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# Predictive value of vaginal IL-6 and TNFα bedside tests repeated until delivery for the prediction of maternal-fetal infection in cases of premature rupture of membranes

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ABSTRACT

*Objective:* Examine the predictive value for maternal-fetal infection of routine bedside tests detecting the proinflammatory cytokines, TNFα and IL-6, in the vaginal secretions of women with premature rupture of the membranes (PROM).
*Study design:* This prospective two-center cohort study included all women hospitalized for PROM over a 2-year period. A bedside test assessed IL-6 and TNFα in vaginal secretions. Both centers routinely tested CRP and leukocytes, assaying both in maternal serum, and analyzed vaginal bacterial flora; all samples were repeated twice weekly until delivery.
*Results:* The study included 689 women. In cases of preterm PROM (PPROM) before 37 weeks (n = 184), a vaginal sample positive for one or more bacteria was the only marker associated with early neonatal infection (OR 5.6, 95%CI; 2.0–15.7). Its sensitivity was 82% (95%CI; 62–94) and its specificity 56% (95%CI; 47–65). All positive markers of infection were associated with the occurrence of chorioamnionitis. In cases of PROM from 37 weeks onward (n = 505), only CRP >5 mg/dL was associated with early neonatal infection (OR = 8.3, 95%CI; 1.1–65.4) or clinical chorioamnionitis (OR = 6.8, 95%CI; 1.5–30.0). The sensitivity of CRP >5 mg/dL was 91% (95%CI; 59–100) and its specificity 45% (95%CI; 40–51) for predicting early neonatal infection, and 89% (95%CI; 65–99) and 46% (95%CI; 41–51), respectively, for predicting clinical chorioamnionitis.
*Conclusion:* The association of vaginal cytokines with maternal-fetal infection is weak and thus prevents their use as a good predictor of maternal-fetal infection. CRP and vaginal samples may be useful for identifying a group of women at low risk of infection.



## Introduction

Premature rupture of the membranes (PROM) occurs in 10.5% of pregnancies and is the principal risk factor for maternal-fetal infection, which is associated with increased neurological morbidity and even neonatal death [1]. In preterm births, the cerebral and pulmonary vulnerability of fetuses with major inflammation strongly increases the risk of sequelae [2–4]. The risk of infection determines maternal management and raises the question, especially after 32–34 weeks of gestation, of immediate delivery (induction of labor or cesarean) to minimize fetal risks, thus increasing prematurity-related neonatal morbidity [5]. Moreover, depending on the obstetric context (previous cesarean), it may increase the risk of cesarean delivery.

Although numerous biomarkers of infection have been studied to assess the risk of maternal and fetal infection in cases of PROM

*Abbreviations:* CI, Confidence interval; CRP, C reactive protein; IL-6, Interleukin 6; OR, Odds ratio; PROM, Preterm rupture of membranes; TNF, Tumor necrosis factor.

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[6], there is no accurate test to predict maternal-fetal infection. The markers most frequently studied, because they are simple, inexpensive, and widely used, are C-reactive protein (CRP), and maternal leukocytosis [7,8]. These assessments have generally been small retrospective series, and the varying intervals between sampling and delivery have made it difficult to interpret their results [9].

The use of proinflammatory cytokines such as interleukin-6 (IL-6) and tumor necrosis factor-α (TNFα) has been studied over the past 15 years in the hope of improving the detection of infection in PROM. The quantitative thresholds used for these tests and their predictiveness for maternal-fetal infection in maternal serum, vaginal secretions, and amniotic fluid vary widely [10,11]. Moreover the use of ELISA testing complicates transposition to clinical practice. For these reasons, we developed, as previously reported, a bedside immunochromatographic test to detect IL-6 in vaginal secretions [12]. The next stage of its development had two steps: first, to include another cytokine, TNFα, to attempt to improve this risk prediction by combining cytokines, and, second, to assess this test in a larger population of women with PROM.

The principal objective of this study was to examine the predictive value for maternal-fetal infection of routine bedside tests that detect the proinflammatory cytokines TNFα and IL-6 in the vaginal secretions of women with premature rupture of the membranes (PROM). The secondary objective was to compare these values to those of the standard markers in routine use.

## Materials and methods

This prospective, two-center cohort study took place between January 2004 and February 2006 in two university hospital centers and was approved by the Patient Protection Committee of Ile-de-France (CRC 03134).

Women were eligible if they provided informed consent and were admitted for PROM after 24 weeks and were not in labor in the 12 h that followed. Women with multiple pregnancies, those younger than 18 years, and those who did not speak French were not included.

