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The combined exposure to intra-amniotic inflammation and neonatal respiratory distress syndrome increases the risk of intraventricular hemorrhage in preterm neonates

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Abstract

OBJECTIVE—To evaluate the impact of combined exposure to intra-amniotic inflammation and neonatal respiratory distress syndrome (RDS) on the development of intraventricular hemorrhage (IVH) in preterm neonates.

METHODS—This retrospective cohort study includes 207 consecutive preterm births (24.0–33.0 weeks of gestation). Intra-amniotic inflammation was defined as an amniotic fluid matrix metalloproteinase-8 concentration >23 ng/mL. According to McMenamin's classification, IVH was defined as grade II or higher when detected by neurosonography within the first weeks of life.

RESULTS—1) IVH was diagnosed in 6.8% (14/207) of neonates in the study population; 2) IVH was frequent among newborns exposed to intra-amniotic inflammation when followed by postnatal RDS (33% [6/18]). The frequency of IVH was 7% (8/115) among neonates exposed either to these conditions— intra-amniotic inflammation or RDS—and 0% (0/64) among those who were not exposed to these conditions; and 3) neonates exposed to intra-amniotic inflammation and postnatal RDS had a significantly higher risk of IVH than those with only intra-

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amniotic inflammation [odds ratio (OR), 4.6; 95% confidence interval (CI), 1.1–19.3) and those with RDS alone (OR, 5.6; 95% CI, 1.0–30.9), after adjusting for gestational age.

CONCLUSION—The combined exposure to intra-amniotic inflammation and postnatal RDS markedly increased the risk of IVH in preterm neonates.

Keywords

brain injury; hypoxic-ischemic injury; intra-amniotic inflammation; periventricular-intraventricular hemorrhage; preterm birth

Introduction

Intraventricular hemorrhage (IVH) remains one of the leading causes of morbidity and mortality in the preterm neonate [1–13]. A substantial body of evidence indicates that IVH is a predictor of periventricular leukomalacia [14–17], neurodevelopmental delay [3, 12, 13, 18] and cerebral palsy [19–23]. Even low-grade IVH is associated with poor neurodevelopmental outcomes [12, 24]. Risk factors that have been linked to the development of IVH include early gestational age at birth [4, 25–28], intrauterine/fetal inflammation [1, 4, 5, 29–33], spontaneous preterm birth [4, 34], early onset of neonatal sepsis [35], respiratory distress syndrome (RDS) [25, 36–39], hypoxia-ischemia [40, 41], and non-use of antenatal corticosteroids [26, 35].

Substantial evidence indicates that intra-amniotic infection/inflammation is causally linked to preterm delivery [5, 42–62]. The inflammatory response has been implicated in the genesis of fetal brain injury in humans [1, 5, 15, 29, 30] as well as in experimental animal models [63–67]. However, some recent studies [4, 68–70] reported that acute histologic chorioamnionitis was not an independent risk factor for the development of IVH. Moreover, reports suggest that antenatal exposure to an inflammatory response accelerates fetal lung maturation [71, 72] and decreases the risk of developing of RDS [73–75]. In a nationwide population-based study, spontaneous preterm infants were at increased risk of cerebral palsy but at decreased risk of RDS [76]. These findings raise the question of how the inflammatory response increases the risk of IVH despite decreasing the risk of RDS, which is known as a risk factor for IVH.

The purpose of this study was to evaluate the relationship among the antenatal inflammatory response, the postnatal occurrence of RDS, and the development of IVH in preterm neonates.

Materials and methods

Study design

This retrospective cohort study includes 207 consecutive singleton preterm neonates who were born in the Seoul National University Hospital from 1995 to 2007 and met the following criteria: 1) gestational age at birth between 24.0 and 33.0 weeks; 2) absence of chromosomal abnormalities and major structural anomalies; 3) collection of amniotic fluid (AF) within 120 hours of delivery, either by abdominal amniocentesis or at the time of

cesarean delivery; and 4) neurosonographic examination within seven days of birth. We reviewed the medical records to determine the clinical and demographic characteristics of the mothers and their neonates. The demographic characteristics included age, parity, and gestational age at birth. The clinical characteristics consisted of antenatal corticosteroid administration, histopathological evaluation of the placenta, cause of preterm birth (spontaneous vs. medically indicated preterm birth) and delivery mode (cesarean vs. vaginal delivery). We also included gender, umbilical arterial blood gas analysis, and Apgar scoring at 1 minute and 5 minutes.

Diagnosis of neonatal respiratory distress syndrome and intraventricular hemorrhage

Neonatal RDS was defined as the presence of respiratory distress, increased oxygen requirement ($\text{FiO}_2 > 0.4$), and diagnostic radiological findings in the absence of evidence of any other causes of respiratory distress as described previously [74].

Neurosonographic examinations were performed as part of routine clinical care in preterm neonates. Sonographic findings of periventricular-intraventricular hemorrhage (PV-IVH) were graded into three categories according to McMenamin's classification [2]: 1) grade I: subependymal hemorrhage with minimal or no IVH; 2) grade II: IVH, but neither lateral ventricle completely filled with blood, with or without mild ventricular dilatation; and 3) grade III: IVH completely filling and distending at least one lateral ventricle. IVH was defined as Grade II or higher by McMenamin's classification of PV-IVH. For a meaningful temporal relationship among intra-amniotic inflammation, neonatal RDS, and the development of IVH, the results of neurosonographic examinations performed within seven days of birth were included.

Definition of intra-amniotic inflammation

Amniotic fluid not used for clinical studies was stored at -70°C for future research purposes. The stored AF was analyzed for matrix metalloproteinase-8 (MMP-8), which was measured with a commercially available enzyme-linked immunosorbent assay (Amersham Pharmacia Biotech, Inc., Amersham, UK), as previously reported [77]. The measurement was not performed in 10 patients because of the lack of available AF. The sensitivity of the test was 0.3 ng/mL. Intra- and inter-assay coefficients of variation for each were $<10\%$. Intra-amniotic inflammation was defined as an elevated AF MMP-8 concentration (>23 ng/mL), as previously reported [77]. The Institutional Review Board of the Seoul National University Hospital approved the collection and use of these samples for research purposes. This University Hospital has a Federal Wide Assurance with the Office for Human Research Protection of the Department of Health and Human Services of the United States.

Diagnosis of acute histologic chorioamnionitis and acute funisitis

Acute histologic chorioamnionitis was defined in the presence of acute inflammatory changes upon examination of the choriodecidua and amnion, respectively; acute funisitis was diagnosed in the presence of neutrophil infiltration into the umbilical vessel walls or Wharton's jelly with the use of criteria previously reported [5, 59].

Statistical analysis

The Mann-Whitney *U*-test was used for comparison of continuous variables. For the dichotomized variables, a Fisher's exact test was performed. Multiple logistic regression analysis was used to examine the relationship of intra-amniotic inflammation and RDS to the probability of the occurrence of IVH after adjusting for variables that had a significant correlation or a tendency ($P < 0.1$) including gestational age at birth, male gender, and acute histologic chorioamnionitis. A probability value < 0.05 was considered as significant. SPSS 21.0 for Windows (IBM, Armonk, NY, USA) was used for statistical analyses.

Results

Characteristics of the study population

Table 1 describes the characteristics and results of the neurosonographic examinations performed in the study population. A total of 258 neurosonographic examinations were performed on 207 neonates (mean 1.3 ± 0.5 neurosonographic examinations/neonate) within seven days of birth; 43 (31%) newborns received multiple scans. IVH was diagnosed in 6.8% (14/207) of neonates in the study population.

Table 2 compares the clinical characteristics according to the development of IVH. Gestational age was significantly lower in neonates with IVH than those without IVH ($P < 0.005$). Neonates with IVH had higher rates of RDS and the presence of histologic chorioamnionitis than those without IVH ($P < 0.05$ for each). Spontaneous preterm birth and male gender were more frequent in neonates with IVH than in those without IVH, but the differences did not reach statistical significance. The rates of antenatal corticosteroid use and cesarean delivery were not significantly different between groups. Among the 197 neonates whose AF was measured for MMP-8 concentrations, neonates with IVH had a significantly higher median AF MMP-8 concentration ($P < 0.05$) and a higher rate of intra-amniotic inflammation ($P < 0.01$) than those without IVH.

