OBSTETRICS

Inflammatory predictors of neurologic disability after preterm premature rupture of membranes

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OBJECTIVE: The maternal-fetal inflammatory response contributes to both preterm premature rupture of membranes (PPROM) and adverse neurological outcomes. Additionally, cytokines associated with fetal placental inflammation can be detrimental to brain development regardless of inciting infection. We investigated whether differential patterns of cytokine markers in maternal and fetal plasma samples reflect subtypes of placental inflammation and neurological outcomes at 6 months in infants born to mothers with PPROM.

STUDY DESIGN: Within a prospective cohort study of 25 women with PPROM, plasma cytokines (interleukin [IL]-1β, IL-6, IL-8, and tumor necrosis factor-α) were measured by enzyme-linked immunosorbent assay from maternal blood samples at rupture and delivery, and from fetal umbilical cord blood samples. Patterns of cytokine expression were correlated with specific placenta pathologies. Infants underwent cranial ultrasound after birth and standardized neurological examinations at 6 months' corrected gestational age. Predictors of inflammation and adverse neurological outcome were assessed by logistic regression, adjusting for gestational age at birth.

RESULTS: Inflammation of the fetal side of the placenta was associated with elevated maternal IL-6 and IL-8 at delivery and fetal IL-1β, IL-6, IL-8, and tumor necrosis factor-α. Worse neurological outcome at 6 months was associated with inflammation of the fetal side of the placenta and shorter duration from rupture of membrane to delivery, independent of gestational age at birth or cranial ultrasound results.

CONCLUSION: Our findings support the connection between fetal inflammation with adverse neurological outcome with PPROM, regardless of cranial ultrasound results. Further longitudinal studies are needed to adequately examine these patterns, and will aid in risk assessment and intervention strategies.

Key words: cerebral palsy, chorioamnionitis, cytokines, funisitis, placenta

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Preterm birth is a major risk factor for long-term neurological disabilities, including cerebral palsy (CP).[1] Despite accounting for 30-40% of preterm deliveries,[2] preterm premature rupture of membranes (PPROM) is understudied in relation to neurologic disability. Infection/inflammation of the placenta and its membranes ("chorioamnionitis") is considered a risk factor for CP, and the main contributor to PPROM.[3-7] Although widely used, antibiotics in PPROM have failed to avert the fetal inflammatory response (FIR),[8] been unsuccessful in improving long-term neurological outcomes,[9-11] or been associated with worse neurologic outcome.[12] These findings highlight the complexity of the maternal-FIR and its alteration in the pathogenesis of abnormal neurological outcome within pregnancies complicated by PPROM.

Reliable clinical signs of placental infection/inflammation are not evident,[13] necessitating alternative methods of early identification before preventive strategies can be developed. Because of

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their involvement in inflammatory processes and their distinct signatures depending on cause, duration, and organ(s) site, cytokines have been proposed as surrogate blood markers of placental inflammation. For instance, when compared to preterm newborns without PPROM, maternal serum and umbilical cord blood interleukin (IL)-8 levels were elevated in PPROM, yet IL-6 levels were only elevated in the cord blood.[14] Additionally, umbilical cord IL-6 levels have been shown to be elevated in PPROM pregnancies when both microbial invasion of the amniotic cavity and histologic chorioamnionitis (HCA) were present, rather than with HCA alone.[15] Interestingly, maternal serum IL-6 levels within PPROM were shown in one study to predict funisitis after antibiotic completion, and to predict preclinical asymptomatic infection in another.[8,16]

Cytokines have likewise been profiled in studies of perinatal infection, inflammation, and CP with varying results.[4,6,17-19] Inconsistencies might result from mixing heterogeneous conditions without sufficient pathological and clinical stratification between the distinct subsets of placental/membrane inflammatory insults, and their various impacts on offsprings' neurooutcome. Animal studies, performed in various species, establish a causal link among placental inflammation, maternal inflammatory mediators (notably, IL-1 and its close and interconnected 'partners' such as IL-6, IL-8, and tumor necrosis factor [TNF]-α, or their equivalent in animals), and perinatal brain injury.[20-26] In fact, IL-1 and TNF-α activation is linked with fetal placental inflammation and perinatal brain injury, even without infection.[23,27] However, there is currently no preclinical experimental animal design adequately modeling the specific combination of gestational age and placental abnormalities seen in human PPROM. Thus, the precise set of inflammatory mediators involved in placental pathologies and perinatal brain injuries in PPROM pregnancies—with presumed baseline inflammation—remain unclear.

