality. We compared maternal characteristics and perinatal outcomes between the derivation and validation cohorts; continuous variables were compared using a non-parametric U-Mann-Whitney test presented as median with 95% Confidence interval. Categorical variables were compared using the Chi-squared or Fisher exact test. Differences were considered statistically significant with a $p<0.05$ with two-sided alternative hypotheses.

Using a machine learning supervised Bayesian classification method (Sparse Bayesian learning), we searched for a trade-off between prediction accuracy and model simplicity using as few input variables as possible. This was achieved by developing different models in the derivation cohort using different input variable combinations with different levels of complexity and retaining those whose accuracy in the validation cohort was deemed clinically useful.

From the probabilistic output of each model, the diagnostic performance was calculated using ROC curves. The AUC was reported and then the optimal cut-off threshold was selected as that maximizing accuracy and used to compute the F1-Score, sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (LR+), and negative likelihood ratio (LR-) for IAI and/or spontaneous delivery within 7 days in the validation cohort.

**Results**

During the study period (2015-2020), 389 women with a diagnosis of PTL were admitted but 288 were finally included (Figure 1). Biological samples of some of these women had previously been used in other studies[13,15].

One hundred and three (35%) women had IAI and/or spontaneously delivered in the following 7 days. Among 89 women delivered within 7 days, 43 had IAI. Finally among 53 women with IAI, 43 delivered before 7 days.

The gestational age at admission (median confidence interval (CI) 95%) was 28.6 (28.1-~~;~~28.9) weeks, the gestational age at delivery was 35 (34-35.8) weeks and the latency from admission to delivery was 36 (25-45) days. Ultrasound (US) transvaginal cervical length at admission was 13.5 (12-15) mm.

Microorganisms isolated in the amniotic fluid ~~with~~ and~~their~~ amniotic fluid interleukin (IL)-6 concentrations are presented in a supplementary appendix (Supplemental appendix S1).

Maternal characteristics and perinatal outcomes according to the occurrence of IAI and/or delivery within 7 days are shown in Table 1. We did not observe any complication related to the invasive procedure.

**Exploratory study**

In 120 women with PTL, we investigated the independence of vaginal amino acids, bacteria and proteins to predict IAI and/or spontaneous delivery within 7 days. There were differences in vaginal amino acids and vaginal proteins concentrations according to the occurrence of IAI and/or spontaneous delivery within 7 days (Supplemental appendix S2 and S3, respectively).

We used a machine learning supervised Bayesian classification method and retained only predictors that obtained a minimum sensitivity of 50% at 70% specificity. Thus, we found vaginal phenylalanine, taurine, serine, proline, vaginal *Lactobacillus*, *Ureaplasma*, *Finegoldia* genus and vaginal IL-6 to be good predictors of the composite outcome.

**Development and validation study**

Non-invasive prediction models were developed using Bayesian classification methods in a cross-validation scenario. Sample size computation established that at least n=172 women were needed to validate the predictor models with enough statistical reproducibility (confidence level >95%, two-sided margin of error <8%). Of the 288 women included, 116

(Nder) were selected for the derivation cohort and 172 (Nval), for the validation cohort, separated by the date of hospital admission.

Differences in the maternal characteristics and perinatal outcomes between women from the derivation and the validation cohorts are shown in Table 2.

According to the selected predictors and the direction of effects, we found 4 non-invasive prediction models for IAI and/or spontaneous delivery within 7 days (Table 3). Models 1 through 4 were ordered by their complexity (number of input variables used and their clinical readiness). Variables are previously normalized using the mean and std values (value_norm = (value-mean)/std), and final output confidence score is passed through a sigmoid ($y = 1 / (1+e^{-x})$).

The regression formula for model 1 was: (1.9094 * vaginal IL6) + (- 0.1795 * US cervical length/vaginal pH) + (0.1503 *maternal CPR/vaginal Lactobacillus genus) + (3.0867 *maternal CPR/ vaginal Lactobacillus genus).

