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Original Articles

Long-term Outcome of Extremely Preterm Infants Following Chorioamnionitis

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Abstract Chorioamnionitis, a risk factor for preterm labour, has been reported to cause a fetal inflammatory response that predisposes the preterm infant to lung and brain injury. This study compared the outcome of 72 infants born below 28 weeks gestation or 1000 g birthweight with chorioamnionitis (Group A, n=18) and without chorioamnionitis (Group B, n=54). There was a higher incidence in Group A of raised serum C-reactive protein (60% vs 32%), raised immature to total neutrophil ratio (53% vs 24%), chronic lung disease (54% vs 43%), periventricular haemorrhage (31% vs 22%), retinopathy of prematurity (23% vs 18%), two-year mortality (33% vs 17%), cerebral palsy (25% vs 11%), and visual impairment (25% vs 16%), but none of these differences reach statistically significance. Further studies with larger cohorts are necessary to confirm the relationship between chorioamnionitis and adverse outcome.

Key words Cerebral palsy; Chorioamnionitis; Chronic lung disease; Prematurity

Introduction

Chorioamnionitis is a major predisposing factor for preterm delivery. It is associated with a three-fold increased risk of preterm birth with intact membranes, and a four-fold increased risk of preterm birth with prelabour rupture of membranes.[1] Bacteria could be cultured in the amniotic fluid in 10-20% of women who deliver after preterm labour,[2] and in 30% with preterm premature rupture of membranes.[3] The fetal inflammatory response syndrome is characterised by chorioamnionitis and elevated pro-inflammatory cytokines (IL-1β, IL-6, IL-8 and tumour necrosis factor α) in the amniotic fluid or fetal blood.[4,5] 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).[6,7] It had also been related to an increased risk of white matter lesion in the brain of preterm infants and cerebral palsy among survivors.[8-10] Therefore in this study, we compared the outcome of a cohort of extremely preterm infants with and without chorioamnionitis, in particular their early pulmonary outcome and late mortality and neurodevelopment at two years of age.

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Material and Methods

Subjects

The study cohort consisted of all infants inborn at Monash Medical Centre or outborn and transferred to Monash Medical Centre in the year 1997 with a gestational age of less than 28 weeks or a birthweight of less than 1000 grams. Excluded from analysis were those with chromosomal disorders or major congenital anomalies. Infants were divided into two groups: Group A, consisting of infants with clinical or histological evidence of chorioamnionitis; and Group B, consisting 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/mm^3), and raised maternal C-reactive protein (CRP, >6 mg/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. All survivors were recalled at two 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. Presence of an inflammatory response or infection. Inflammatory response was assessed by the maximum CRP values on the first three days after birth, an elevated CRP being defined as a value above 6 mg/l. Infection was assessed by the immature to total neutrophil ratio (IT ratio) and microbiological results of cultures (blood, cerebrospinal fluid, surface swabs, and gastric aspirate) taken on the first 3 days after birth, an elevated IT ratio being defined as a value above 0.2.
2. Short-term outcome. These included RDS defined by the characteristic radiological changes, CLD defined by supplemental oxygen at 36 weeks post-conceptional age, necrotising enterocolitis (NEC) defined by the radiological finding of intramural bowel gas, retinopathy of prematurity (ROP) assessed by routine ophthalmological examination beginning at Day 28 or 32 weeks post-conceptional age, and periventricular haemorrhage and leukomalacia (PVH and PVL) assessed by routine cerebral ultrasonographic examination beginning within the first week after birth.
3. Long-term outcome. These included the two-year mortality rate and sensorineural outcome among survivors: cerebral palsy, developmental delay (defined as a Mental Developmental Index MDI on the Bayley Scales of Infant Development of 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

Demographic Data

Seventy-two infants met the inclusion criteria (four with major congenital anomalies were excluded). Eighteen infants had chorioamnionitis (Group A) and fifty-four infants did not (Group B). The incidence of chorioamnionitis in our cohort of preterm births below 28 weeks gestation or 1000 grams birthweight was therefore twenty-five percent. There were no significant differences between the two groups in gestational age, birthweight, gender, Apgar scores, and antenatal corticosteroid therapy (Table 1).

