

Chorioamnionitis and Outcome in Extremely Preterm Infants

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Abstract

Introduction: Chorioamnionitis is a risk factor for preterm delivery. Intrauterine infection leads to the fetal inflammatory response which is characterised by elevated cytokine levels. Chorioamnionitis is reported to cause accelerated but abnormal lung maturation, resulting in decreased incidence of respiratory distress syndrome (RDS) but increased chronic lung disease (CLD), and predisposes the infant to cerebral injury. **Objective:** To investigate the relation between chorioamnionitis and RDS, CLD, cerebral lesions, neurodevelopmental outcome and mortality in a cohort of extremely premature infants. **Materials and Methods:** Infants born between 1997 and 2001 with a gestational age of less than 28 weeks or a birth weight of less than 1000 g were divided into two groups: Group 1 with evidence of chorioamnionitis and Group 2 without. Outcomes of these two groups of infants were compared. **Results:** A total of 388 infants were included (105 in Group 1 and 283 in Group 2). Chorioamnionitis was significantly associated with an increased risk of extreme preterm delivery. Group 1 showed a trend towards an increased incidence of CLD and mortality, while the incidence of periventricular leukomalacia, retinopathy of prematurity (ROP) and necrotising enterocolitis (NEC) were similar between the two groups. Subgroup analysis of 2-year neurodevelopmental outcome showed an increased trend towards cerebral palsy and visual impairment, while the incidence of developmental delay and hearing impairment are similar between the two groups. **Conclusion:** Extremely preterm infants with chorioamnionitis showed a trend towards an increased incidence of CLD, mortality, cerebral palsy and visual impairment, but a decreased risk of RDS.

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Key words: Cerebral palsy, Chorioamnionitis, Chronic lung disease, Prematurity

Introduction

Preterm delivery is associated with considerable morbidity and mortality, especially among the 1% to 2% infants delivered at less than 32 weeks.¹ Chorioamnionitis is a major predisposing factor for preterm delivery. It is associated with a 3-fold increased risk of preterm birth with intact membranes, and a 4-fold increased risk of preterm birth with prelabour rupture of membranes.² Chorioamnionitis is present in up to 45% of pregnancies with preterm labour and delivery at 23 to 26 weeks' gestation and 18% at 27 to 30 weeks' gestation.³⁻⁵ Intra-uterine infection causes an inflammatory response in both mother and fetus. On the maternal side, chorioamnionitis triggers cytokine release, which in turn leads to premature rupture of membranes, cervical ripening and uterine contractions.⁶ On the fetal side, chorioamnionitis causes the fetal inflammatory response syndrome, characterised by raised pro-inflammatory cytokines (IL-1 β , IL-6, IL-8 and tumour

necrosis factor α) in the amniotic fluid or fetal blood.^{5,7} This is postulated to cause accelerated but abnormal maturation of the fetal lung, leading to a decreased incidence of respiratory distress syndrome (RDS), but an increased incidence of chronic lung disease (CLD).^{8,9} It had also been related to an increased risk of white matter lesions in the brain of preterm infants among survivors.¹⁰⁻¹² In this study, we investigated the relationship between chorioamnionitis and the development of RDS, CLD, cerebral lesions and mortality in a cohort of extremely preterm infants.

Materials and Methods

Subjects

The study cohort consisted of all infants at Monash Medical Centre in the 5-year period between 1 January 1997 and 31 December 2001, with a gestational age of less than 28 weeks or a birth weight of less than 1000 g. Infants

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with chromosomal disorders or major congenital anomalies were excluded. Infants were divided into two groups: *Group 1* consisted of all infants with clinical or histological evidence of chorioamnionitis, and *Group 2* consisted of all other infants with no evidence of chorioamnionitis. Clinical chorioamnionitis was defined as the presence of maternal fever above 37.8°C and two or more of the following signs and symptoms: maternal tachycardia (>100/min), fetal tachycardia (>160/min), uterine tenderness, foul-smelling amniotic fluid, maternal leukocytosis (>15,000/mm³) and raised maternal C-reactive protein (CRP, >6mg/L). Histological chorioamnionitis was defined as the presence of acute inflammatory changes on examination of the placenta, membranes or umbilical cord.