The regression formula for model 2 was: (3.1253 * vaginal IL6) + (0.1953 *maternal CPR / vaginal lactic acid).

The regression formula for model 3 was: (0.5759 * maternal CPR) + (0.1555 * vaginal pH) + (2.6738 * vaginal IL6) + (-0.1516 * US cervical length/vaginal pH).

Finally, the regression formula for model 4 was: (-0.1008 * US cervical length) + (0.6078 * maternal CPR) + (2.7981 * vaginal IL6).

The diagnostic performance of these four different models for predicting IAI and/or spontaneous delivery within 7 days is shown in Figure 2~~1~~ (receiver operating characteristic [ROC] curves).

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All four models presented a similar overall performance, with an area under the ROC curve (AUC) ranging from 82.2% (±3.1 95% CI) to 85.2% (±3.1 95% CI) and F1-Scores ranging from 76.9% (±3.4 95% CI) to 78.4% (±3.5 95% CI).

**Comment**

**Main finding**

With the use of technologies feasible for point-of-care systems we developed non-invasive prediction models of IAI and/or early delivery with good accuracy. This information might help clinicians to select women with PTL at high risk of poor outcomes who might benefit from, among other interventions, hospital admission for close follow-up, or amniocentesis to rule in/out an IAI/inflammatory status.

**Results in the Context of What is Known**

We found vaginal IL-6 and maternal CRP to be independent predictors of the occurrence of IAI and/or spontaneous delivery within 7 days. Different authors have explored proteins in the cervico-vaginal fluid and/or maternal plasma as predictors of IAI and/or sPTD. Thus, Holst et al[11] proposed a good predictor model of IAI using cervical proteins such as IL-17 and monocyte chemotactic protein-1 (MCP-1). Kim et al[12] found nulliparity, cervico-vaginal L-8, MIP-1β and maternal CRP as predictors of IAI and/or spontaneous delivery before 48h. However, none of these studies validated the results as we did. To our knowledge, Coombs et al[10] were the only group who developed and validated different prediction models of IAI with acceptable accuracy using ELISA techniques. These models included a combination of cervico-vaginal IL-6, GRO-alpha (CXCL1), alpha-fetoprotein, and insulin-like growth factor binding protein 1. Despite their validation, they did not test their findings using techniques feasible for rapid diagnosis, thereby limiting their clinical use in the clinical setting.

Concerning the influence of the ascending microbial environment, we found an inverse relation between vaginal lactic acid concentration and vaginal *Lactobacillus* genus load and the occurrence of IAI and/or spontaneous delivery within 7 days. In addition, a higher vaginal pH

was observed in women with IAI and/or spontaneous delivery within 7 days. In this line, Hitti et al[30][29] reported a high expression of vaginal cytokines, an abnormal vaginal Gram stain, absence of hydrogen peroxide-producing *Lactobacillus* and the presence of anaerobic vaginal microbiota in the vaginal cultures of women with IAI. The main differences with our findings are that we sequenced the vaginal microbiota instead of performing cultures, and this allows diagnosing a multitude of microorganisms considered cultivable or not cultivable. In addition, we reproduced the same results using feasible targeted qPCR techniques thereby strengthening our findings. Recently, Chan et al[30][1] showed vaginal depletion of *Lactobacillus* species and high bacterial diversity leads to increased cervico-vaginal inflammatory markers such as IL-8, IL-6 and IL-1β and increased risk of sPTD. The strength of our study is that we found different models that act as a surrogate of not only early delivery but also the occurrence of IAI using a non-invasive approach.

**Clinical implications**

This study provides evidence supporting the development of point-of-care non-invasive systems to improve the clinical management of PTL. Discriminating high and low risk groups would allow efficient use of resources and targeted use of steroids and antibiotics, which when used indiscriminately may negatively affect long-term neurodevelopmental outcome[31][2],[32][3], but in selected cases may avoid serious complications[7].