As recommended, the diagnosis of rupture of the membrane was based on maternal history (including the exact time of amniotic fluid loss) and sterile speculum examination completed by a para-clinical test of diagnosis if necessary [13].

It was an observational study only and management of PROM was left to the discretion of the physician. French protocols after 34 weeks gestation recommend antibiotics and labor induction in cases of colonization by *Streptococcus agalactiae*.

### Factors studied

Leukocytosis and CRP were determined twice a week, along with bacterial analysis of the vaginal flora. The vaginal samples were taken on simple swabs (Dacron® swab) for bacteriological examination (direct examination and culturing). The vaginal sample was considered positive if the culture identified one or more of the following bacteria, which are the most frequently responsible for maternal-fetal infection [14]: *Escherichia coli, Streptococcus agalactiae, Enterococcus*, or other Gram-negative bacilli or gram-positive cocci.

The IL-6 test was the qualitative immunochromatographic bedside test developed and previously reported by our team [12]. The bedside test for TNFα was based on the same principle; it used an anti-TNFα monoclonal antibody combined with a marker (clone

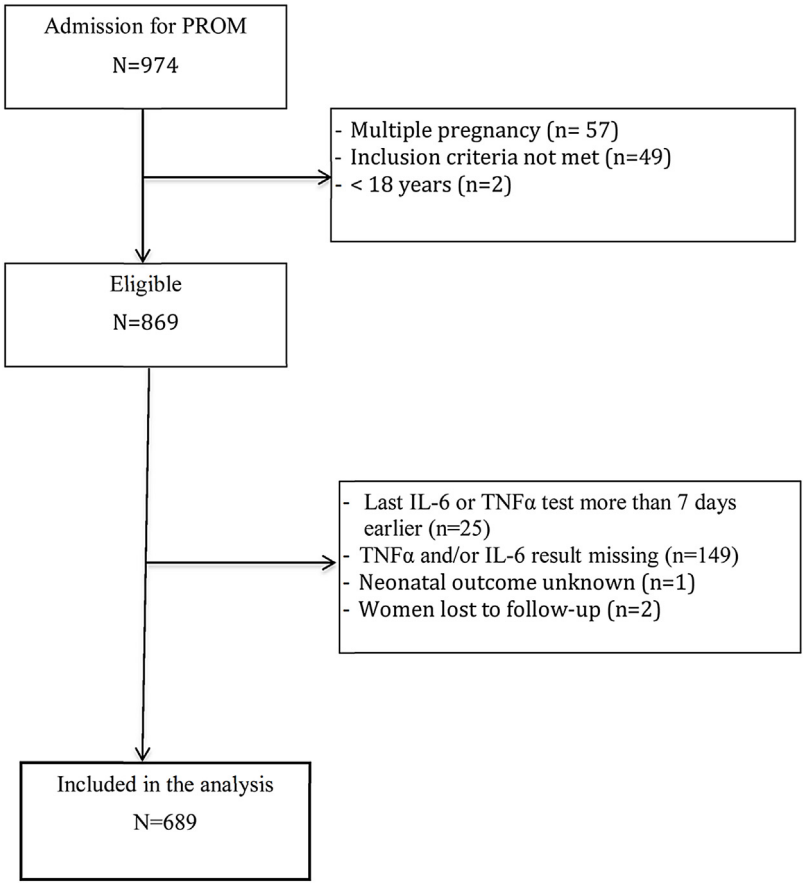


**Fig. 1.** Flow chart.

B-C7) and a fixed antibody that recognized the anti-TNFα antibody (clone B-F7). The sensitivity studies showed that the threshold of positivity was 50 μg/L for both IL-6 and TNFα. The results of the qualitative tests for both cytokines were treated as dichotomous variables: positive or negative.

A nurse performed and read the results of the bedside test in the labor ward in real time. The obstetric team was blinded to IL-6 and TNFα results but not to the routine markers of infection (CRP, leukocytosis, and bacteriological results of vaginal samples).

The other factors studied were maternal individual and obstetric characteristics, gestational age at PROM, time since PROM, prescription of antibiotics at admission, type of management (active or expectant, as defined above).

## Variables of interest

Early neonatal infection was defined by infection occurring within 72 h of birth. The final diagnosis of neonatal sepsis was reassessed after analysis of the neonate's clinical course and laboratory findings through discharge by a pediatrician unaware of the IL-6 and TNFα test results.

Clinical chorioamnionitis was defined by a maternal temperature greater than 38 °C, associated with uterine contractions or fetal tachycardia greater than 160 bpm and the absence of any other cause of hyperthermia.