The relationship among intra-amniotic inflammation, respiratory distress syndrome and intraventricular hemorrhage

Table 3 shows the multivariate analysis for the factors that had a probability of < 0.1 in the univariate analysis. Because the presence of intra-amniotic inflammation was closely linked to the occurrence of histologic chorioamnionitis, they were included separately in the multivariate analysis model. When acute histologic chorioamnionitis was included in the multivariate analysis, gestational age at birth was an independent risk factor for the development of IVH [odds ratio (OR), 0.70; 95% confidence interval (CI), 0.52–0.94]. When the presence of intra-amniotic inflammation was included in the analysis, this factor (OR, 7.88; 95% CI, 1.63–38.1) and the occurrence of RDS (OR, 6.50; 95% CI, 1.69–25.1) were independent risk factors for the development of IVH. When these two variables (intra-amniotic inflammation and acute histologic chorioamnionitis) were included together in the multivariate analysis, the presence of intra-amniotic inflammation (OR, 25.3; 95% CI, 1.89–338.6) and the occurrence of RDS (OR, 4.63; 95% CI, 1.10–19.4) were independently associated with the development of IVH.

The effect of combined exposure to intra-amniotic inflammation and postnatal respiratory distress syndrome on intraventricular hemorrhage

Table 4 presents the clinical characteristics and outcomes of the study population according to the presence or absence of intra-amniotic inflammation and the occurrence of neonatal RDS. IVH was frequent among newborns exposed to intra-amniotic inflammation when followed by postnatal RDS (33% [6/18]). The frequency of IVH was 7% (8/115) among neonates exposed to either of these conditions (intra-amniotic inflammation or RDS) and 0% (0/64) among those who were not exposed to these conditions. Figure 1 displays the comparison of rates of IVH among groups according to the presence or absence of intra-amniotic inflammation and the occurrence of RDS. Neonates who were exposed to both of these conditions had a significantly higher risk of IVH than those with RDS alone (OR, 5.63; 95% CI, 1.03–30.87) and those with intra-amniotic inflammation alone (OR, 4.56; 95% CI, 1.08–19.26) after adjusting for the gestational age at birth.

Discussion

Principal findings of this study

IVH occurred in 6.8% (14/207) of the preterm singleton neonates (gestational age between 24.0–33.0 weeks). Intra-amniotic inflammation and neonatal RDS were independent risk factors for the development of IVH. Moreover, the impact of the combined exposure to intra-amniotic inflammation and postnatal RDS was considerably greater than that of separate exposures to either of the conditions.

Intra-amniotic inflammation and intraventricular hemorrhage

Strong evidence suggests that prenatal exposure to an inflammatory response is associated with the development of IVH [1, 5, 29–34, 78, 79]. The germinal matrix zone of preterm neonates is supported by a single cell layer of endothelium without muscle coats and is prone to hemorrhage [80]. The inflammatory response exerts a direct neurotoxic effect [81–83], leads to circulatory disturbances [80], and induces adhesion of leukocytes to fragile vessels [84], all of which may increase the risk of IVH. The findings of our study support the view that prenatal exposure to an inflammatory response plays an important role in the development of IVH in preterm neonates.

Prenatal exposure to an inflammatory response can be determined by elevated concentrations of AF inflammatory mediators [49, 54, 77, 85–104], AF white blood cell count [105–107], the presence of inflammatory changes in the fetal membranes or acute histologic chorioamnionitis [59, 78, 106, 108–111], and the concentration of cytokines in umbilical cord blood [1, 48, 112–116]. In the study herein, intra-amniotic inflammation was defined as an elevated AF MMP-8 concentration (>23 ng/mL); previous studies have shown that the concentration of AF MMP-8 is an excellent marker of intra-amniotic infection and/or inflammation, the fetal inflammatory response syndrome, impeding preterm delivery, and neonatal morbidity [54, 57, 58, 77, 86, 100, 117]. The measurement of AF cytokine concentrations has some advantages over other tools: 1) histologic examination cannot be performed antenatally and the results may not be available in time for clinical management

decisions; 2) histologic chorioamnionitis is frequently found in patients with active labor [118]; and 3) amniocentesis is frequently used for the assessment of lung maturity.

Recent reports have claimed that acute histologic chorioamnionitis is not an independent risk factor for the development of IVH [4, 68–70], which is consistent with our findings (Table 3). However, upon further analysis, we found that intra-amniotic inflammation was an independent risk factor, even after adjusting for other confounding variables. Acute histologic chorioamnionitis represents a maternal inflammatory response in the fetal membranes in response to chemotactic stimuli within the amniotic cavity [108]. Therefore, intra-amniotic inflammation is a better index of the risk of fetal systemic inflammation than histologic chorioamnionitis. Indeed, most neutrophils in the amniotic cavity are of fetal origin [59, 119].

The relationship between neonatal respiratory distress syndrome and intraventricular hemorrhage

Several studies support that RDS is a risk factor for the development of IVH [25, 36, 37, 120, 121]. RDS is associated with the fluctuation of cerebral blood flow [122–124], low blood pressure [125], use of mechanical ventilation and hypoxia-ischemia [126], all of which increase the risk of IVH. In the current study, neonates with RDS had an OR of 3.71 (95% CI, 1.20–11.5; $P < 0.05$) for the development of IVH. Moreover, neonates with combined exposure to intra-amniotic inflammation and subsequent RDS had a significantly higher risk of developing IVH than those with intra-amniotic inflammation alone, even after adjusting for gestational age at birth.

Does intra-amniotic inflammation sensitize the immature brain to postnatal injury?

An important question is whether combined exposure to intra-amniotic inflammation and postnatal RDS has a synergistic effect on the development of IVH and brain damage in preterm neonates. Our findings showed that combined exposure of intra-amniotic inflammation and postnatal RDS markedly increases the risk for the development of IVH. Such findings are in keeping with the results of other studies in humans [127] as well as in animals [128–135]. In animal models, the impact of combined exposure to inflammation and postnatal hypoxic-ischemic injury was greater than when either of the conditions was induced separately [128–135]. Indeed, Nelson and Grether [127] found that the risk of unexplained spastic cerebral palsy in neonates with exposure to both infection/birth-asphyxiating conditions was considerably greater than the risk in those with either condition alone, and was 78 times greater than the risk in those without exposure to either of these conditions.

Other factors related to the development of intraventricular hemorrhage

In the study herein, corticosteroid use and cesarean delivery did not show protective effects against the development of IVH. Roberts and Dalziel [136] demonstrated that antenatal corticosteroid treatment decreases the occurrence of cerebroventricular hemorrhage in their meta-analysis, which included 2,872 infants from 13 studies. However, we could not detect this effect in the present study. Possible explanations for this finding include: 1) the small sample size in our study population; 2) selection bias of corticosteroid use (in the study

period, all patients were eligible for receiving antenatal steroids); 3) there was no adjustment for an incomplete course of steroids.

Whether cesarean delivery can prevent IVH is controversial. Although some have reported that cesarean delivery prevents the development of IVH [137–140], other investigators [4, 26, 78, 141] have found that the risk of IVH was not influenced by mode of delivery. In our study, the mode of delivery was not associated with the development of IVH.

Clinical implications

The major clinical implication of our study is that the presence or absence of intra-amniotic inflammation contributes to the estimate of risk for the subsequent development of IVH. Moreover, if RDS occurs in the neonates who were exposed to intra-amniotic inflammation, the risk of IVH is greatly increased. It seems paradoxical that intra-amniotic inflammation decreases the risk of neonatal RDS but increases the risk of IVH. In the current study, neonates with intra-amniotic inflammation had a significantly lower rate of the occurrence of RDS than those without intra-amniotic inflammation [21% (18/84) in the intra-amniotic inflammation group vs. 43% (49/113) in the non-intra-amniotic inflammation group; $P < 0.001$ after adjusting for gestational age at birth]. However, although 9% (18/197) of preterm neonates were affected by combined intra-amniotic inflammation and postnatal RDS, 43% (6/14) of cases with IVH were from those neonates. These findings suggest that preterm neonates exposed to intra-amniotic inflammation and subsequent postnatal RDS are at great risk of the development of IVH and require evaluation and more intense surveillance.

In conclusion, antenatal intra-amniotic inflammation and postnatal RDS are independent risk factors for the development of IVH, and the combined exposure of these two factors markedly increases the risk of IVH in preterm neonates.