Data Collection

Perinatal and neonatal data were prospectively collected. Detailed analysis of long-term outcome was also performed on one particular subgroup which included all infants in both Groups 1 and 2 in the year 1997. All survivors in the 1997 subgroup were recalled at 2 years of age, corrected for prematurity, when a detailed physical and developmental assessment (Bayley Scales of Infant Development) as well as ophthalmology and audiology examinations were performed.

Outcome Measures

The two groups of infants were compared for (1) mortality, defined as death before hospital discharge; (2) morbidity including (a) RDS, defined by characteristic radiological changes; (b) CLD, defined by need of supplemental oxygen at 36 weeks post-conceptual age; (c) necrotising enterocolitis (NEC), defined by the radiological finding of intramural bowel gas; (d) retinopathy of prematurity (ROP), assessed by routine ophthalmologic examination beginning at day 28 or 32 weeks post-conceptual age; and (e) periventricular haemorrhage and leukomalacia (PVH and

PVL), assessed by routine cerebral ultrasonographic examination beginning within the first week after birth, and at regular intervals thereafter; and (3) neuro-developmental outcome of all survivors from the 1997 subgroup, including cerebral palsy, developmental delay [defined as Mental Developmental Index (MDI) on the Bayley Scales of Infant Development below 70], visual impairment (defined as bilateral blindness), and hearing impairment (defined as hearing loss that required a hearing aid).

Statistical Methods

Statistical analyses were performed using the Student's *t*-test, chi-square test, Mann-Whitney U test and Fisher's exact test (Sigmastat).

Results

A total of 388 infants fulfilled the inclusion criteria, with 105 infants in Group 1 and 283 infants in Group 2. The percentage of infants with chorioamnionitis was 27%. There was no significant difference in gender, birth weight, and Apgar score at 1 minute between the 2 groups (Table I), but gestational age and Apgar score at 5 minutes is significantly lower in Group 1. Infants in Group 1 had a higher hospital mortality rate, a lower incidence of RDS, and a higher incidence of CLD compared with infants in Group 2, but the differences did not reach statistical significance (Table II). There was no difference in the incidence of PVL, PVH, ROP or NEC between the 2 groups. In the 1997 subgroup, infants in Group 1 had a higher 2-year mortality rate and a higher incidence of cerebral palsy and visual impairment compared with infants in Group 2 but, again, the differences did not reach statistical significance (Table III). There was no difference in the incidence of developmental delay, in the MDI scores, and in the incidence of hearing impairment between the 2 groups.

TABLE I: DEMOGRAPHIC DATA

	Group 1 (n = 105)	Group 2 (n = 283)	P value
Gestational age (week, median + 25 th and 75 th quartiles)	25 (24,26)	26 (25,28)	0.001
Birth weight (gram, median + 25 th and 75 th quartiles)	778 (618,888)	780 (605,910)	0.98
Antenatal corticosteroids	91/102 (89%)	226/275 (82%)	0.13
Gender (male:female)	59:46	154:129	0.84
Apgar score at 1 min (median + 25 th and 75 th quartile)	4 (1,6)	4 (2,6)	0.16
Apgar score at 5 min (median + 25 th and 75 th quartile)	7 (4,8)	8 (6,9)	0.006

TABLE II: HOSPITAL OUTCOME

	Group 1	Group 2	P value
Death	34/105 (32%)	74/283 (26%)	0.28
RDS	60/87 (69%)	189/249 (76%)	0.26
CLD	36/86 (42%)	88/248 (36%)	0.36
PVL	6/77 (7.8%)	16/230 (7.0%)	1.00
PVH >Grade 3	7/77 (9.1%)	22/228 (9.7%)	0.94
ROP >Stage 3	3/70 (4.3%)	12/187 (6.4%)	0.70
NEC	3/83 (3.6%)	10/245 (4.1%)	0.89

CLD: chronic lung disease; NEC: necrotising enterocolitis; PVH: periventricular haemorrhage; PVL: periventricular leukomalacia; RDS: respiratory distress syndrome

The different denominators denote the number of infants surviving at the time the outcome assessment was carried out.