To our knowledge, this is the first time that the vaginal microbial environment, its metabolome expression and vaginal inflammation mediated by proteins have been explored in the same cohort of women with PTL to predict the occurrence of IAI and/or early delivery. Moreover, this was done using feasible techniques that provide a rapid result and can be implemented in the clinical setting. These non-invasive models might help in the clinical-decision making to decide whether a woman with symptoms of PTL and intact membranes needs to be transferred to a referral center with neonatal intensive care units or not. In centers as ours that include the performance of an amniocentesis as part of the clinical management of women with PTL below 34 weeks, they constitute an alternative to the invasive procedure in the low-risk population of IAI and/or delivery within 7 days. Thus, they might help to optimize antenatal clinical management regarding hospital admission or antenatal steroids administration. However, we

believe these non-invasive models do not substitute the amniocentesis as gold standard of IAI in the high-risk group. Amniotic fluid microbiological information allows confirming IAI suspicion and to adjust antenatal antibiotic treatment and maternal and neonatal management according to the type of microorganism isolated. All four models showed good accuracy for use as screening tools to predict IAI and/or early delivery. All 4 models included vaginal IL-6 that can currently be tested using an automated immunoanalyzer, providing a highly reliable result in 18 minutes. Interestingly, model 4 only included 3 predictors, with maternal CRP and US transvaginal cervical length parameters already being implemented in most clinical settings for the management of PTL.

**Research implications**

Further research is warranted to prospectively evaluate the diagnostic performance of the different prediction models after clinical implementation in cohorts different from ours.

**Strengths and limitations**

The strengths were that the models were validated in an independent cohort and that diagnosis of IAI was based on microbial cultures or PCR targeting the 16S ribosomal RNA gene sequence.

As limitations of this study, we acknowledge that biomarkers were tested from frozen samples. In addition, it was not designed to evaluate whether our prediction models improve perinatal outcomes.

**Conclusion**

We report the use of high-dimensional biology provided by –omics approaches to develop non-invasive approaches for rapid testing prediction models of poor outcomes in women with PTL.

**Tables**

**Table 1** Maternal and perinatal outcomes according to the occurrence of IAI and/or spontaneous delivery within 7 days

**Table 2** Maternal and perinatal outcomes of the women in the derivation and validation cohorts

**Table 3** Diagnostic performances of different machine learning predictor models based on the occurrence of IAI and/or spontaneous delivery within 7 days after admission

## Figures

**Figure 1** Flow-chart of entire study population

**Figure 2** Full receiver operating characteristic (ROC) curves of prediction machine learning models

## Supplementary Materials

S1 Microorganisms isolated in the amniotic fluid

S2 Vaginal amino acid concentrations according to the occurrence of IAI and/or spontaneous delivery within 7 days after admission

S3 Protein concentrations according to the occurrence of IAI and/or spontaneous delivery within 7 days

## Author contributions

Conceptualization: TC, EG

Methodology: MCC, MC, SM, XF, VA, XPBA, JB

Investigation: TC, SF, MP, ABS, DB, CM, CR, JP

Funding acquisition: TC

Supervision: TC, EG

Writing – original draft: TC, XPBA

Writing – review & editing: TC, EG

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**Con formato:** Sin Resaltar

**Con formato:** Español (España)

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**Table 1 Maternal and perinatal outcomes according to the occurrence of IAI and/or spontaneous delivery within 7 days**