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References

1. Gomez R, Romero R, Ghezzi F, Yoon BH, Mazor M, Berry SM. The fetal inflammatory response syndrome. *Am J Obstet Gynecol.* 1998; 179:194–202. [PubMed: 9704787]
2. McMenamin JB, Shackelford GD, Volpe JJ. Outcome of neonatal intraventricular hemorrhage with periventricular echodense lesions. *Ann Neurol.* 1984; 15:285–90. [PubMed: 6721450]
3. Ment LR, Vohr B, Allan W, Katz KH, Schneider KC, Westerveld M, et al. Change in cognitive function over time in very low-birth-weight infants. *JAMA.* 2003; 289:705–11. [PubMed: 12585948]
4. Vergani P, Locatelli A, Doria V, Assi F, Paterlini G, Pezzullo JC, et al. Intraventricular hemorrhage and periventricular leukomalacia in preterm infants. *Obstet Gynecol.* 2004; 104:225–31. [PubMed: 15291991]

5. Yoon BH, Romero R, Kim CJ, Jun JK, Gomez R, Choi JH, et al. Amniotic fluid interleukin-6: a sensitive test for antenatal diagnosis of acute inflammatory lesions of preterm placenta and prediction of perinatal morbidity. *Am J Obstet Gynecol.* 1995; 172:960–70. [PubMed: 7892891]
6. Vohr B, Ment LR. Intraventricular hemorrhage in the preterm infant. *Early Hum Dev.* 1996; 44:1–16. [PubMed: 8821891]
7. Perlman JM, Broyles RS, Rogers CG. Neonatal neurologic characteristics of preterm twin infants <1,250 gm birth weight. *Pediatr Neurol.* 1997; 17:322–6. [PubMed: 9436796]
8. Baschat AA, Gembruch U, Viscardi RM, Gortner L, Harman CR. Antenatal prediction of intraventricular hemorrhage in fetal growth restriction: what is the role of Doppler? *Ultrasound Obstet Gynecol.* 2002; 19:334–9. [PubMed: 11952960]
9. Valcamonico A, Accorsi P, Sanzeni C, Martelli P, La Boria P, Cavazza A, et al. Mid- and long-term outcome of extremely low birth weight (ELBW) infants: an analysis of prognostic factors. *J Matern Fetal Neonatal Med.* 2007; 20:465–71. [PubMed: 17674256]
10. Kent AL, Wright IM, Abdel-Latif ME, New South W. Australian Capital Territory Neonatal Intensive Care Units Audit G. Mortality and adverse neurologic outcomes are greater in preterm male infants. *Pediatrics.* 2012; 129:124–31. [PubMed: 22184652]
11. Stoll BJ, Hansen NI, Bell EF, Shankaran S, Laptook AR, Walsh MC, et al. Neonatal outcomes of extremely preterm infants from the NICHD Neonatal Research Network. *Pediatrics.* 2010; 126:443–56. [PubMed: 20732945]
12. Mukerji A, Shah V, Shah PS. Periventricular/Intraventricular Hemorrhage and Neurodevelopmental Outcomes: A Meta-analysis. *Pediatrics.* 2015; 136:1132–43. [PubMed: 26598455]
13. Payne AH, Hintz SR, Hibbs AM, Walsh MC, Vohr BR, Bann CM, et al. Neurodevelopmental outcomes of extremely low-gestational-age neonates with low-grade periventricular-intraventricular hemorrhage. *JAMA Pediatr.* 2013; 167:451–9. [PubMed: 23460139]
14. Larroque B, Marret S, Ancel PY, Arnaud C, Marpeau L, Supernant K, et al. White matter damage and intraventricular hemorrhage in very preterm infants: the EPIPAGE study. *J Pediatr.* 2003; 143:477–83. [PubMed: 14571224]
15. Yoon BH, Romero R, Yang SH, Jun JK, Kim IO, Choi JH, et al. Interleukin-6 concentrations in umbilical cord plasma are elevated in neonates with white matter lesions associated with periventricular leukomalacia. *Am J Obstet Gynecol.* 1996; 174:1433–40. [PubMed: 9065108]
16. Armstrong DL, Sauls CD, Goddard-Finegold J. Neuropathologic findings in short-term survivors of intraventricular hemorrhage. *Am J Dis Child.* 1987; 141:617–21. [PubMed: 3578185]
17. Volpe JJ. Intraventricular hemorrhage and brain injury in the premature infant. *Neuropathology and pathogenesis.* *Clin Perinatol.* 1989; 16:361–86. [PubMed: 2663307]
18. Vohr BR, Wright LL, Dusick AM, Mele L, Verter J, Steichen JJ, et al. Neurodevelopmental and functional outcomes of extremely low birth weight infants in the National Institute of Child Health and Human Development Neonatal Research Network, 1993–1994. *Pediatrics.* 2000; 105:1216–26. [PubMed: 10835060]
19. Futagi Y, Toribe Y, Ogawa K, Suzuki Y. Neurodevelopmental outcome in children with intraventricular hemorrhage. *Pediatr Neurol.* 2006; 34:219–24. [PubMed: 16504792]
20. Vohr B, Allan WC, Scott DT, Katz KH, Schneider KC, Makuch RW, et al. Early-onset intraventricular hemorrhage in preterm neonates: incidence of neurodevelopmental handicap. *Semin Perinatol.* 1999; 23:212–7. [PubMed: 10405190]
21. Allan WC, Vohr B, Makuch RW, Katz KH, Ment LR. Antecedents of cerebral palsy in a multicenter trial of indomethacin for intraventricular hemorrhage. *Arch Pediatr Adolesc Med.* 1997; 151:580–5. [PubMed: 9193243]
22. Dunin-Wasowicz D, Rowecka-Trzebicka K, Milewska-Bobula B, Kassur-Siemenska B, Bauer A, Idzik M, et al. Risk factors for cerebral palsy in very low-birthweight infants in the 1980s and 1990s. *J Child Neurol.* 2000; 15:417–20. [PubMed: 10868787]
23. Msall ME, Buck GM, Rogers BT, Merke D, Catanzaro NL, Zorn WA. Risk factors for major neurodevelopmental impairments and need for special education resources in extremely premature infants. *J Pediatr.* 1991; 119:606–14. [PubMed: 1919894]