TABLE III: LONG-TERM OUTCOME

	Group 1	Group 2	P value
Mortality	6/18 (33%)	9/54 (17%)	0.18
Cerebral palsy	3/12 (25%)	5/43 (12%)	0.35
Developmental delay	3/12 (25%)	11/43 (26%)	1.00
MDI score (median, quartiles)	83 (67, 91)	80 (65, 92)	0.83
Visual impairment	3/12 (25%)	7/43 (16%)	0.43
Hearing impairment	0/12 (0%)	1/43 (2%)	1.00

Discussion

Numerous studies have investigated the effect of chorioamnionitis and the resultant inflammatory response on neonatal outcome.⁷⁻¹³ Preterm delivery following chorioamnionitis has been associated with higher incidence of CLD,⁹ cystic PVL^{10,11} and cerebral palsy.¹² In recent years, biochemical studies have revealed a number of cytokines involved in the inflammatory process and speculated on possible mechanisms of action of these cytokines on different organ systems of the fetus.¹³⁻¹⁵ However, most of these studies have included more mature preterm infants of 28 to 35 weeks' gestation or birth weight of up to 1500 g. There had been few studies published solely on extremely preterm or extremely-low-birth-weight (ELBW) infants. These infants have a higher baseline mortality and morbidity rate and, as their immune system is more immature, their response to chorioamnionitis might not be the same as the more mature preterm infants. Most published studies only reported 1 or 2 specific outcomes, like CLD or cerebral palsy. There had been few studies looking at multiple outcome variables of chorioamnionitis, and entities like ROP or NEC have seldom been studied. In this study, we compared the short-term mortality and morbidity in a complete cohort of extremely preterm infants born less than 28 weeks' gestation or 1000 g birth weight with and without chorioamnionitis.

Chorioamnionitis and Preterm Delivery

Chorioamnionitis is defined by intrauterine bacterial infection affecting tissues of either fetal-maternal origin (choriodecidual space and placenta) or fetal origin (chorioamniotic membrane, amniotic fluid and umbilical cord).¹³ It was reported back in the 1950s that leukocytosis of the amniotic fluid or chorioamnion was associated with premature membrane rupture and labour.¹⁴ Positive bacterial culture from amniotic fluid of women in preterm labour with intact membranes was first reported in 1977.¹⁵ Since then, many studies have shown strong correlation between chorioamnionitis and premature delivery especially before 30 weeks' gestation.¹⁶ Recent studies on cytokine levels in amniotic fluid and fetal blood have provided evidence that an inflammatory response in both the mother and fetus is responsible for preterm delivery.⁷ Bacterial invasion of the choriodecidual space releases endotoxins and exotoxins, leading to cytokine release (TNF- α , IL-1 α , IL-1 β , IL6, IL8, and granulocyte colony-stimulating factor), thus stimulating prostaglandin production and metalloprotease synthesis.^{4,17,18} Metalloproteases cause softening of the cervix and rupture of chorioamniotic membranes, while prostaglandins stimulate uterine contractions.^{19,20} At the same time, inflammatory response in the fetus causes cytokine release and increases fetal adrenal cortisol production, which also contributes to preterm delivery.^{21,22}

In our cohort, 27% of our preterm infants had clinical or histological evidence of chorioamnionitis, which agrees with the percentage published in other studies. Also, the gestational age of infants in Group 1 was significantly lower than in Group 2, suggesting that chorioamnionitis may carry a higher risk of extremely preterm delivery. Although the median gestational ages differ only by 1 week, the clinical trends observed in this study might be related to and can be explained by the lower gestation in Group 1. Further studies using amniotic or fetal blood cytokine levels would be useful as an objective measure to identify those infants with intrauterine infection and inflammatory response in-utero.