Inflammatory Response and Infection

A higher percentage of infants in Group A had an elevated serum CRP level or an elevated IT ratio (Table 2). However, the differences did not reach statistical significance. Three infants in Group A had a positive surface culture or gastric aspirate (Ureaplasma urealyticum, Escherichiae coli and Staphylococcus aureus respectively). Five infants in Group B had a positive surface culture

Table 1 Demographic data

	Group A (n=18)	Group B (n=54)	p-value
Gestational age (week, mean±SD)	25.4±1.8	26.4±2.2	0.10
Birthweight (gram, mean±SD)	832±226	781±185	0.34
Gender (male:female)	11:7	25:29	0.41
Apgar score at 1 min (mean±SD)	4.6±2.6	4.8±2.4	1.00
Apgar score at 5 min (mean±SD)	5.7±3.3	7.2±2.3	0.14
Antenatal corticosteroid therapy	17 (94%)	43 (80%)	0.27

(Group B Streptococcus in two, and one each had Ureaplasma urealyticum and Group B Streptococcus, Group G Streptococcus, and Candida albicans). No infant had a positive blood or cerebrospinal fluid culture.

Short-term Outcome

No difference was found in the incidence of RDS between the two groups. The median duration of oxygen therapy was greater in Group A, and the incidence of CLD, ROP and PVH was higher (Table 2). None of the differences reached statistical significance. Only one infant had severe PVH (intraventricular haemorrhage with ventricular dilatation or intracerebral haemorrhage). There was no difference in the incidence of PVL and NEC between the two groups.

Long-term Outcome

Complete follow-up data were available in all but one of the forty-four two-year survivors. There was a trend towards an increased two-year mortality and an increased incidence of cerebral palsy and visual impairment in Group A (Table 3). However, none of the differences reached statistical significance.

Discussion

Chorioamnionitis, defined as intrauterine bacterial infection affecting tissues of either fetal-maternal origin (choriodecidual space and placenta), or fetal origin (chorioamniotic membrane, amniotic fluid and umbilical cord), is strongly associated with preterm delivery prior to 30 weeks gestation.[11] Twenty-five percent of our extremely preterm cohort had clinical or histological chorioamnionitis. Preterm delivery following chorioamnionitis has been reported to be associated with a higher neonatal mortality,[12] and a higher incidence of CLD,[7] cystic PVL,[13] and cerebral palsy.[14] Previous studies published on outcome of chorioamnionitis had included preterm infants up to 35 weeks gestation or 2000 grams birthweight. Furthermore, most studies selectively reported only one or two specific outcomes associated with chorioamnionitis. The aim of this study was to compare the short-term morbidity and two-year mortality and neurodevelopment of a complete cohort of extremely preterm infants born below 28 weeks gestation or 1000 grams birthweight with and without chorioamnionitis.

In our study, there is a trend towards a higher rate of

Table 2 Short-term outcome

	Group A	Group B	p-value
Elevated CRP	9/15 (60%)	16/49 (32%)	0.11
Elevated IT ratio	8/15 (53%)	12/49 (24%)	0.06
RDS	12/14 (85%)	43/51 (84%)	1.00
CLD	7/13 (54%)	21/49 (43%)	0.54
O_2 therapy (days, median, quartiles)	101 (18, 158)	30 (8, 82)	0.16
ROP	3/13 (23%)	9/49 (18%)	0.70
PVH	4/13 (31%)	11/49 (22%)	0.72
PVL	0/13 (0%)	3/49 (6%)	1.00
NEC	0/14 (0%)	1/49 (2%)	1.00

The different denominators are because some infants died prior to admission to the neonatal intensive care unit or in the early neonatal period, and data are not available at the time of the laboratory test or clinical diagnosis.

Table 3 Long-term outcome

	Group A	Group B	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

CLD, PVH and ROP diagnosed before hospital discharge, and a trend towards a higher mortality and rate of cerebral palsy and visual impairment at two years of age. Failure to reach statistical significance might be due to the relatively small number of infants in the study and the relatively low incidence of these outcomes. Alternatively, it might indicate that extremely preterm infants have a different response to chorioamnionitis. Further studies with larger cohorts are necessary to confirm the association between chorioamnionitis and long-term adverse outcome in extreme prematurity.