Recently, we identified 2 distinct pathological patterns in PPROM placentas; almost 40% did not show evidence of HCA, suggesting a noninflammatory mechanism of preterm rupture.[28] Additionally, we and others have demonstrated isolated funisitis without evidence of chorioamnionitis.[28,29] We therefore hypothesize that patterns of cytokine elevation and clinical characteristics will associate with patterns of maternal placental inflammation (chorioamnionitis) and fetal side placental inflammation (funisitis and chorionic plate vasculitis). We further hypothesize that patterns of cytokine elevation combined with placental pathologic findings and maternal and fetal clinical characteristics will emerge, which will inform our overall goal to create a high-risk multivariate index predictor model for perinatal brain injury in pregnancies complicated by PPROM. In this study, we investigate whether plasma titers of key placenta- and neuro-inflammatory markers (IL-1β, IL-6, IL-8, and TNF-α, targeted from preclinical and human epidemiological studies) in maternal samples at time of rupture of membranes (ROM) as well as maternal and fetal samples at delivery are associated with placental inflammatory patterns and adverse neurological outcomes at 6 months' corrected gestational age in preterm infants born to mothers with PPROM. It is of the utmost importance to improve knowledge and diagnostic markers in this group, to ultimately aid in the development of new therapeutic approaches to beneficially modulate the maternal-FIR and prevent adverse neurological outcome.

Materials and Methods

Women with PPROM were enrolled in a prospective cohort study at the University of Colorado Hospital from July 1, 2010, through June 30, 2012. PPROM was confirmed by standard clinical characteristics of alkaline pH, ferning, and pooling of amniotic fluid in the vagina on speculum examination in women >24 or <34 weeks' gestation prior to labor onset. Clinical chorioamnionitis (CCA) was defined as $\geq$2 of the following: maternal fever >38.0°C, maternal tachycardia (>100 beats/min), fetal tachycardia (>160 beats/min), maternal fundal tenderness, maternal leukocytosis (white blood cell count [WBC] >15,000), and purulent vaginal discharge or amniocentesis with findings consistent with chorioamnionitis (glucose <15 mg/dL, WBC >30/μL, leukocyte esterase positive, Gram stain with bacteria, or >6 WBC/high-power field).[30] Multiple gestations and nonviable pregnancies were excluded. Research was conducted in accordance with the 2004 Declaration of Helsinki, with signed informed consent (Colorado Multiple Institutional Review Board study 09-1107).

Demographic and clinical data were abstracted from the University of Colorado Hospital perinatal database by a trained research assistant using a standardized protocol; ambiguity of maternal data was clarified by a maternal-fetal medicine specialist (V.D.W.) and for neonatal data by a neonatal neurologist (J.A-W.). Data included: (1) demographics (maternal age, parity, and ethnicity; newborn sex and birthweight; prenatal care onset); (2) maternal predictors (underlying condition requiring medical supervision, gestational diabetes, pregnancy-induced hypertension, preeclampsia, chorioamnionitis); (3) intrapartum predictors (category II or III fetal heart tracing leading to cesarean delivery, emergency cesarean delivery); and (4) neonatal complications (respiratory distress, hemodynamic failure, sepsis, hypoglycemia, seizures). Although smoking, autoimmune disease, pregestational diabetes, and infertility/assisted reproduction were also predictors of interest, there were no cases within our cohort.

Cytokine analysis

Maternal venous blood samples were collected at time of enrollment and within 2 hours after delivery. Fetal cord blood venous samples were obtained immediately after delivery by trained perinatal research nurses with experience in venous cord blood collection. Samples were collected in EDTA tubes and centrifuged for 20 minutes at 1600*g* at 4° C, then transferred and centrifuged again. Platelet-poor plasma was aliquotted, frozen, and sent for cytokine analysis (IL-1β, IL-6, IL-8, and TNF-α)