| | IAI and/or delivery ≤7d n=103 | No-IAI/delivery > 7d n=185 | *p* |
|---|---|---|---|
| Maternal age | 33.3 (32.4- 34.2) | 31.6 (29.4- 33.5) | 0.0558 |
| BMI | 23.5 (22.5-25.1) | 22.4 (21.6-22.8) | 0.0411 |
| Caucasian ethnicity | 69 (67) | 131 (71) | 0.652 |
| Nulliparity | 62 (60) | 108 (58) | 0.764 |
| Smoking | 1 (0.97) | 2 (1.08) | 0.936 |
| Conization | 2 (1.9) | 2 (1.08) | 0.550 |
| Uterine malformation | 2 (1.9) | 2 (1.08) | 0.550 |
| Maternal disease | 41 (39.8) | 87 (47) | 0.237 |
| US Cervical length (mm) | 12 (8- 15) | 15 (13- 15) | 0.0024 |
| Maternal C-reactive protein (mg/L) | 1.9 (1.24- 2.6) | 0.49 (0.36- 0.6) | <0.0001 |
| Maternal white blood cells (x$10^9$/L) | 14480 (13800- 15838) | 11700 (11152- 12366) | <0.0001 |
| Gestational age at admission (weeks) | 28.1 (26.9- 28.9) | 28.7 (28.1- 29.3) | 0.3508 |
| Gestational age at amniocentesis | 28.1 (26.9- 28.9) | 28.7 (28.2- 29.3) | 0.3592 |
| Amniotic fluid IL-6 (ng/mL) | 67.53 (35.6- 127.2) | 1.09 (0.96- 1.35) | <0.0001 |
| Vaginal pH (absolute value) | 5.52 (5.32- 5.76) | 4.93 (4.74- 5.13) | <0.0001 |
| Vaginal IL-6 (pg/mL) | 965 (598.4- 1322.9) | 27 (18- 41.9) | <0.0001 |
| Vaginal lactic acid (mg/L) | 94 (64.3- 115.6) | 118 (107.3- 131) | 0.0459 |
| Vaginal *Lactobacillus* genus (log number copies gene/ng total DNA) | 6.39 (6.04- 6.57) | 6.65 (6.53-6.77) | 0.0347 |
| Gestational age at delivery (weeks) | 28.7 (27.3- 29.7) | 38 (37.4- 38.3) | <0.0001 |
| Latency to delivery (days) | 2 (1.1- 3) | 60 (52.5- 65) | <0.0001 |
| Clinical chorioamnionitis at delivery | 48/100 (48) | 10/171 (5.8) | <0.0001 |

Continuous variables were compared using a nonparametric Mann Whitney U test presented as medians (95% confidence interval). Categorical variables were compared using Chi-square or Fisher exact tests and presented as number (%).

**Table 2 Maternal and perinatal outcomes of the women in the derivation and validation cohorts**

| | Derivation cohort N=116 | Validation cohort N=172 | *p* |
|---|---|---|---|
| Maternal age | 32.9 (29.5-35.5) | 32.5 (31.5- 33.6) | 0.6015 |
| BMI | 22.8 (21.9-23.5) | 22.5 (21.7-23.3) | 0.098 |
| Ethnicity | | | 0.001 |
| -Caucasian | 82 (71) | 118 (68.6) | |
| -~~Magreb~~Maghreb | ~~18 (15.5)~~ | ~~18 (10.4)~~ | |
| ~~-Hispanic~~ | ~~4 (3.4)~~ | ~~25 (14.5)~~ | |
| ~~-Afrocaribbean~~ | ~~1 (0.86)~~ | ~~2 (1.16)~~ | |
| ~~-Sudasia~~ | ~~2 (1.72)~~ | ~~8 (4.65)~~ | |
| ~~-Africa~~ | ~~9 (7.75)~~ | ~~1 (0.58)~~ | |
| -Hispanic | 18 (15.5) | 18 (10.4) | |
| -Afrocaribbean | 4 (3.4) | 25 (14.5) | |
| -Sudasia | 1 (0.86) | 2 (1.16) | |
| -Africa | 2 (1.72) | 8 (4.65) | |
| | 9 (7.75) | 1 (0.58) | |
| Nulliparity | 68 (59) | 102 (59) | 0.908 |
| Smoking | 3 (2.6) | 0 | 0.033 |
| Conization | 0 | 4 (2.3) | 0.098 |
| Uterine malformation | 4 (3.4) | 0 | 0.014 |