Inflammatory Response in the Fetus

Experimentally, intra-amniotic endotoxin induces granulocyte recruitment to the chorioamnion and pro-inflammatory cytokine expression in inflammatory cells in the amniotic fluid.[15] The finding that neonatal cytokine levels, but not maternal serum levels, correlate with the presence and severity of chorioamnionitis and umbilical vasculitis, supports the hypothesis that the cytokine cascade leading to fetal inflammation has fetal origins.[16] Amniotic fluid or neonatal cytokine levels were not available in our study, but almost all infants had CRP and IT ratio measured within the first three days after birth. Although more infants in the chorioamnionitis group had raised CRP and IT ratio, the differences did not reach statistically significance when compared to the group without chorioamnionitis. Most of the mothers in the former group received antibiotics and all their infants were started on antibiotics immediately after birth. This might have blunted their neonatal inflammatory response in the presence of chorioamnionitis. Positive microbiological culture was found in only a small number of our infants with chorioamnionitis. A previous study had shown that only one in three cases of intra-amniotic inflammation were culture positive.[11]

Chorioamnionitis and Pulmonary Outcome

Animal experiments had shown that the fetal lung responds to intra-amniotic endotoxin or cytokine with 'clinical maturation' as assessed by measurements of lung volume and compliance, gas exchange, and induction of surfactant lipids and proteins.[17-19] Cytokines bind to receptors in type-2 alveolar cells and induces the transcription factors required for the differentiation of the surfactant system independent of the effect of corticosteroids. Although previous studies had shown that chorioamnionitis decreases the incidence of RDS,[7,20] this was not confirmed in the present series of infants born less than 28 weeks gestation, probably because the response is different in extreme prematurity.

Experimental chorioamnionitis in sheep causes an injury that mimics many aspects of the injury sequence resulting in CLD in the preterm lung. Activated granulocytes are found in the fetal lung fluid, and there is increased apoptosis and cell proliferation and robust pro-inflammatory cytokine expression in the lung parenchyma. The end result is larger and fewer alveoli,[19,21] as is found in preterm lungs with CLD. The present study showed a trend towards an increase in CLD with chorioamnionitis, which is in agreement with previous studies performed on more mature preterm infants.[6,7] It is probable 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.

Chorioamnionitis and Mortality

Two studies had reported that chorioamnionitis reduces mortality in preterm infants born less than 27 and 32 weeks gestation respectively.[22,23] In our study, no significant difference in mortality was found in the chorioamnionitis group. The association between chorioamnionitis and mortality is complex, and confounding variables, such as the higher frequency of antenatal corticosteroid therapy in our present cohort compared to the previous studies, could have explained the difference in the findings.

Chorioamnionitis and Brain Injury

A rapidly expanding body of evidence implicates diverse pro-inflammatory cytokines as potential mediators of perinatal brain injury.[24] Experimentally induced intrauterine infection results in white matter lesions in rats and rabbits.[25,26] This was postulated to be due to elevated levels of inflammatory cytokines in the brain, which are neurotoxic.[27] Cytokines either cross the blood-brain barrier or are released by microglial cells within the white matter to cause damage to the periventricular region of the brain. Studies on term human infants had shown a definite association between cerebral palsy and clinical and histological chorioamnionitis[28] and pro-inflammatory cytokines and chemokines in neonatal blood.[29] In term infants, the association of infection and asphyxia with cerebral palsy is much stronger than either factor alone, suggesting a synergistic effect.[30] In support of the clinical data, experiments in newborn rats showed that bacterial endotoxin dramatically sensitises cerebral white matter to hypoxia-ischaemia.[31]

In preterm infants, elevated cytokine levels in amniotic fluid and fetal blood are predictive of PVL and cerebral

palsy.[8,9] Clinical studies had shown an association between chorioamnionitis in preterm infants and PVL and cerebral palsy.[14,32-35] A meta-analysis of seventeen published studies concluded that clinical chorioamnionitis in preterm infants is associated with PVL (RR 3.0, 95% CI 2.2-4.0) and cerebral palsy (RR 1.9, 95% CI 1.4-2.5), whereas histological chorioamnionitis is only associated with PVL but not cerebral palsy.[36] These studies included preterm infants up to 35 weeks gestation and 2000 grams birthweight. One study with a cohort of infants born at 24-29 weeks gestation, very similar to that of our present study, did not find a significantly higher incidence of cerebral palsy, developmental delay, hearing or visual impairment in the chorioamnionitis group.[37] Another study also reported no adverse effect on cognitive or psychomotor development.[38] The present study only showed a trend towards a higher rate of cerebral palsy in the group with chorioamnionitis, and the differences did not reach statistical significance. A larger cohort of extremely preterm infants needs to be studied to confirm any adverse effect of chorioamnionitis on long-term neurodevelopmental outcome.

Conclusions

This study investigated the association of chorioamnionitis with short-term and long-term morbidity and mortality in a cohort of infants born below 28 weeks gestation. There was a trend towards increased mortality, CLD, PVH, cerebral palsy, ROP and visual impairment among preterm infants with chorioamnionitis. Further studies with larger cohorts are necessary to confirm the association between chorioamnionitis and adverse long-term outcome in extremely preterm infants.

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