Chorioamnionitis and RDS

In animal studies, intra-amniotic inflammation causes 'clinical maturation' of the lung.^{23,24} Intra-amniotic cytokine induces surfactant proteins SP-A and SP-B, and increases surfactant phospholipids in the immature lung.²⁵ Exposure to endotoxin in fetal sheep resulted in increased lung compliance, gas exchange and lung gas volumes; increased levels of mRNA for surfactant proteins; and increased percentage of fully processed SP-B.^{25,26} Cytokines bind to receptors in Type 2 alveolar cells and induce the transcription factors required for differentiation of the surfactant system, independent of the effect of glucocorticoids.²⁷ In humans, chorioamnionitis has been reported to be associated with a decreased RDS rate and an increased CLD rate in a study of 58 preterm infants with a birth weight of below 2000 g.⁹ In our study, we found a decreased incidence of RDS in the chorioamnionitis group, which agrees with previous studies, but the difference did not reach statistical significance. This may be because the lungs of our infants were still extremely immature at below 28 weeks. Therefore, although chorioamnionitis may accelerate alveoli maturation, its effect on preventing clinical RDS may not be as significant as in more mature preterm infants.

Chorioamnionitis and CLD

In our study, the CLD rate is 37%, which is comparable to that reported in previous studies (20% to 40%).²⁸ Although it is uncommon nowadays to see the classical CLD following exposure to high pressure ventilation and high oxygen concentrations, around 30% of preterm infants with minimal RDS requiring only gentle mechanical ventilation developed CLD.²⁹ An increasing number of epidemiological and biochemical studies have implicated chorioamnionitis and the fetal inflammatory response in the development of CLD in these infants. Experimental chorioamnionitis in sheep causes an injury that closely mimics the injury sequence in CLD. There is accumulation of granulocytes in the lung fluid, increased apoptosis in the lung parenchyma, and pro-inflammatory cytokine expression in lung tissue,

leading to formation of larger and fewer alveoli.^{25,30} In humans, epidemiological studies had demonstrated an increase in CLD rate with chorioamnionitis in preterm infants. Significantly higher levels of IL6, TNF- α , IL-1 β and IL8 were found in the amniotic fluid of infants who developed CLD.⁸ Higher levels of cytokines IL1, IL6, IL8 and TNF- α were found in the bronchoalveolar lavage of infants who subsequently developed CLD,³¹ while levels of anti-inflammatory cytokines IL-10 and IL-12 were found to be lower.^{32,33} A meta-analysis found that infants colonised with ureaplasma urealyticum, a common organism found in chorioamnionitis, are at risk of subsequently developing CLD.³⁴ Recent studies suggested that cytokines may also affect the alveolar capillary integrity by activating inflammatory cells to release proteolytic enzymes or by priming phagocytes to release toxic oxygen radicals.³⁵ The underlying pathophysiology for lung injury in chorioamnionitis is postulated to be due to a persistent inflammatory response in the immature airways, resulting in a disturbance of normal alveolar and pulmonary vascular development.^{8,35,36} It has also been postulated that chorioamnionitis initiates an injury response that 'primes' the fetal lung to have an amplified inflammatory reaction to the initiation of mechanical ventilation after preterm birth.²⁹ In our study, the incidence of CLD is increased in the chorioamnionitis group, which agrees with other studies, but the difference did not reach statistical significance. This may be due to the high baseline risk of CLD in these extremely preterm infants and the multifactorial nature of CLD.