24. Patra K, Wilson-Costello D, Taylor HG, Mercuri-Minich N, Hack M. Grades I–II intraventricular hemorrhage in extremely low birth weight infants: effects on neurodevelopment. *J Pediatr*. 2006; 149:169–73. [PubMed: 16887428]
25. Gleissner M, Jorch G, Avenarius S. Risk factors for intraventricular hemorrhage in a birth cohort of 3721 premature infants. *J Perinat Med*. 2000; 28:104–10. [PubMed: 10875094]
26. Riskin A, Riskin-Mashiah S, Bader D, Kugelman A, Lerner-Geva L, Boyko V, et al. Delivery mode and severe intraventricular hemorrhage in single, very low birth weight, vertex infants. *Obstet Gynecol*. 2008; 112:21–8. [PubMed: 18591303]
27. Bhandari V, Buhimschi CS, Han CS, Lee SY, Pettker CM, Campbell KH, et al. Cord blood erythropoietin and interleukin-6 for prediction of intraventricular hemorrhage in the preterm neonate. *J Matern Fetal Neonatal Med*. 2011; 24:673–9. [PubMed: 20937006]
28. Kwak HM, Shin MY, Cha HH, Choi SJ, Lee JH, Kim JS, et al. The efficacy of cefazolin plus macrolide (erythromycin or clarithromycin) versus cefazolin alone in neonatal morbidity and placental inflammation for women with preterm premature rupture of membranes. *Placenta*. 2013; 34:346–52. [PubMed: 23465535]
29. Viscardi RM, Hashmi N, Gross GW, Sun CC, Rodriguez A, Fairchild KD. Incidence of invasive ureaplasma in VLBW infants: relationship to severe intraventricular hemorrhage. *J Perinatol*. 2008; 28:759–65. [PubMed: 18596706]
30. Kasper DC, Mechtler TP, Bohm J, Petricevic L, Gleiss A, Spargser J, et al. In utero exposure to *Ureaplasma* spp. is associated with increased rate of bronchopulmonary dysplasia and intraventricular hemorrhage in preterm infants. *J Perinat Med*. 2011; 39:331–6. [PubMed: 21526978]
31. Martinez E, Figueroa R, Garry D, Visintainer P, Patel K, Verma U, et al. Elevated Amniotic Fluid Interleukin-6 as a Predictor of Neonatal Periventricular Leukomalacia and Intraventricular Hemorrhage. *J Matern Fetal Investig*. 1998; 8:101–7.
32. Thomas W, Speer CP. Chorioamnionitis: important risk factor or innocent bystander for neonatal outcome? *Neonatology*. 2011; 99:177–87. [PubMed: 20881433]
33. Leviton A, Dammann O, Durum SK. The adaptive immune response in neonatal cerebral white matter damage. *Ann Neurol*. 2005; 58:821–8. [PubMed: 16250014]
34. Verma U, Tejani N, Klein S, Reale MR, Beneck D, Figueroa R, et al. Obstetric antecedents of intraventricular hemorrhage and periventricular leukomalacia in the low-birth-weight neonate. *Am J Obstet Gynecol*. 1997; 176:275–81. [PubMed: 9065168]
35. Linder N, Haskin O, Levit O, Klinger G, Prince T, Naor N, et al. Risk factors for intraventricular hemorrhage in very low birth weight premature infants: a retrospective case-control study. *Pediatrics*. 2003; 111:e590–5. [PubMed: 12728115]
36. Morales WJ, Koerten J. Obstetric management and intraventricular hemorrhage in very-low-birth-weight infants. *Obstet Gynecol*. 1986; 68:35–40. [PubMed: 3725257]
37. Ferrari B, Tonni G, Luzietti R, Ciarlini G, Vadora E, Merialdi A. Neonatal complications and risk of intraventricular-periventricular hemorrhage. *Clin Exp Obstet Gynecol*. 1992; 19:253–8. [PubMed: 1294347]
38. Levene MI, Fawer CL, Lamont RF. Risk factors in the development of intraventricular haemorrhage in the preterm neonate. *Arch Dis Child*. 1982; 57:410–7. [PubMed: 7092304]
39. Heljic S, Maksic H, Buljina A. Hemorrhagic and hypoxic-ischemic brain lesions in premature infants on artificial ventilation. *Med Arh*. 2000; 54:265–7. [PubMed: 11219899]
40. Hill A, Volpe JJ. Seizures, hypoxic-ischemic brain injury, and intraventricular hemorrhage in the newborn. *Ann Neurol*. 1981; 10:109–21. [PubMed: 7283398]
41. Lou HC. Perinatal hypoxic-ischaemic brain damage and intraventricular haemorrhage. *Baillieres Clin Obstet Gynaecol*. 1988; 2:213–20. [PubMed: 3046802]
42. Gomez R, Romero R, Edwin SS, David C. Pathogenesis of preterm labor and preterm premature rupture of membranes associated with intraamniotic infection. *Infect Dis Clin North Am*. 1997; 11:135–76. [PubMed: 9067790]
43. Romero R, Mazor M, Wu YK, Sirtori M, Oyarzun E, Mitchell MD, et al. Infection in the pathogenesis of preterm labor. *Semin Perinatol*. 1988; 12:262–79. [PubMed: 3065940]

44. Romero R, Sirtori M, Oyarzun E, Avila C, Mazor M, Callahan R, et al. Infection and labor. VI. Prevalence, microbiology, and clinical significance of intraamniotic infection in women with preterm labor and intact membranes. *Am J Obstet Gynecol.* 1989; 161:817–24. [PubMed: 2675611]
45. Romero R, Shamma F, Avila C, Jimenez C, Callahan R, Nores J, et al. Infection and labor. VI. Prevalence, microbiology, and clinical significance of intraamniotic infection in twin gestations with preterm labor. *Am J Obstet Gynecol.* 1990; 163:757–61. [PubMed: 2403156]
46. Angus SR, Segel SY, Hsu CD, Locksmith GJ, Clark P, Sammel MD, et al. Amniotic fluid matrix metalloproteinase-8 indicates intra-amniotic infection. *Am J Obstet Gynecol.* 2001; 185:1232–8. [PubMed: 11717662]
47. Gibbs RS, Romero R, Hillier SL, Eschenbach DA, Sweet RL. A review of premature birth and subclinical infection. *Am J Obstet Gynecol.* 1992; 166:1515–28. [PubMed: 1595807]
48. Yoon BH, Romero R, Park JS, Chang JW, Kim YA, Kim JC, et al. Microbial invasion of the amniotic cavity with *Ureaplasma urealyticum* is associated with a robust host response in fetal, amniotic, and maternal compartments. *Am J Obstet Gynecol.* 1998; 179:1254–60. [PubMed: 9822511]
49. Yoon BH, Romero R, Moon JB, Shim SS, Kim M, Kim G, et al. Clinical significance of intra-amniotic inflammation in patients with preterm labor and intact membranes. *Am J Obstet Gynecol.* 2001; 185:1130–6. [PubMed: 11717646]
50. Yoon BH, Romero R, Kim M, Kim EC, Kim T, Park JS, et al. Clinical implications of detection of *Ureaplasma urealyticum* in the amniotic cavity with the polymerase chain reaction. *Am J Obstet Gynecol.* 2000; 183:1130–7. [PubMed: 11084554]
51. Yoon BH, Chang JW, Romero R. Isolation of *Ureaplasma urealyticum* from the amniotic cavity and adverse outcome in preterm labor. *Obstet Gynecol.* 1998; 92:77–82. [PubMed: 9649098]
52. Oh KJ, Lee SE, Jung H, Kim G, Romero R, Yoon BH. Detection of ureaplasmas by the polymerase chain reaction in the amniotic fluid of patients with cervical insufficiency. *J Perinat Med.* 2010; 38:261–8. [PubMed: 20192887]
53. Gomez-Lopez N, Romero R, Plazyo O, Panaitescu B, Furcron AE, Miller D, et al. Intra-Amniotic Administration of HMGB1 Induces Spontaneous Preterm Labor and Birth. *Am J Reprod Immunol.* 2016; 75:3–7. [PubMed: 26781934]
54. Kim SM, Romero R, Lee J, Chaemsaitong P, Lee MW, Chaiyasit N, et al. About one-half of early spontaneous preterm deliveries can be identified by a rapid matrix metalloproteinase-8 (MMP-8) bedside test at the time of mid-trimester genetic amniocentesis. *J Matern Fetal Neonatal Med.* 2016; 29:2414–21. [PubMed: 26643648]
55. Romero R, Dey SK, Fisher SJ. Preterm labor: one syndrome, many causes. *Science.* 2014; 345:760–5. [PubMed: 25124429]
56. Romero R, Espinoza J, Goncalves LF, Kusanovic JP, Friel LA, Nien JK. Inflammation in preterm and term labour and delivery. *Semin Fetal Neonatal Med.* 2006; 11:317–26. [PubMed: 16839830]
57. Shim SS, Romero R, Hong JS, Park CW, Jun JK, Kim BI, et al. Clinical significance of intra-amniotic inflammation in patients with preterm premature rupture of membranes. *Am J Obstet Gynecol.* 2004; 191:1339–45. [PubMed: 15507963]
58. Maymon E, Romero R, Chaiworapongsa T, Berman S, Conoscenti G, Gomez R, et al. Amniotic fluid matrix metalloproteinase-8 in preterm labor with intact membranes. *Am J Obstet Gynecol.* 2001; 185:1149–55. [PubMed: 11717649]
59. Kim CJ, Romero R, Chaemsaitong P, Chaiyasit N, Yoon BH, Kim YM. Acute chorioamnionitis and funisitis: definition, pathologic features, and clinical significance. *Am J Obstet Gynecol.* 2015; 213:S29–52. [PubMed: 26428501]
60. Romero R, Mazaki-Tovi S, Vaisbuch E, Kusanovic JP, Chaiworapongsa T, Gomez R, et al. Metabolomics in premature labor: a novel approach to identify patients at risk for preterm delivery. *J Matern Fetal Neonatal Med.* 2010; 23:1344–59. [PubMed: 20504069]
61. Romero R, Espinoza J, Kusanovic JP, Gotsch F, Hassan S, Erez O, et al. The preterm parturition syndrome. *BJOG.* 2006; 113(Suppl 3):17–42.

