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Accuracy of several maternal seric markers for predicting histological chorioamnionitis after preterm premature rupture of membranes: a prospective and multicentric study

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ABSTRACT

Objective: To assess and compare several maternal seric markers for the prediction of histological chorioamnionitis (HCA) after preterm premature rupture of membranes (PPROM). Study design A prospective and multicentric observational study was undertaken, including six French tertiary referral centres. Pregnant women over 18 years, with PPROM between 22 + 0 and 36 + 6 WG were enrolled. A blood sample was obtained before delivery and analysed for C-Reactive Protein (CRP), InterCellular Adhesion Molecule-1 (ICAM-1), Interleukin-6 (IL-6), Interleukin-8 (IL-8), Matrix-Metalloproteinase 8 and 9 (MMP-8, MMP-9), Triggering receptor on myeloid cells (TREM-1), and Human Neutrophile Peptides (HNP). HCA was determined by histological examination distinguishing maternal from fetal inflammatory response. Placental analyses and biological assays were performed in duplicate. Comparison of maternal seric markers levels in women with or *vs.* without HCA was performed, using a non-parametric Receiver Operating Characteristic.

Results: 295 women were kept for analysis. The prevalence of HCA was 42.7% (126/295). The concentrations of MMP-8, MMP-9, HNP and CRP were higher in HCA *vs.* the non-HCA group ($P < 0.05$) whereas the concentrations of ICAM- 1, IL-6, IL-8 were not different ($P > 0.05$). The ROC curve with the largest AUC was for CRP (AUC; 0.70; 95% CI; 0.64–0.77) and it was significantly higher than those for MMP-8, MMP-9, or HNP ($P < 0.03$).

Conclusion: CRP was the best maternal marker for predicting HCA in women with PPROM.

Introduction

Preterm premature rupture of membranes (PPROM) is a major problem in perinatal medicine; it is responsible for 30% of preterm deliveries and affects 1–4.5% of pregnancies [1,2]. PPROM is defined by a breach of the amniotic cavity before 37 weeks of gestation (WG), which generates a high risk of chorioamnionitis, due to vaginal bacteria. Histological chorioamnionitis (HCA) is

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associated with severe maternal, fetal and neonatal infections. Moreover, the fetal inflammatory response in HCA is strongly associated with cerebral injuries such as periventricular leucomalacia in the newborn even in the absence of fetal infection [3–5]. HCA is thus used in most studies as the gold standard for defining chorioamnionitis after birth.

Because there is currently no single option to prevent chorioamnionitis, obstetrical management after PPROM varies widely. Immediate delivery is the most effective intervention for shielding the fetus from chorioamnionitis, but preterm delivery, particularly before 32 WG, is still associated with a high risk of neonatal morbidity and mortality [4].

C-reactive protein is an inflammatory marker used worldwide, including in obstetrics by numerous teams for early diagnosis of HCA [6]. Its clinical usefulness, however, is controversial [6,7]. Other markers have thus been studied for the prediction of chorioamnionitis, such as Intercellular Adhesion Molecule-1 (ICAM-1), Interleukin 6 (IL-6), Interleukin 8 (IL-8), Matrix-Metalloprotease 8 (MMP-8), Matrix-Metalloprotease 9 (MMP-9), Human Neutrophil Peptides (HNP), and Triggering Receptor of Myeloid cells-1 (TREM-1) [8–16]. Previous studies have described a relation between their levels in amniotic fluid or umbilical cord tissue at birth and the presence of HCA [8–16]. Nonetheless, the studies assessing the predictive value of these maternal blood markers for predicting HCA are limited, none has discussed the results according to the localization of the inflammatory response and on the basis of the now well-established criteria, issued by the Society for Pediatric Pathology.

The aim of the present study was to assess and compare ICAM-1, IL-6, IL-8, MMP-8, MMP-9, HNP, and TREM-1 levels in maternal blood to that of the C-reactive protein (CRP), assessed on the day of birth, to determine the best marker for predicting HCA.

Materials and methods

This prospective and multicentre study took place between 2007 and 2009 in six French tertiary referral centres. The relevant institutional review board (Comité de Protection des Personnes dans la Recherche Biomédicale Sud-Est III) approved the study, and each participant signed a written consent form.