Histological chorioamnionitis was defined as the more or less linear aggregation along tissue planes of neutrophil polymorphs of maternal origin in the subchorionic fibrin, chorion or amnion of the peripheral membranes, or the fetal plate of the placenta.

We assessed also the following factors: death in the delivery room; transfer to NICU, neonatal neurological complications (periventricular leukomalacia, defined either by hyperechoic cerebral lesions persisting past 14 days of life or by hypoechoic lesions and/or the persistence of cerebral hyperechogenicity, all grouped together as white matter diseases); and intraventricular hemorrhage, diagnosed by transfontanellar ultrasound and classified into 4 grades (Papile's criteria) [15]. Respiratory complications were also recorded (bronchopulmonary dysplasia, defined by oxygen dependence at 28 days of life and after 36 weeks) [16], as well as gastrointestinal complications (necrotizing enterocolitis stage ≥ 2, by Bell's criteria) [17].

## Statistical analysis

We considered only the results of samples taken in the 7 days before delivery to preserve the potential temporal relation between the test results and outcomes. Chi-squared or Fisher's exact tests were used to compare categorical variables. We constructed Receiver Operating Characteristics (ROC) curves for the continuous variables used to predict clinical chorioamnionitis or neonatal infection (CRP and leukocytes) and calculated the areas

**Table 1**
Characteristics of women admitted for premature rupture of the membranes.

| | PPROM <37 WG n = 184 | PROM ≥37 WG n = 505 |
|---|---|---|
| Maternal characteristics and outcomes | | |
| Region of birth | | |
| France/Europe | 91 (51) | 331 (66.1) |
| North Africa | 25 (14) | 59 (11.8) |
| Sub–Saharan Africa | 34 (18.9) | 57 (11.4) |
| Other | 29 (16.7) | 54 (10.8) |
| Gestational age at PROM (Weeks, med, complete range) | 31 (23–36) | 39.3 (37.0–41.6) |
| Interval between PROM and delivery | | |
| <24hours | 32 (17.4) | 155 (30.7) |
| 1–7 days | 105 (57) | 348 (68.9) |
| >7 days | 47 (25.5) | 2 (0.4) |
| Nulliparas | 109 (59.2) | 226 (45.0) |
| Induction of labor | 24 (13.1) | 134 (26.6) |
| Mode of delivery Vaginal delivery | 113 (61.4) | 402 (79.6) |
| Cesarean during labor | 33 (18.0) | 90 (17.8) |
| Cesarean before labor | 38 (20.7) | 13 (2.6) |
| Clinical chorioamnionitis | 34 (18.5) | 20 (4.0) |
| Histological chorioamnionitis | | |
| Yes | 90 (59.6) | 83 (22.1) |
| No | 61 (40.4) | 293 (77.9) |
| Gestational age at birth | | |
| 24–32 | 78 (42.4) | 0 |
| 33–36 | 95 (51.6) | 0 |
| ≥37 | 11 (6.0) | 505 (100) |
| Neonatal Outcomes | | |
| Birth weight, (grams, median, complete range) | 2065 (530–3780) | 3320 (1730–4960) |
| Transfert in Intensive Care Unit | 100 (54.4) | 10 (2.0) |
| Probable or certain neonatal infection | 29 (15.8) | 16 (3.2) |
| Intra-utero fetal death | 2 (1.1) | 0 |
| Neonatal death | 6 (3.3) | 0 |
| Bronchopulmonary dysplasia (28 d)[a] | 22 (12.5) | 0 |
| Necrotizing enterocolitis [b] | 2 (1.1) | 0 |
| Intraventricular hemorrhage grade 1/2[b] | 31 (17.0) | 1 (0.2) |
| Intraventricular hemorrhage grade 3/4[b] | 2 (1.1) | 0 |
| Periventricular leukomalacia[b] | 8 (4.3) | 1 (0.2) |

All percentage are for available data (rate of missing data <2% except for histological chorioamnionitis); $p < 0{,}01$ between the two groups for all factors studied except for intra-utero fetal death, necrotizing enterocolitis and intraventricular hemorrhage grade 3/4 (fisher exact test, p = 0.07); a: exclusion of in utero and neonatal death; b: exclusion of in utero fetal death.

under the curve (AUC) with Delong and Clark-Pearson's algorithm; CRP and leukocyte levels were then studied as dichotomous, by choosing a sensitivity of 85% for both to optimize the detection of true positives.

The associations and predictive values of each marker with neonatal infection and clinical chorioamnionitis were measured separately for the births before 37 weeks and those at 37 weeks or more (because the relation between gestational age and maternal-fetal infection was not linear).

We used STATA SE software v. 12.1 (College Station, TX) for the statistical analyses.