**Con formato:** Ninguno, Espacio Antes: 0 pto, No conservar con el siguiente, No conservar líneas juntas

**Con formato:** Ninguno, Espacio Antes: 0 pto, No conservar con el siguiente, No conservar líneas juntas

**Con formato:** Ninguno, Espacio Antes: 0 pto, No conservar con el siguiente, No conservar líneas juntas

| | | | |
|---|---|---|---|
| Maternal disease | 49 (42.2) | 79 (46) | 0.537 |
| Cervical length (mm) | 15 (11.9- 15) | 13 (12- 15) | 0.9349 |
| Maternal C-reactive protein (mg/L) | 0.74 (0.52- 0.92) | 0.79 (0.55- 1.02) | 0.6325 |
| Maternal white blood cells ($x10^9$/L) | 12315 (11430- 12703) | 12800 (12112- 13920) | 0.1676 |
| Gestational age at admission (weeks) | 28.1 (26.9- 28.7) | 28.9 (28.1- 29.6) | 0.0778 |
| Gestational age at amniocentesis | 28.2 (26.9- 28.7) | 28.9 (28.1- 29.6) | 0.0468 |
| Amniotic fluid IL-6 (ng/mL) | 1.5 (1.2- 4.5) | 4.3 (1.6- 9.8) | 0.4521 |
| IAI | 20 (17) | 33 (19) | 0.676 |
| Vaginal IL-6 (pg/mL) | 102.5 (79.2- 228.3) | 36.5 (22- 79.5) | 0.0220 |
| Vaginal pH (absolute value) | 5.09 (4.69- 5.26) | 5.23 (5.06- 5.38) | 0.2828 |
| Vaginal lactic acid (mg/L) | 112.5 (85.9- 136.04) | 114.5 (94.6- 123.4) | 0.8332 |
| Vaginal *Lactobacillus* genus (log number copies gene/ng total DNA) | 6.61 (6.09- 6.86) | 6.56 (6.42-6.65) | 0.8219 |
| Gestational age at delivery (weeks) | 37.3 (35.3- 38) | 33.4 (31.8- 34.8) | 0.0008 |
| IAI and/or spontaneous delivery ≤7d | 32 (28) | 71 (41) | 0.017 |
| Spontaneous delivery ≤7 d | 28/112 (25) | 61/167 (37) | 0.043 |

Continuous variables were compared using a nonparametric Mann Whitney U test presented as medians (95% Confidence interval). Categorical variables were compared using Chi-square or Fisher exact tests and presented as number (%).

**Table 3 Diagnostic performances of different machine learning predictor models based on the occurrence of IAI and/or spontaneous delivery within 7 days after admission**

| | IAI and/or spontaneous delivery ≤ 7 days | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Number of variables | Predictors used | AUC (95% CI) | F1 (95% CI) | Sensitivity n (%) | Specificity n (%) | PPV n (%) | NPV n (%) | LR+ | LR- |
| Model 1 | 4 | IL-6, ~~CPR~~CRP, lactic acid, *Lactobacillus* genus | 85.2(+-3.1) | 78.4(+-3.5) | 84.5 | 78.2 | 73.2 | 87.8 | 3.88 | 0.2 |
| Model 2 | 3 | IL-6, ~~CPR~~CRP, lactic acid | 84.7(+-3.0) | 77.7(+-3.2) | 85.9 | 75.2 | 70.9 | 88.4 | 3.47 | 0.19 |
| Model 3 | 4 | IL-6, ~~CPR~~CRP, pH, cervical length | 83.3(+-3.1) | 77.1(+-3.8) | 76.1 | 85.1 | 78.3 | 83.5 | 5.12 | 0.28 |
| Model 4 | 3 | IL-6, ~~CPR~~CRP, cervical length | 82.2(+-3.1) | 76.9(+-3.4) | 84.5 | 75.2 | 70.6 | 87.4 | 3.41 | 0.21 |

Vaginal IL-6 (pg/mL), US Cervical length (mm), maternal C-reactive protein (CRP) concentrations (mg/L), vaginal pH as absolute value; vaginal lactic acid as mg/L; vaginal *Lactobacillus* genus as log number copies gene/ng total DNA. AUC: Area under curve; F1: F1-score; PPV: Positive predictive value; NPV: Negative predictive value; LR: Likelihood ratio; IAI: Intra-amniotic infection.