62. DiGiulio DB, Romero R, Amogan HP, Kusanovic JP, Bik EM, Gotsch F, et al. Microbial prevalence, diversity and abundance in amniotic fluid during preterm labor: a molecular and culture-based investigation. *PLoS One*. 2008; 3:e3056. [PubMed: 18725970]
63. Yoon BH, Kim CJ, Romero R, Jun JK, Park KH, Choi ST, et al. Experimentally induced intrauterine infection causes fetal brain white matter lesions in rabbits. *Am J Obstet Gynecol*. 1997; 177:797–802. [PubMed: 9369822]
64. Dean JM, Farrag D, Zahkhouk SA, El Zawahry EY, Hagberg H, Kjellmer I, et al. Cerebellar white matter injury following systemic endotoxemia in preterm fetal sheep. *Neuroscience*. 2009; 160:606–15. [PubMed: 19285118]
65. Gavilanes AW, Strackx E, Kramer BW, Gantert M, Van den Hove D, Steinbusch H, et al. Chorioamnionitis induced by intraamniotic lipopolysaccharide resulted in an interval-dependent increase in central nervous system injury in the fetal sheep. *Am J Obstet Gynecol*. 2009; 200:437e1–8. [PubMed: 19217590]
66. Lodygensky GA, West T, Stump M, Holtzman DM, Inder TE, Neil JJ. In vivo MRI analysis of an inflammatory injury in the developing brain. *Brain Behav Immun*. 2010; 24:759–67. [PubMed: 19945527]
67. Wallace K, Veerisetty S, Paul I, May W, Miguel-Hidalgo JJ, Bennett W. Prenatal infection decreases calbindin, decreases Purkinje cell volume and density and produces long-term motor deficits in Sprague-Dawley rats. *Dev Neurosci*. 2010; 32:302–12. [PubMed: 20948182]
68. Sarkar S, Kaplan C, Wiswell TE, Spitzer AR. Histological chorioamnionitis and the risk of early intraventricular hemorrhage in infants born < or =28 weeks gestation. *J Perinatol*. 2005; 25:749–52. [PubMed: 16237461]
69. Richardson BS, Wakim E, daSilva O, Walton J. Preterm histologic chorioamnionitis: impact on cord gas and pH values and neonatal outcome. *Am J Obstet Gynecol*. 2006; 195:1357–65. [PubMed: 16677589]
70. Rocha G, Proenca E, Quintas C, Rodrigues T, Guimaraes H. Chorioamnionitis and brain damage in the preterm newborn. *J Matern Fetal Neonatal Med*. 2007; 20:745–9. [PubMed: 17763276]
71. Moss TJ, Nitsos I, Kramer BW, Ikegami M, Newnham JP, Jobe AH. Intra-amniotic endotoxin induces lung maturation by direct effects on the developing respiratory tract in preterm sheep. *Am J Obstet Gynecol*. 2002; 187:1059–65. [PubMed: 12389005]
72. Jobe AH, Newnham JP, Willet KE, Moss TJ, Gore Ervin M, Padbury JF, et al. Endotoxin-induced lung maturation in preterm lambs is not mediated by cortisol. *Am J Respir Crit Care Med*. 2000; 162:1656–61. [PubMed: 11069792]
73. Shimoya K, Taniguchi T, Matsuzaki N, Moriyama A, Murata Y, Kitajima H, et al. Chorioamnionitis decreased incidence of respiratory distress syndrome by elevating fetal interleukin-6 serum concentration. *Hum Reprod*. 2000; 15:2234–40. [PubMed: 11006206]
74. Lee J, Seong HS, Kim BJ, Jun JK, Romero R, Yoon BH. Evidence to support that spontaneous preterm labor is adaptive in nature: neonatal RDS is more common in ‘indicated’ than in ‘spontaneous’ preterm birth. *J Perinat Med*. 2009; 37:53–8. [PubMed: 19099368]
75. Tsuda H, Takahashi Y, Iwagaki S, Kawabata I, Hayakawa H, Kotani T, et al. Intra-amniotic infection increases amniotic lamellar body count before 34 weeks of gestation. *J Matern Fetal Neonatal Med*. 2010; 23:1230–6. [PubMed: 20158396]
76. Morken NH, Kallen K, Jacobsson B. Outcomes of preterm children according to type of delivery onset: a nationwide population-based study. *Paediatr Perinat Epidemiol*. 2007; 21:458–64. [PubMed: 17697076]
77. 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; 185:1156–61. [PubMed: 11717650]
78. Salafia CM, Minior VK, Rosenkrantz TS, Pezzullo JC, Popek EJ, Cusick W, et al. Maternal, placental, and neonatal associations with early germinal matrix/intraventricular hemorrhage in infants born before 32 weeks’ gestation. *Am J Perinatol*. 1995; 12:429–36. [PubMed: 8579656]
79. Huleihel M, Golan H, Hallak M. Intrauterine infection/inflammation during pregnancy and offspring brain damages: possible mechanisms involved. *Reprod Biol Endocrinol*. 2004; 2:17. [PubMed: 15104793]

80. Ugwumadu A. Infection and fetal neurologic injury. *Curr Opin Obstet Gynecol.* 2006; 18:106–11. [PubMed: 16601469]
81. Perlman JM. White matter injury in the preterm infant: an important determination of abnormal neurodevelopment outcome. *Early Hum Dev.* 1998; 53:99–120. [PubMed: 10195704]
82. Andrews T, Zhang P, Bhat NR. TNFalpha potentiates IFNgamma-induced cell death in oligodendrocyte progenitors. *J Neurosci Res.* 1998; 54:574–83. [PubMed: 9843148]
83. Kahn MA, De Vellis J. Regulation of an oligodendrocyte progenitor cell line by the interleukin-6 family of cytokines. *Glia.* 1994; 12:87–98. [PubMed: 7532622]
84. Hurwitz AA, Lyman WD, Guida MP, Calderon TM, Berman JW. Tumor necrosis factor alpha induces adhesion molecule expression on human fetal astrocytes. *J Exp Med.* 1992; 176:1631–6. [PubMed: 1281214]
85. Cobo T, Palacio M, Martinez-Terron M, Navarro-Sastre A, Bosch J, Filella X, et al. Clinical and inflammatory markers in amniotic fluid as predictors of adverse outcomes in preterm premature rupture of membranes. *Am J Obstet Gynecol.* 2011; 205:126e1–8. [PubMed: 21621184]
86. Kim KW, Romero R, Park HS, Park CW, Shim SS, Jun JK, et al. A rapid matrix metalloproteinase-8 bedside test for the detection of intraamniotic inflammation in women with preterm premature rupture of membranes. *Am J Obstet Gynecol.* 2007; 197:292e1–5. [PubMed: 17826425]
87. Jacobsson B, Mattsby-Baltzer I, Andersch B, Bokstrom H, Holst RM, Nikolaitchouk N, et al. Microbial invasion and cytokine response in amniotic fluid in a Swedish population of women with preterm prelabor rupture of membranes. *Acta Obstet Gynecol Scand.* 2003; 82:423–31. [PubMed: 12752072]
88. Jacobsson B, Mattsby-Baltzer I, Andersch B, Bokstrom H, Holst RM, Wennerholm UB, et al. Microbial invasion and cytokine response in amniotic fluid in a Swedish population of women in preterm labor. *Acta Obstet Gynecol Scand.* 2003; 82:120–8. [PubMed: 12648172]
89. Yoon BH, Jun JK, Romero R, Park KH, Gomez R, Choi J-H, et al. Amniotic fluid inflammatory cytokines (interleukin-6, interleukin-1 β , and tumor necrosis factor- α), neonatal brain white matter lesions, and cerebral palsy. *Am J Obstet Gynecol.* 1997; 177:19–26. [PubMed: 9240577]
90. Oh KJ, Park KH, Kim SN, Jeong EH, Lee SY, Yoon HY. Predictive value of intra-amniotic and serum markers for inflammatory lesions of preterm placenta. *Placenta.* 2011; 32:732–6. [PubMed: 21839511]
91. Lee SE, Romero R, Jung H, Park CW, Park JS, Yoon BH. The intensity of the fetal inflammatory response in intraamniotic inflammation with and without microbial invasion of the amniotic cavity. *Am J Obstet Gynecol.* 2007; 197:294e1–6. [PubMed: 17826426]
92. Lee SE, Romero R, Park CW, Jun JK, Yoon BH. The frequency and significance of intraamniotic inflammation in patients with cervical insufficiency. *Am J Obstet Gynecol.* 2008; 198:633e1–8. [PubMed: 18342290]
93. Nien JK, Yoon BH, Espinoza J, Kusanovic JP, Erez O, Soto E, et al. A rapid MMP-8 bedside test for the detection of intra-amniotic inflammation identifies patients at risk for imminent preterm delivery. *Am J Obstet Gynecol.* 2006; 195:1025–30. [PubMed: 17000236]
94. Gotsch F, Romero R, Chaiworapongsa T, Erez O, Vaisbuch E, Espinoza J, et al. Evidence of the involvement of caspase-1 under physiologic and pathologic cellular stress during human pregnancy: a link between the inflammasome and parturition. *J Matern Fetal Neonatal Med.* 2008; 21:605–16. [PubMed: 18828051]
95. Holst RM, Laurini R, Jacobsson B, Samuelsson E, Savman K, Doverhag C, et al. Expression of cytokines and chemokines in cervical and amniotic fluid: relationship to histological chorioamnionitis. *J Matern Fetal Neonatal Med.* 2007; 20:885–93. [PubMed: 18050018]
96. Mazaki-Tovi S, Romero R, Kusanovic JP, Erez O, Gotsch F, Mittal P, et al. Visfatin/Pre-B cell colony-enhancing factor in amniotic fluid in normal pregnancy, spontaneous labor at term, preterm labor and prelabor rupture of membranes: an association with subclinical intrauterine infection in preterm parturition. *J Perinat Med.* 2008; 36:485–96. [PubMed: 18598235]
97. Bashiri A, Horowitz S, Huleihel M, Hackmon R, Dukler D, Mazor M. Elevated concentrations of interleukin-6 in intra-amniotic infection with *Ureaplasma urealyticum* in asymptomatic women during genetic amniocentesis. *Acta Obstet Gynecol Scand.* 1999; 78:379–82. [PubMed: 10326880]