## Results

The analysis covered 689 women of whom 184 had a PPROM (before 37 weeks' gestation) and 505 a PROM from 37 weeks onward (Fig. 1). Table 1 summarizes their social and demographic characteristics and obstetric and neonatal outcomes. The two groups differed for all characteristics considered and all outcomes. Specifically, women who had a PPROM were less frequently from France or Europe, had a longer interval between membrane rupture and delivery, and had a higher rate of clinical or histological chorioamnionitis. Similarly neonates born to women with PPROM had a higher rate of neonatal infection.

The associations between the quantitative variables (CRP and leukocytes) and neonatal infection or clinical chorioamnionitis were studied with ROC curves (Fig. 1). The area under the curve for CRP was not significantly greater than that for leukocytosis (AUC = 0.8 (0.8–0.9) vs 0.7 (0.6–0.7), $P = 0.07$). To compare the standard markers, we chose thresholds of 5 mg/dL for CRP and 9300 g/L for leukocytes, both corresponding to a sensitivity around 85% for the overall study population (Fig. 2). The association between the different markers and either early neonatal infection or clinical chorioamnionitis was then studied for each gestational-age stratum (before 37 weeks, Table 2a; at and after 37 weeks, Table 2b).

Before 37 weeks (n = 184), a vaginal sample positive for one or more bacteria was the only factor associated with a neonatal infection (OR 5.6, 95%CI; 2.0–15.7). However, all the markers studied except leukocytes were associated with clinical chorioamnionitis before 37 weeks (Table 2a).

At and after 37 weeks, CRP >5 mg/dL was the only marker associated with a neonatal infection (aOR = 8.3, 95%CI; 1.1–65.4) or clinical chorioamnionitis (aOR = 6.8, 95%CI; 1.5-30.0) (Table 2b).

Results of the histological examination of the placenta were available for 527 women. IL6 and TNFα were associated with histological chorioamnionitis before but not after 37 weeks (supplemental tables S1a and S1b).

Before 37 weeks: 1) a bacteria-positive vaginal sample had a sensitivity of 82% (95%CI; 62–94) and a specificity of 56% (95%CI; 47–65) and provided the best compromise between sensitivity and specificity for predicting neonatal infection among the markers studied; 2) an IL–6 + sample had a sensitivity of 77% (95%CI; 59–89) and a specificity of 54% (95%CI; 45–62) for predicting clinical chorioamnionitis; TNFα + had a sensitivity of 47% (95%CI; 30–65) and a specificity of 81% (95%CI; 73–87); 3) Combining factors did not improve predictive values for neonatal infection substantially: CRP >5 mg/dl and vaginal sample + together predicted neonatal infection with sensitivity, specificity, PPV, and NPV of respectively 54, 80, 18, and 96%. Similarly, positive results for TNFα and IL-6 predicted neonatal infection with sensitivity, specificity, PPV, and NPV of respectively 24, 87, 12, and 94%.

At and after 37 weeks, the sensitivity of CRP >5 mg/L was 91% (95%CI; 59–100) and its specificity 45% (95%CI; 40–51) for predicting neonatal infection, and 89% (95%CI, 65–99) and 46% (95%CI; 41–51), respectively, for clinical chorioamnionitis (Table 3). Overall, both CRP >5 mg/dL and leukocytes >9300/mL had good negative predictive values of around 90%.

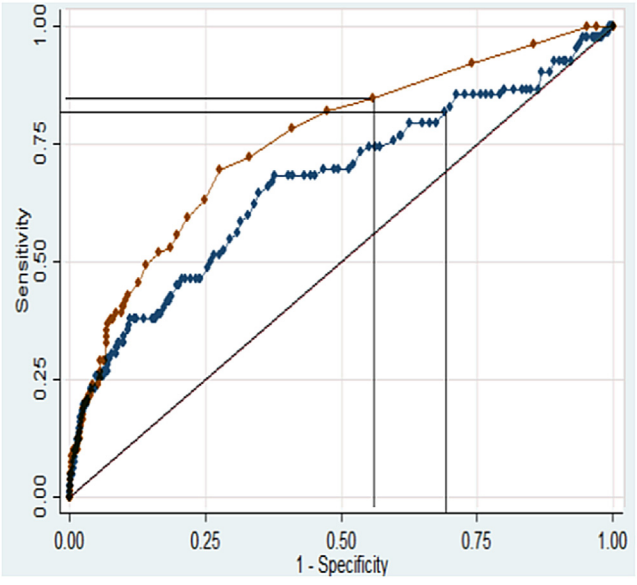


**Fig. 2.** ROC curve assessing the prediction of neonatal infection by maternal CRP (brown) and leukocytes (blue). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

## Comment

The association of vaginal cytokines with maternal-fetal infection is weak and does not allow their use as a good predictor of maternal-fetal infection. CRP and vaginal samples may be useful for identifying a group of women at low risk of infection who may benefit from expectant management when this is needed.