Population

All women aged over 18 years with confirmed PPROM between 22 + 0 and 36 + 6 WG were eligible for inclusion. PPROM was defined as the presence of leakage of fluid from the vagina and a positive result or slightly positive test for Insulin-like growth factor-binding protein 1 at two different test times.

In keeping with the practices prevalent at the time of the study, all women were hospitalized after PPROM diagnosis until delivery. Corticosteroids and tocolysis were systematically proposed before 34 WG. Antibiotics were systematically administered before 36 WG or in the presence of vaginal group B streptococcus. Expectant management was recommended until 30 WG and scheduled delivery after 34 WG. Between 30 and 34 WG the maternal management was left to the discretion of the physician.

The neonates were included at birth. In multiple pregnancies, the newborn without PPROM was excluded. Almost all neonates had bacterial cultures (peripheral samples: gastric fluid, ear, umbilical, meconial and tracheal samples; central samples: blood or spinal fluid samples) and inflammatory blood marker tests. The absence of infection was defined by a negative bacterial sample + a normal CRP + a normal clinical examination. Bacterial colonisation was defined by a positive peripheral bacterial sample + a negative central bacterial sample + a normal CRP. Probable infection was defined by a positive or a negative peripheral bacterial sample + a negative central bacterial sample + elevated CRP level. Infection was defined by a positive central bacterial sample. Newborns were monitored for the study until discharge from the hospital.

Laboratory procedures

Maternal peripheral blood samples were drawn every 48 h during hospitalisation and at least once the day of delivery. The samples were collected into 7-mL pre-chilled heparin-treated glass tubes. The samples were then transferred to the immunology laboratory in dry ice. The tubes were centrifuged for 10 min at 3000 g and the serum collected, flash-frozen and immediately stored at −80 °C until analysis.

The assays of IL-6 and IL-8 were based on a multiplex technique and used the Bio-Plex Pro human Cytokine kit from Bio-Rad (x MAP technology, Luminex Corporation, Austin, Texas, USA) [17,18]. The concentrations of ICAM-1, TREM-1, MMP-8, MMP-9, and HNP were determined with enzyme-linked immunosorbent assay (ELISA) kits (respectively, KHS5412: Invitrogen, Carlsbad, New Mexico, USA, DTRM10B: R&D Systems, Minneapolis, Minnesota, USA, DMP800 and DMP900: R&D Systems, Minneapolis, Minnesota, USA, and the HNP1-3 ELISA kit: 019HK317 for Human Neutrophil Peptides, CliniSciences, Nanterre, France).

CRP levels were measured by an automated latex particle-enhanced immunonephelometric assay with a Siemens BN ProSpec analyser system and reagents from Siemens (Siemens, Erlangen, Germany). In accordance with the manufacturer's protocol, the assays were run in duplicate, and the median values were provided. The samples were tested with the same lot of each kit and internal controls were used to assess the reliability of the assays.

Histological examinations

All placentas underwent histological examination. In the twin pregnancies, the umbilical cord of the fetus with PPROM was marked to localize the placenta – or the part of it – and the membranes for the examination.

Microscopic analyses were carried out after fixation, inclusion and standard Hemalum-Phloxine-Saffron or Hemalum-Eosin-Saffron dyeing. The microscopic readings were based on criteria established by the Amniotic Fluid Infection Nosology committee of the Perinatal Section of the Society for Pediatric Pathology distinguishing maternal from fetal inflammatory response [19,20].

All slides were read twice: once by a anatomopathologist of the hospital and another by a pathologist of the reference laboratory (Lyon Sud laboratory). In case of disagreement, another pathologist from the latter laboratory performed a third examination. Samples were considered positive for HCA whenever there were either maternal or fetal signs of inflammation on histological analysis. The grade and the stage of maternal or fetal inflammatory responses were collected because they are potentially useful for predicting clinical outcome.

Statistical considerations

The characteristics of the two groups of women (with and without HCA) were compared with the Chi-square test for categorical variables and the Wilcoxon rank test for continuous variables. The final value of the marker assayed on the day of birth was considered for the statistical analysis. A non-parametric Receiver Operating Characteristic (ROC) curve was constructed for each marker. The Areas Under the Curve (AUC) and its 95%