**Figure 1 Flow-chart of entire study population**

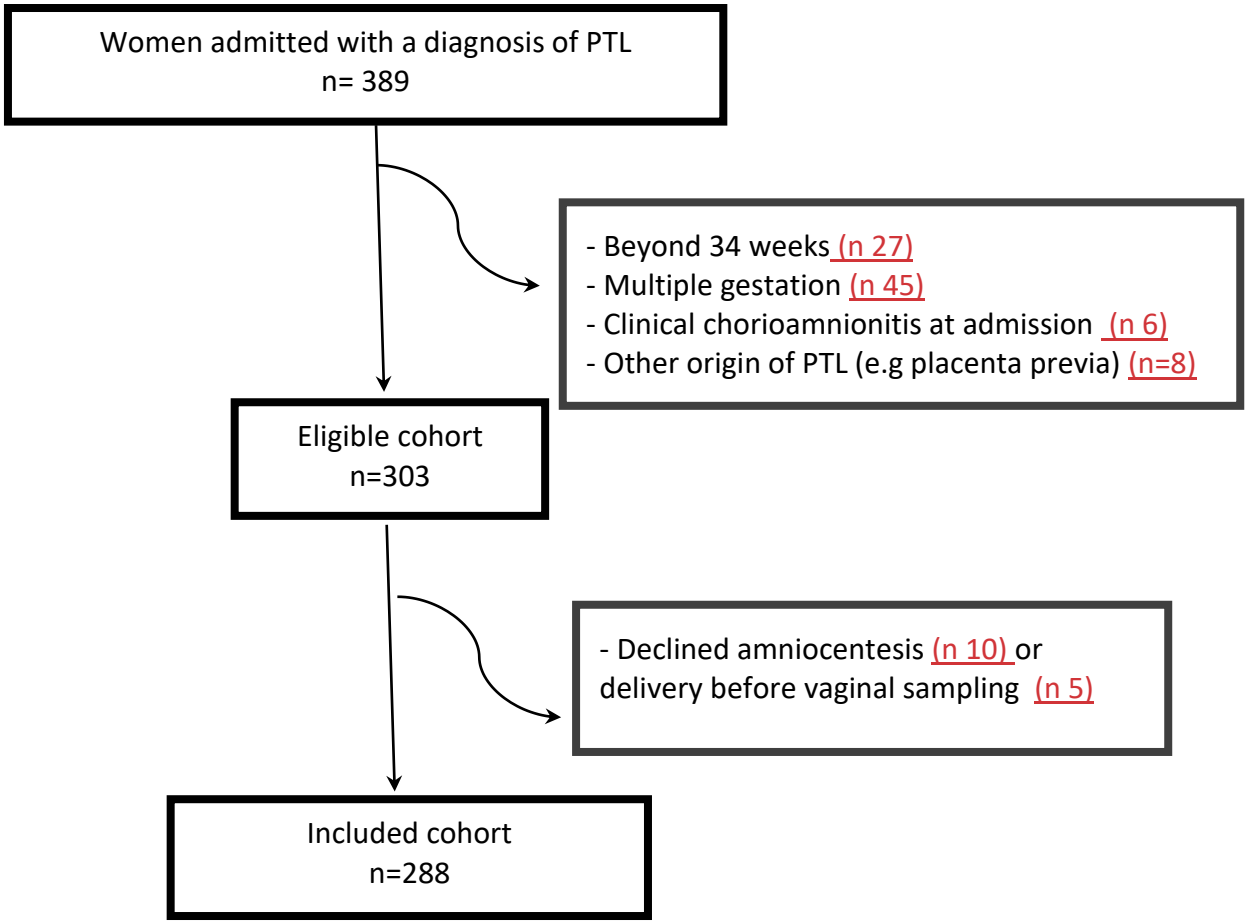

**Figure 2 Full receiver operating characteristic (ROC) curves of prediction machine learning models**