The study was prospective and enabled us to collect exhaustive information intended specifically to answer the questions in this study. Another strength of this work was the repetition of sampling throughout management, from admission to delivery. This procedure enabled us to use only samples taken in the seven days before delivery and thus ensured a consistent temporal relation between the markers and the onset of the infection. The short median interval between PROM and delivery, in particular from 37 weeks onward, is explained by the occurrence of PROM at and after 37 weeks for 75% of the women (median interval between PROM and delivery at and after 37 weeks: 1 day, range 0–15).

One noticeable methodological point is that the threshold used for CRP and leukocytes were chosen based on ROC curves calculated from the entire population and not before or at and after 37 weeks. We used this procedure because we made the choice to use the same cutoff for all analyses to make them more understandable and useful. We stratified the analysis (birth before or at and after 37 weeks) because of the possibility that medical practices and management might differ as a function of gestational age and thereby modify the association between the markers and maternal-fetal infection.

There are however two main methodological issues. The obstetric team knew the routinely used laboratory results (CRP, leukocytes, and analysis of vaginal bacterial flora) and could use this information to diagnose clinical chorioamnionitis. The consequences of the availability of this information on the predictive values are unclear. It might have artificially increased the predictive value of CRP for clinical chorioamnionitis, or,

**Table 2a**
Association between markers of infection and neonatal infection or clinical chorioamnionitis in cases of PPROM before 37 weeks.

| PPROM< 37 weeks (n = 184) | Neonatal infection (n = 29) | | | Clinical chorioamnionitis (n = 34) | | |
|---|---|---|---|---|---|---|
| | n | (%) | crude OR | n | (%) | crude OR |
| IL-6 positive | 17 | (59) | 1.4 [0.6–3.1] | 26 | (76) | 3.8 [1.6–9.0]* |
| TNFα positive | 10 | (34) | 1.8 [0.8–4.2] | 16 | (47) | 3.7 [1.7–8.2]* |
| Leukocytes >9300 g/L | 27 | (93) | 2.7 [0.6–12.2] | 32 | (94) | 3.4 [0.8–15.0] |
| CRP > 5 mg/dL | 23 | (79) | 1.7 [0.7–4.6] | 29 | (85) | 2.9 [1.0–7.9]* |
| Bacteria-positive vaginal sample | 22 | (81) | 5.6 [2.0–15.7]* | 22 | (67) | 2.3 [1.0–5.2]* |

*$P < 0.05$, missing data: vaginal sample (10%), CRP (8%), leukocytes (1%); bacterial species if positive (n = 80): *Streptococcus agalactiae* (28%), *Escherichia coli* (45%), other Gram-negative bacilli (25%), other Gram-positive cocci (19%).

**Table 2b**
Association between infectious markers and neonatal infection or clinical chorioamnionitis in cases of PROM at or after 37 weeks.

| PROM≥ 37 weeks (n = 505) | Neonatal infection (n = 16) | | | Clinical chorioamnionitis (n = 20) | | |
|---|---|---|---|---|---|---|
| | n | (%) | crude OR | n | (%) | crude OR |
| IL-6 positive | 6 | (38) | 0.9 [0.3–2.6] | 8 | (40) | 1.0 [0.4–2.6] |
| TNFα positive | 3 | (19) | 1.4 [0.4–4.9] | 3 | (15) | 1.0 [0.3–3.6] |
| Leukocytes >9300 g/L | 8 | (62) | 0.8 [0.3–2.5] | 15 | (79) | 2.0 [0.6–6.1] |
| CRP >5 mg/dL | 10 | (91) | 8.3 [1.1–65.4]* | 16 | (89) | 6.8 [1.5–30.0]* |
| Bacteria-positive vaginal sample | 8 | (53) | 2.6 [0.9–7.2] | 7 | (35) | 1.2 [0.5–3.0] |

*$P < 0.05$, missing data: vaginal sample (4%), CRP (27%), leukocytes (18%); bacterial species if positive (n = 157): *Streptococcus agalactiae* (48%), *Escherichia coli* (26%), other Gram-negative bacilli (19%), other Gram-positive cocci (18%).