98. Biggio JR Jr, Ramsey PS, Cliver SP, Lyon MD, Goldenberg RL, Wenstrom KD. Midtrimester amniotic fluid matrix metalloproteinase-8 (MMP-8) levels above the 90th percentile are a marker for subsequent preterm premature rupture of membranes. *Am J Obstet Gynecol.* 2005; 192:109–13. [PubMed: 15672011]
99. Fortunato SJ, Menon R. Screening of novel matrix metalloproteinases (MMPs) in human fetal membranes. *J Assist Reprod Genet.* 2002; 19:483–6. [PubMed: 12416653]
100. Maymon E, Romero R, Pacora P, Gomez R, Athayde N, Edwin S, et al. Human neutrophil collagenase (matrix metalloproteinase 8) in parturition, premature rupture of the membranes, and intrauterine infection. *Am J Obstet Gynecol.* 2000; 183:94–9. [PubMed: 10920315]
101. Andrews WW, Hauth JC, Goldenberg RL, Gomez R, Romero R, Cassell GH. Amniotic fluid interleukin-6: correlation with upper genital tract microbial colonization and gestational age in women delivered after spontaneous labor versus indicated delivery. *Am J Obstet Gynecol.* 1995; 173:606–12. [PubMed: 7645642]
102. Coultrip LL, Lien JM, Gomez R, Kapernick P, Khoury A, Grossman JH. The value of amniotic fluid interleukin-6 determination in patients with preterm labor and intact membranes in the detection of microbial invasion of the amniotic cavity. *Am J Obstet Gynecol.* 1994; 171:901–11. [PubMed: 7943100]
103. Romero R, Yoon BH, Kenney JS, Gomez R, Allison AC, Sehgal PB. Amniotic fluid interleukin-6 determinations are of diagnostic and prognostic value in preterm labor. *Am J Reprod Immunol.* 1993; 30:167–83. [PubMed: 8311926]
104. Park CW, Lee SM, Park JS, Jun JK, Romero R, Yoon BH. The antenatal identification of funisitis with a rapid MMP-8 bedside test. *J Perinat Med.* 2008; 36:497–502. [PubMed: 19127606]
105. Romero R, Quintero R, Nore J, Avila C, Mazor M, Hanaoka S, et al. Amniotic fluid white blood cell count: a rapid and simple test to diagnose microbial invasion of the amniotic cavity and predict preterm delivery. *Am J Obstet Gynecol.* 1991; 165:821–30. [PubMed: 1951538]
106. Yoon BH, Jun JK, Park KH, Syn HC, Gomez R, Romero R. Serum C-reactive protein, white blood cell count, and amniotic fluid white blood cell count in women with preterm premature rupture of membranes. *Obstet Gynecol.* 1996; 88:1034–40. [PubMed: 8942849]
107. Yoon BH, Yang SH, Jun JK, Park KH, Kim CJ, Romero R. Maternal blood C-reactive protein, white blood cell count, and temperature in preterm labor: a comparison with amniotic fluid white blood cell count. *Obstet Gynecol.* 1996; 87:231–7. [PubMed: 8559530]
108. Pacora P, Chaiworapongsa T, Maymon E, Kim YM, Gomez R, Yoon BH, et al. Funisitis and chorionic vasculitis: the histological counterpart of the fetal inflammatory response syndrome. *J Matern Fetal Neonatal Med.* 2002; 11:18–25. [PubMed: 12380603]
109. Kim CJ, Yoon BH, Park SS, Kim MH, Chi JG. Acute funisitis of preterm but not term placentas is associated with severe fetal inflammatory response. *Hum Pathol.* 2001; 32:623–9. [PubMed: 11431717]
110. Park CW, Moon KC, Park JS, Jun JK, Romero R, Yoon BH. The involvement of human amnion in histologic chorioamnionitis is an indicator that a fetal and an intra-amniotic inflammatory response is more likely and severe: clinical implications. *Placenta.* 2009; 30:56–61. [PubMed: 19046766]
111. Romero R, Salafia CM, Athanassiadis AP, Hanaoka S, Mazor M, Sepulveda W, et al. The relationship between acute inflammatory lesions of the preterm placenta and amniotic fluid microbiology. *Am J Obstet Gynecol.* 1992; 166:1382–8. [PubMed: 1595794]
112. Smulian JC, Vintzileos AM, Lai YL, Santiago J, Shen-Schwarz S, Campbell WA. Maternal chorioamnionitis and umbilical vein interleukin-6 levels for identifying early neonatal sepsis. *J Matern Fetal Med.* 1999; 8:88–94. [PubMed: 10338061]
113. Yoon BH, Romero R, Shim JY, Shim SS, Kim CJ, Jun JK. C-reactive protein in umbilical cord blood: a simple and widely available clinical method to assess the risk of amniotic fluid infection and funisitis. *J Matern Fetal Neonatal Med.* 2003; 14:85–90. [PubMed: 14629087]
114. Yoon BH, Romero R, Park JS, Kim M, Oh SY, Kim CJ, et al. The relationship among inflammatory lesions of the umbilical cord (funisitis), umbilical cord plasma interleukin 6 concentration, amniotic fluid infection, and neonatal sepsis. *Am J Obstet Gynecol.* 2000; 183:1124–9. [PubMed: 11084553]