Chorioamnionitis and Brain Injury

Cerebral white matter injury in the preterm neonate is a major precursor of subsequent cerebral palsy and cognitive deficit. Pro-inflammatory cytokines have been implicated as potential mediators of perinatal brain injury.³⁷ Experimentally-induced intrauterine infection or injection of bacterial endotoxin resulted in expression of cytokines in the fetal brains of kitten, rats and rabbits, producing white matter lesions similar to PVL.³⁸⁻⁴⁰ In humans, many studies have been done to investigate the relationship between intrauterine infection, PVL and cerebral palsy, with conflicting results. Cytokine studies have shown elevated IL-6 and IL-1 β in preterm infants with PVL.⁴¹ Elevated levels of IL-6, IL-8 as well as an increased rate of funisitis were also found in infants who developed cerebral palsy.¹⁰ However, other studies did not find an association between elevated cytokine levels and PVL or cranial ultrasonographic abnormalities.^{42,43} A recent meta-analysis showed that clinical chorioamnionitis is significantly associated with both cerebral palsy and cystic PVL, while histological chorioamnionitis is significantly associated with cystic PVL.⁴⁴ In term infants, epidemiological studies

suggest a strong association (odds ratio, 9-10) between clinical as well as histological chorioamnionitis and cerebral palsy.⁴⁵ In general, the association between cerebral injury and chorioamnionitis is much more definite in term compared to preterm neonates. In addition, the combination of infection and birth asphyxia with cerebral palsy is much stronger than either factor alone, suggesting a synergistic effect.⁴⁶ This is supported by animal experiments when low dose of bacteria endotoxin administered to newborn rats dramatically sensitises cerebral white and grey matter to hypoxia ischaemia.⁴⁷

The mechanism of cytokine induced white matter injury is still under investigation. Postulated mechanisms include disruption of the blood brain barrier, penetration of cytokines into the brain through circumventricular organs, or in-situ synthesis in the central nervous system by microglia, astrocytes, endothelial cells and neurons.^{37,48} Cytokines may indirectly cause cerebral injury by systemic effects like hypotension and disseminated intravascular coagulation. They may also cause damage to oligodendrocytes, astrocytes, and myelin directly, or stimulate production of other cytokines such as platelet activating factor, which may lead to cellular damage.^{2,49} Currently, research is being done to investigate the role of anti-inflammatory agents to reduce the inflammatory response, as well as methods to eradicate intrauterine infection. Our study shows no significant difference in PVL in the chorioamnionitis group. In our subgroup analysis, there was a statistically non-significant trend towards an increased rate of cerebral palsy in the chorioamnionitis group. This result is different from that obtained in some previous studies. However, in support of our findings, a recent study measured the levels of cytokines, chemokines and other proteins in blood sampled from preterm infants 2 days after birth, and found no association between cytokine and chemokine levels and the subsequent development of cerebral palsy.⁵⁰ One possibility is that the inflammatory response in extremely preterm infants may be different. Pro-inflammatory cytokines may not play such an important role in the pathophysiology of brain injury in extremely preterm compared to more mature infants.⁵¹ Also, the number of infants in our subgroup analysis is small, and larger studies on neurodevelopmental outcome, preferably following these infants up to school age, are required to investigate the long-term sequelae of chorioamnionitis in extremely preterm infants.

Chorioamnionitis and Mortality

Although chorioamnionitis is associated with an increased incidence of pulmonary and neurological morbidity, 2 studies had reported that chorioamnionitis reduces mortality in preterm infants delivered at less than 27 and 32 weeks' gestation, respectively.^{52,53} In our study, there was no

significant difference in mortality between the 2 groups. However, many confounding variables can affect the mortality rate of these extremely premature infants. Further larger scale studies or meta-analysis that include other studies with similar cohorts is required to confirm the association between chorioamnionitis and mortality.

Conclusion

This study investigated the association of chorioamnionitis with mortality and morbidity in a cohort of extremely preterm infants born below 28 weeks' gestation or less than 1000 g. There was a trend towards increased mortality, CLD, and ROP and decreased RDS among preterm infants with chorioamnionitis. A subgroup analysis on 2-year neurodevelopmental outcome shows a trend towards an increased risk of cerebral palsy and visual impairment with chorioamnionitis. Further studies that include data on cytokines and long-term neurodevelopmental outcome are necessary to document the inflammatory response of the fetus to chorioamnionitis and to confirm the association between chorioamnionitis and adverse outcome in extremely preterm infants.

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