**Table 3**
Predictive values of markers of infection studied for neonatal infection or clinical chorioamnionitis.

| | | IL-6 + | TNFα + | Leukocytes >9300 g/L) | CRP (>5 mg/dL) | Vaginal sample + |
|---|---|---|---|---|---|---|
| PPROM<37 weeks (n = 184) | | | | | | |
| Neonatal infection | Se% (95% CI) | 59% (39–77) | 35% (18–54) | 93% (77–99) | 79% (60–92) | 82% (62–94) |
| | Sp% (95% CI) | 49% (41–58) | 77% (69–84) | 17% (11–24) | 31% (24–40) | 56% (47–65) |
| | PPV% (CI 95%) | 19% (11–29) | 23% (12–39) | 19% (13–26) | 20% (13–28) | 28% (18–39) |
| | NPV% (95% CI) | 86% (76–92) | 85% (78–91) | 92% (75–99) | 88% (75–95) | 94% (86–98) |
| Clinical chorioamnionitis | Se% (95% CI) | 77% (59–89) | 47% (30–65) | 94% (80–99) | 85% (69–95) | 67% (48–82) |
| | Sp% (95% CI) | 54% (45–62) | 81% (73–87) | 17% (12–25) | 33% (25–42) | 54% (45–63) |
| | PPV% (95% CI) | 29% (20–39) | 37% (23–53) | 22% (16–30) | 25% (17–33) | 28% (18–39) |
| | NPV% (95% CI) | 90% (82–96) | 86% (79–92) | 92% (75–99) | 90% (78–97) | 86% (77–93) |
| PPROM>37 weeks (n = 505) | | | | | | |
| Neonatal infection | Se% (95% CI) | 38% (15–65) | 19% (4–46) | 62% (32–86) | 91% (59–100) | 53% (27–79) |
| | Sp% (95% CI) | 61% (56–65) | 86% (82–89) | 34% (29–39) | 45% (40–51) | 69% (65–73) |
| | PPV% (95% CI) | 3% (1–6) | 4% (1–11] | 3% (1–6) | 5% (2–9) | 5% (2–10) |
| | NPV% (95% CI) | 97% (94–99) | 97% (95–98) | 97% (92–99) | 99% (97–100) | 98% (96–99) |
| Clinical chorioamnionitis | Se% (95% CI) | 40% (19–64) | 15% (3–38) | 79% (54–94) | 89% (65–99) | 35% (15–59) |
| | Sp% (95% CI) | 61% (56–65) | 86% (82–89) | 35% (30–39) | 46% (41–51) | 69% (64–73) |
| | PPV% (95% CI) | 4% (2–8) | 4% (1–11) | 5% (3–9) | 8% (4–12) | 5% (2–9) |
| | NPV% (95% CI) | 96% (94–98) | 96% (94–98) | 97% (93–99) | 99% (96–100) | 96% (94–98) |

Se: Sensitivity, Sp: Specificity, PPV: Positive Predictive Value, NPV: Negative Predictive Value.

inversely, decreased it because clinical decisions were based on it and may have led to preventing cases of clinical chorioamnionitis. On the other hand, it is improbable that this knowledge affected the predictive values for neonatal infection because the diagnosis was based solely on pediatric clinical and laboratory criteria.

The CRP value was missing for more than 30% of the women with PROM at and after 37 weeks. Unlike bacteriology from vaginal samples, which was systematically performed, CRP was not always measured when PROM occurred at 37 weeks or later: 96% of the women for whom CRP is not available are in that group (Tables 2a and 2b). The prevalence of maternal-fetal infection in this population was very low (3% neonatal infections, 4% clinical chorioamnionitis).

Contrary to our expectations and the results of our previous study [12], IL-6 and TNFα were only associated with clinical chorioamnionitis before 37 weeks and no association was observed with early neonatal infection. One hypothesis regarding the difference between preterm PROM and PROM from 37 weeks onward is that the interval between membrane rupture and delivery is much longer in cases of PPROM and thus leaves more time for a subclinical infection to emerge clinically. The studies published thus far report an association between proinflammatory cytokines, including IL-6, and maternal-fetal infection or subsequent neonatal infection complications [11,18–23]. The protocols were prospective, but sometimes used invasive techniques (for example, routine amniocentesis), as proinflammatory cytokines

were measured mainly in amniotic fluid or maternal serum. Other studies have analyzed the relations between vaginal cytokines and infection or preterm delivery in cases of PPROM [24–28]. They have some positive results but included fewer subjects and used heterogeneous thresholds for positive results; consequently, their quantitative measurement methods are not easily reproducible.