115. Chaiworapongsa T, Romero R, Kim JC, Kim YM, Blackwell SC, Yoon BH, et al. Evidence for fetal involvement in the pathologic process of clinical chorioamnionitis. *Am J Obstet Gynecol.* 2002; 186:1178–82. [PubMed: 12066094]
116. Romero R, Chaemsathong P, Docheva N, Korzeniewski SJ, Tarca AL, Bhatti G, et al. Clinical chorioamnionitis at term V: umbilical cord plasma cytokine profile in the context of a systemic maternal inflammatory response. *J Perinat Med.* 2016; 44:53–76. [PubMed: 26360486]
117. Moon JB, Kim JC, Yoon BH, Romero R, Kim G, Oh SY, et al. Amniotic fluid matrix metalloproteinase-8 and the development of cerebral palsy. *J Perinat Med.* 2002; 30:301–6. [PubMed: 12235718]
118. Seong HS, Lee SE, Kang JH, Romero R, Yoon BH. The frequency of microbial invasion of the amniotic cavity and histologic chorioamnionitis in women at term with intact membranes in the presence or absence of labor. *Am J Obstet Gynecol.* 2008; 199:375e1–5. [PubMed: 18928978]
119. Sampson JE, Theve RP, Blatman RN, Shipp TD, Bianchi DW, Ward BE, et al. Fetal origin of amniotic fluid polymorphonuclear leukocytes. *Am J Obstet Gynecol.* 1997; 176:77–81. [PubMed: 9024093]
120. Sun H, Zhou Y, Xiong H, Kang W, Xu B, Liu D, et al. Prognosis of very preterm infants with severe respiratory distress syndrome receiving mechanical ventilation. *Lung.* 2015; 193:249–54. [PubMed: 25583617]
121. Vergani P, Patane L, Doria P, Borroni C, Cappellini A, Pezzullo JC, et al. Risk factors for neonatal intraventricular haemorrhage in spontaneous prematurity at 32 weeks gestation or less. *Placenta.* 2000; 21:402–7. [PubMed: 10833376]
122. Mullaart RA, Hopman JC, Rotteveel JJ, Daniels O, Stoelinga GB, De Haan AF. Cerebral blood flow fluctuation in neonatal respiratory distress and periventricular haemorrhage. *Early Hum Dev.* 1994; 37:179–85. [PubMed: 7925076]
123. Kusuda S, Ito Y, Kim TJ, Miyagi N, Shishida N, Tanaka Y. Cerebral hemodynamics after exogenous surfactant administration for respiratory distress syndrome in piglet model. *J Perinat Med.* 2000; 28:363–71. [PubMed: 11125926]
124. Perlman JM, Goodman S, Kreusser KL, Volpe JJ. Reduction in intraventricular hemorrhage by elimination of fluctuating cerebral blood-flow velocity in preterm infants with respiratory distress syndrome. *N Engl J Med.* 1985; 312:1353–7. [PubMed: 3887165]
125. Lemmers PM, Toet M, van Schelven LJ, van Bel F. Cerebral oxygenation and cerebral oxygen extraction in the preterm infant: the impact of respiratory distress syndrome. *Exp Brain Res.* 2006; 173:458–67. [PubMed: 16506004]
126. Lauterbach MD, Raz S, Sander CJ. Neonatal hypoxic risk in preterm birth infants: the influence of sex and severity of respiratory distress on cognitive recovery. *Neuropsychology.* 2001; 15:411–20. [PubMed: 11499996]
127. Nelson KB, Grether JK. Potentially asphyxiating conditions and spastic cerebral palsy in infants of normal birth weight. *Am J Obstet Gynecol.* 1998; 179:507–13. [PubMed: 9731861]
128. Girard S, Kadhim H, Beaudet N, Sarret P, Sebire G. Developmental motor deficits induced by combined fetal exposure to lipopolysaccharide and early neonatal hypoxia/ischemia: a novel animal model for cerebral palsy in very premature infants. *Neuroscience.* 2009; 158:673–82. [PubMed: 19010395]
129. Larouche A, Roy M, Kadhim H, Tsanaclis AM, Fortin D, Sebire G. Neuronal injuries induced by perinatal hypoxic-ischemic insults are potentiated by prenatal exposure to lipopolysaccharide: animal model for perinatally acquired encephalopathy. *Dev Neurosci.* 2005; 27:134–42. [PubMed: 16046847]
130. Eklind S, Mallard C, Leverin AL, Gilland E, Blomgren K, Mattsby-Baltzer I, et al. Bacterial endotoxin sensitizes the immature brain to hypoxic--ischaemic injury. *Eur J Neurosci.* 2001; 13:1101–6. [PubMed: 11285007]
131. Hagberg H, Peebles D, Mallard C. Models of white matter injury: comparison of infectious, hypoxic-ischemic, and excitotoxic insults. *Ment Retard Dev Disabil Res Rev.* 2002; 8:30–8. [PubMed: 11921384]
132. Choi EK, Park D, Kim TK, Lee SH, Bae DK, Yang G, et al. Animal models of periventricular leukomalacia. *Lab Anim Res.* 2011; 27:77–84. [PubMed: 21826166]

133. Brochu ME, Girard S, Lavoie K, Sebire G. Developmental regulation of the neuroinflammatory responses to LPS and/or hypoxia-ischemia between preterm and term neonates: An experimental study. *J Neuroinflammation*. 2011; 8:55. [PubMed: 21599903]
134. Maxwell JR, Denson JL, Joste NE, Robinson S, Jantzie LL. Combined in utero hypoxia-ischemia and lipopolysaccharide administration in rats induces chorioamnionitis and a fetal inflammatory response syndrome. *Placenta*. 2015; 36:1378–84. [PubMed: 26601766]
135. Jantzie LL, Corbett CJ, Berglass J, Firl DJ, Flores J, Mannix R, et al. Complex pattern of interaction between in utero hypoxia-ischemia and intra-amniotic inflammation disrupts brain development and motor function. *J Neuroinflammation*. 2014; 11:131. [PubMed: 25082427]
136. Roberts D, Dalziel S. Antenatal corticosteroids for accelerating fetal lung maturation for women at risk of preterm birth. *Cochrane Database Syst Rev*. 2006:CD004454. [PubMed: 16856047]
137. Deulofeut R, Sola A, Lee B, Buchter S, Rahman M, Rogido M. The impact of vaginal delivery in premature infants weighing less than 1,251 grams. *Obstet Gynecol*. 2005; 105:525–31. [PubMed: 15738019]
138. Ment LR, Oh W, Ehrenkranz RA, Philip AG, Duncan CC, Makuch RW. Antenatal steroids, delivery mode, and intraventricular hemorrhage in preterm infants. *Am J Obstet Gynecol*. 1995; 172:795–800. [PubMed: 7892866]
139. Dani C, Poggi C, Bertini G, Pratesi S, Di Tommaso M, Scarselli G, et al. Method of delivery and intraventricular haemorrhage in extremely preterm infants. *J Matern Fetal Neonatal Med*. 2010; 23:1419–23. [PubMed: 20236026]
140. Minguez-Milio JA, Alcazar JL, Auba M, Ruiz-Zambrana A, Minguez J. Perinatal outcome and long-term follow-up of extremely low birth weight infants depending on the mode of delivery. *J Matern Fetal Neonatal Med*. 2011; 24:1235–8. [PubMed: 21381880]
141. Malloy MH, Onstad L, Wright E. The effect of cesarean delivery on birth outcome in very low birth weight infants. National Institute of Child Health and Human Development Neonatal Research Network. *Obstet Gynecol*. 1991; 77:498–503. [PubMed: 2002969]

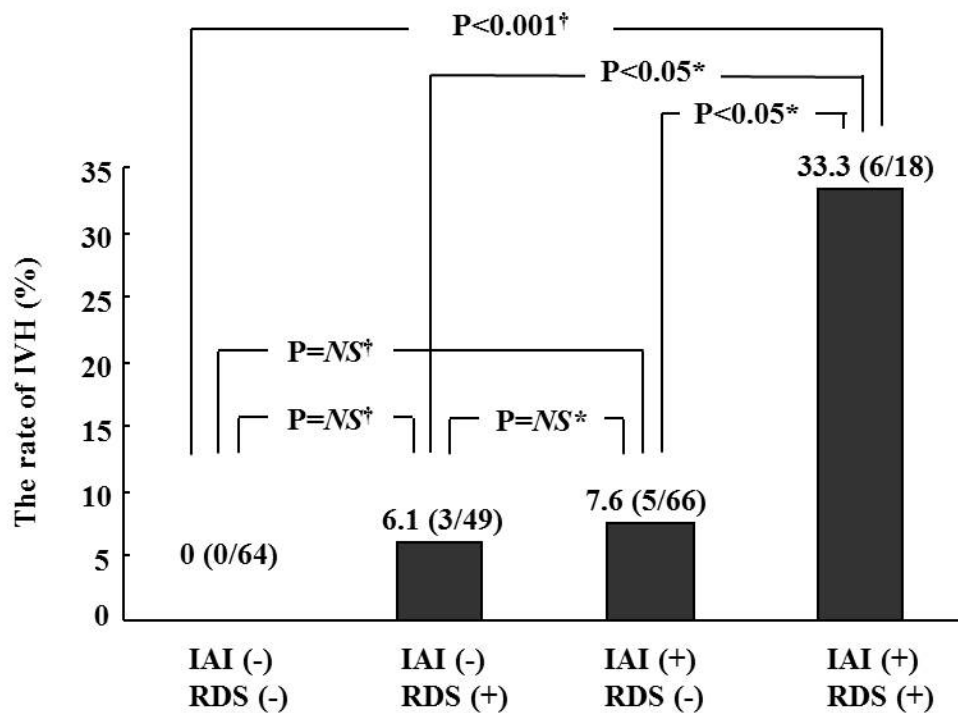


Figure 1. The rate of the development of intraventricular hemorrhage (IVH) according to the presence or absence of intra-amniotic inflammation and the occurrence of neonatal respiratory distress syndrome (RDS)

Intra-amniotic inflammation was defined as an elevated amniotic fluid MMP-8 concentration (>23 ng/mL). *Each P-value was adjusted for gestational age at birth. †P-value could not be adjusted for gestational age at birth because no patient without intra-amniotic inflammation and RDS had an IVH. Patients with intra-amniotic inflammation and neonatal RDS had a higher risk of the development of IVH than those with neonatal RDS alone [odds ratio (OR), 5.63; 95% CI, 1.03–30.9; $P<0.05$] and those with intra-amniotic inflammation alone [OR, 4.56; 95% CI, 1.08–19.3; $P<0.05$] after adjusting for gestational age at birth.