Our study reports the association of bacteriologic analyses of vaginal samples with maternal-fetal infection in PROM, especially before 37 weeks. This is the largest cohort analyzing the association between vaginal bacterial colonization and the onset of either neonatal infection or clinical chorioamnionitis in women with PROM. A review pointed out the need for research into perinatal vertical transmission of vaginal bacteria [29]. The presence of bacteria in the vagina of women with PROM does not always entail their proliferation in the amniotic fluid or contamination of the newborn; transmission rates have not yet been clearly elucidated [30]. The remaining question is that of the usefulness of treatment by appropriate antibiotics when vaginal samples are positive for bacteria in cases of PROM; this question should, especially in cases of long latency and PPROM, be specifically studied.

CRP is one of the most commonly used markers of infection in many countries for women with PROM. Its usefulness is nonetheless controversial: the studies on this subject are very heterogeneous in their methods, inclusion criteria, and results [8]. In our study, CRP >5 mg/L was associated with neonatal infection and clinical chorioamnionitis at and after 37 weeks. The negative predictive value was high. The larger population in our cohort and the repetition of sampling until delivery probably explain these predictive values, which are higher than those usually found [8].

## Conclusion

The association of vaginal cytokines with maternal-fetal infection is weak and thus prevents their use as a good predictor of maternal-fetal infection.

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## Conflict of interest statement

The authors declare no conflict of interest.

## Condensation

The association of vaginal cytokines with maternal-fetal infection is weak and does not allow their use as a good predictor of maternal-fetal infection.

## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ejogrb.2017.01.013.