Table 1

Characteristics of neurosonographic examinations performed in the study population

Characteristics	Number of neonates (n=207)
No. of neurosonographic examinations	
1	79.2% (164)
2	17.9% (37)
3	1.9% (4)
4	1.0% (2)
Postnatal day at neurosonographic examination	
3days	63.8% (132)
4–7days	21.3% (44)
Both (3days and 4–7days)	15.0% (31)
Grade of PV-IVH by neurosonographic examination *	
0	67.6% (140)
I	25.6% (53)
II	4.8% (10)
III	1.9% (4)
IVH [†]	
Yes	6.8% (14)
No	93.2% (193)

Values are given as % (n).

* Sonographic finding of PV-IVH was graded by McMenamin's classification.

[†] IVH was defined as PV-IVH of grade II or higher by McMenamin's classification.

IVH: intraventricular hemorrhage; PV-IVH: periventricular-intraventricular hemorrhage.

Table 2

Clinical characteristics of the study population according to the development of intraventricular hemorrhage

Characteristics	IVH (–) (n=193)	IVH (+) (n=14)	P-value
Maternal age (years)	31.4 ± 4.4	31.6 ± 4.3	NS
Nulliparity	48.2% (93/193)	35.7% (5/14)	NS
Gestational age at birth (weeks)	29.8 ± 2.2	27.8 ± 2.3	0.003
Antenatal corticosteroid use	74.1% (143/193)	92.9% (13/14)	NS
Cesarean delivery	69.4% (134/193)	64.3% (9/14)	NS
Spontaneous preterm birth	55.4% (107/193)	78.6% (11/14)	NS
Male	46.1% (89/193)	71.4% (10/14)	0.095
Birthweight, grams	1282 ± 427	1086 ± 456	0.095
Fetal growth restriction	26.9% (52/193)	21.4% (3/14)	NS
Apgar score <4 at 1min	27.5% (53/193)	42.9% (6/14)	NS
Apgar score <7 at 5min	44.6% (86/193)	64.3% (9/14)	NS
Umbilical arterial pH <7.15 [*]	10.2% (18/176)	15.4% (2/13)	NS
Respiratory distress syndrome (RDS)	32.6% (63/193)	64.3% (9/14)	0.022
AF MMP-8 concentration (ng/mL) [†]	6.0 (0.3–6386.3)	358.0 (0.3–4202.7)	0.011
AF MMP-8 >23 ng/mL [†]	39.9% (73/183)	78.6% (11/14)	0.009
Acute histologic chorioamnionitis [‡]	41.4% (72/174)	75.0% (9/12)	0.033
Acute funisitis [§]	20.5% (36/176)	41.7% (5/12)	NS

Values are given as mean ± standard deviation or median (range) or % (n/N).

^{*} Eighteen patients whose umbilical arterial pH was not measured were excluded from the analysis.

[†] MMP-8 concentration was not measured in 10 patients because of the lack of remaining AF.

[‡] Twenty-one patients were excluded because their placental histologic examinations were not performed.

[§] Nineteen patients were excluded because the presence or absence of funisitis was not determined.

AF: amniotic fluid; IVH: intraventricular hemorrhage; MMP-8: matrix metalloproteinase-8; NS, not significant.

Table 3

Relationship among various variables with the development of intraventricular hemorrhage analyzed by overall logistic regression

	Univariate analysis		Multivariate analysis	
	OR (95% CI)	OR (95% CI)*	OR (95% CI) [†]	OR (95% CI) [‡]
Gestational age at birth (weeks)	0.69 (0.55–0.88)	0.70 (0.52–0.94)	0.87 (0.66–1.16)	0.77 (0.56–1.05)
Male gender	2.92 (0.89–9.64)	2.62 (0.69–10.0)	3.30 (0.90–12.1)	1.98 (0.49–7.96)
Respiratory distress syndrome	3.71 (1.20–11.5)	2.70 (0.69–10.5)	6.50 (1.69–25.1)	4.63 (1.10–19.4)
Acute histologic chorioamnionitis	4.25 (1.11–16.2)	3.80 (0.84–17.3)		0.70 (0.11–4.43)
Intra-amniotic inflammation (defined as AF MMP-8 concentration >23 ng/mL)	5.53 (1.49–20.5)		7.88 (1.63–30.1)	25.3 (1.89–338.6)

* All variables of P<0.1 in the univariate analysis except the presence or absence of intraamniotic inflammation were included.

[†] All variables of P<0.1 in the univariate analysis except the presence or absence of histologic chorioamnionitis were included.

[‡] All variables of P<0.1 in the univariate analysis were included.

AF: amniotic fluid; MMP-8: matrix metalloproteinase-8; OR: odds ratio.

Clinical characteristics and outcomes of the study population according to the presence or absence of intra-amniotic inflammation and the occurrence of neonatal respiratory distress syndrome

Characteristics	IAI (-)/RDS (-) (n=64)	IAI (-)/RDS (+) (n=49)	IAI (+)/RDS (-) (n=66)	IAI (+)/RDS (+) (n=18)	P-value*
Gestational age at birth (weeks)	31.0 ± 2.4 [§]	29.5 ± 4.2 ^{††}	29.3 ± 2.4 ^{††}	27.1 ± 4.8	<.001
Birthweight, gram	1255 ± 457	1245 ± 434	1349 ± 422	1086 ± 391	NS
Fetal growth restriction	54.7% (35/64) ^{†,‡,§}	24.5% (12/49) ^{**††}	7.6% (5/66)	0% (0/18)	<.001
Cesarean delivery	85.9% (55/64) ^{‡,§}	91.8% (45/49) ^{**††}	43.9% (29/66)	38.9% (7/11)	<.001
Spontaneous preterm birth	34.4% (22/64) ^{‡,§}	24.5% (12/49) ^{**††}	95.5% (63/66)	100% (18/18)	<.001
Male	48.4% (31/64)	46.9% (23/49)	47.0% (31/66)	50.0% (9/18)	NS
Apgar score <4 at 1 minute	25.0% (16/64)	40.8% (20/49) ^{**††}	16.7% (11/66) ^{‡†}	50.0% (9/18)	.005
Apgar score <7 at 5 minutes	37.5% (24/64) ^{†,§}	61.2% (30/49) ^{**††}	36.4% (24/66) ^{‡†}	66.7% (12/18)	.007
Umbilical arterial pH <7.15	11.7% (7/60)	22.4% (11/49) ^{**}	1.9% (1/54)	5.9% (1/17)	.009
Intraventricular hemorrhage	0% (0/64) [§]	6.1% (3/49) ^{††}	7.6% (5/66) ^{‡†}	33.3% (6/18)	<.001

Values are given as mean ± standard deviation or % (n/N).

* P value for the overall group comparison.

[†] Significant difference between IAI (-)/RDS (-) and IAI (-)/RDS (+)

[‡] Significant difference between IAI (-)/RDS (-) and IAI (+)/RDS (-)

[§] Significant difference between IAI (-)/RDS (-) and IAI (+)/RDS (+)

^{**} Significant difference between IAI (-)/RDS (+) and IAI (+)/RDS (-)

^{††} Significant difference between IAI (-)/RDS (+) and IAI (+)/RDS (+)

^{‡†} Significant difference between IAI (+)/RDS (-) and IAI (+)/RDS (+)

IAI: intra-amniotic inflammation; NS: not significant; RDS, respiratory distress syndrome.