## References

[1] Chan GJ, Lee AC, Baqui AH, Tan J, Black RE. Risk of early-onset neonatal infection with maternal infection or colonization: a global systematic review and meta-analysis. PLoS Med 2013;10(8):e1001502.
[2] Lee SM, Park JW, Kim BJ, Park CW, Park JS, Jun JK, et al. Acute histologic chorioamnionitis is a risk factor for adverse neonatal outcome in late preterm birth after preterm premature rupture of membranes. PLoS One 2013;8(12):e79941.
[3] van Vliet EO, de Kieviet JF, Oosterlaan J, van Elburg RM. Perinatal infections and neurodevelopmental outcome in very preterm and very low-birth-weight infants: a meta-analysis. JAMA Pediatrics 2013;167(7):662–8.
[4] Kacerovsky M, Musilova I, Andrys C, Hornychova H, Pliskova L, Kostal M, et al. Prelabor rupture of membranes between 34 and 37 weeks: the intraamniotic inflammatory response and neonatal outcomes. Am J Obstet Gynecol 2014210 (4) 325 e1-e10.
[5] Morris JM, Roberts CL, Bowen JR, Patterson JA, Bond DM, Algert CS, et al. Immediate delivery compared with expectant management after preterm prelabour rupture of the membranes close to term (PPROMT trial): a randomised controlled trial. Lancet 2016;387(10017):444–52.
[6] Popowski T, Goffinet F, Maillard F, Schmitz T, Leroy S, Kayem G. Maternal markers for detecting early-onset neonatal infection and chorioamnionitis in cases of premature rupture of membranes at or after 34 weeks of gestation: a two-center prospective study. BMC Pregnancy Childbirth 2011;11(1):26.
[7] van der Heyden JL, van Teeffelen SS, Coolen AC, Halbertsma FJ, Aardenburg R, Mertens HJ, et al. Is it useful to measure C-reactive protein and leukocytes in patients with prelabor rupture of membranes? Am J Perinatol 2010;27 (7):543–7.
[8] van de Laar R, van der Ham DP, Oei SG, Willekes C, Weiner CP, Mol BW. Accuracy of C-reactive protein determination in predicting chorioamnionitis and neonatal infection in pregnant women with premature rupture of membranes: a systematic review. Eur J Obstet Gynecol Reprod Biol 2009;147 (2):124–9.
[9] Popowski T, Goffinet F, Batteux F, Maillard F, Kayem G. Prediction of maternofetal infection in preterm premature rupture of membranes: Serum maternal markers. Gynecol Obstet Fertil 2011;39(5):302–8.
[10] Lewis DF, Barrilleaux PS, Wang Y, Adair CD, Baier J, Kruger T. Detection of interleukin-6 in maternal plasma predicts neonatal and infectious complications in preterm premature rupture of membranes. Am J Perinatol 2001;18 (7):387–91.
[11] Jun JK, Yoon BH, Romero R, Kim M, Moon JB, Ki SH, et al. Interleukin 6 determinations in cervical fluid have diagnostic and prognostic value in preterm premature rupture of membranes. Am J Obstet Gynecol 2000;183 (4):868–73.
[12] Kayem G, Goffinet F, Batteux F, Jarreau PH, Weill B, Cabrol D. Detection of interleukin-6 in vaginal secretions of women with preterm premature rupture of membranes and its association with neonatal infection: a rapid immunochromatographic test. Am J Obstet Gynecol 2005;192(1):140–5.
[13] RCOG. Preterm prelabour rupture of membranes (Green-top guideline No . 44). R Coll Obstetricians Gynaecol 2017. . [Internet]. Available from: https://www.rcog.org.uk/en/guidelines-research-services/guidelines/gtg44/.
[14] Cape A, Tuomala RE, Taylor C, Puopolo KM. Peripartum bacteremia in the era of group B streptococcus prophylaxis. Obstet Gynecol 2013;121(4):812–8.
[15] Papile LA, Munsick-Bruno G, Schaefer A. Relationship of cerebral intraventricular hemorrhage and early childhood neurologic handicaps. J Pediatr 1983;103 (2):273–7.
[16] Jobe AH, Bancalari E. Bronchopulmonary dysplasia. Am J Respir Crit Care Med 2001;163(7):1723–9.
[17] Bell MJ, Ternberg JL, Feigin RD, Keating JP, Marshall R, Barton L, et al. Neonatal necrotizing enterocolitis: therapeutic decisions based upon clinical staging. Ann Surg 1978;187(1):1–7.
[18] Romero R, Sepulveda W, Kenney JS, Archer LE, Allison AC, Sehgal PB. Interleukin 6 determination in the detection of microbial invasion of the amniotic cavity. Ciba Found Symp 1992;167:205–20 discussion 20-3.
[19] Yoon BH, Romero R, Jun JK, Park KH, Park JD, Ghezzi F, et al. Amniotic fluid cytokines (interleukin-6, tumor necrosis factor-alpha, interleukin-1 beta, and interleukin-8) and the risk for the development of bronchopulmonary dysplasia. Am J Obstet Gynecol 1997;177(4):825–30.
[20] Park JS, Romero R, Yoon BH, Moon JB, Oh SY, Han SY, et al. The relationship between amniotic fluid matrix metalloproteinase-8 and funisitis. Am J Obstet Gynecol 2001;185(5):1156–61.
[21] Goncalves LF, Chaiworapongsa T, Romero R. Intrauterine infection and prematurity. Ment Retard Dev Disabil Res Rev 2002;8(1):3–13.
[22] Park KH, Chaiworapongsa T, Kim YM, Espinoza J, Yoshimatsu J, Edwin S, et al. Matrix metalloproteinase 3 in parturition, premature rupture of the membranes, and microbial invasion of the amniotic cavity. J Perinat Med 2003;31(1):12–22.
[23] Yoon BH, Romero R, Moon J, Chaiworapongsa T, Espinoza J, Kim YM, et al. Differences in the fetal interleukin-6 response to microbial invasion of the amniotic cavity between term and preterm gestation. J Matern Fetal Neonatal Med 2003;13(1):32–8.
[24] Sakai M, Sasaki Y, Yoneda S, Kasahara T, Arai T, Okada M, et al. Elevated interleukin-8 in cervical mucus as an indicator for treatment to prevent premature birth and preterm, pre-labor rupture of membranes: a prospective study. Am J Reprod Immunol 2004;51(3):220–5.
[25] Torbe A, Czajka R, Kordek A, Rzepka R, Kwiatkowski S, Rudnicki J. Value of vaginal fluid proinflammatory cytokines for the prediction of early-onset neonatal infection in preterm premature rupture of the membranes. J Interferon Cytokine Res 2007;27(5):393–8.
[26] Vousden N, Chandiramani M, Seed P, Shennan A. Interleukin-6 bedside testing in women at high risk of preterm birth. J Matern Fetal Neonatal Med 2011;24 (10):1301–4.

[27] Ryu A, Park KH, Oh KJ, Lee SY, Jeong EH, Park JW. Predictive value of combined cervicovaginal cytokines and gestational age at sampling for intra-amniotic infection in preterm premature rupture of membranes. Acta Obstet Gynecol Scand 2013;92(5):517–24.
[28] Dorfeuille N, Morin V, Tetu A, Demers S, Laforest G, Gouin K, et al. Vaginal fluid inflammatory biomarkers and the risk of adverse neonatal outcomes in women with PPROM. Am J Perinatol 2016;33(3):1003–7.
[29] Seale J, Millar M. Perinatal vertical transmission of antibiotic-resistant bacteria: a systematic review and proposed research strategy. BJOG 2014;121(8):923–8.
[30] Carroll SG, Papaioannou S, Ntumazah IL, Philpott-Howard J, Nicolaides KH. Lower genital tract swabs in the prediction of intrauterine infection in preterm prelabour rupture of the membranes. Br J Obstetrics Gynaecol. 1996;103 (1):54–9.