ORIGINAL ARTICLE

Impact of chorioamnionitis on short- and long-term outcomes in very low birth weight preterm infants: the Neonatal Research Network Japan

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Abstract
Objective: To evaluate the short- and long-term outcomes among very low birth weight (VLBW) preterm infants after histologic chorioamnionitis (HCA).

Methods: We performed a retrospective analysis of 5849 single infants (birth weight <1500 g) born at a gestational age between 22 + 0 and 33 + 6 weeks. Clinical data were obtained from the Neonatal Research Network Japan between 2003 and 2007. Multivariable logistic regression analyses were performed to assess the effect of HCA on short- and long-term outcome.

Results: According to logistic regression analysis, HCA was associated with lower incidence of respiratory distress syndrome (odds ratio [OR] = 0.54; p < 0.001), increased chronic lung disease (OR = 1.68; p < 0.001) and sepsis (OR = 1.71; p < 0.001) and as a short-term outcomes. There was no significant association with intraventricular hemorrhage (OR = 1.11; p = 0.33), periventricular leukomalacia (OR = 1.07; p = 0.70) and death before discharge (OR = 0.97; p = 0.084). HCA was associated with increased home oxygen therapy (OR = 3.09; p < 0.001), but not with cerebral palsy (CP; OR = 0.91; p = 0.63), develop quotient <70 (OR = 1.27; p = 0.17), visual impairment (OR = 1.08; p = 0.77), severe hearing impairment (OR = 1.28; p = 0.62) and death (OR = 0.98; p = 0.91) before three years of age.

Conclusions: In this retrospective population-based study in Japan, HCA was not a risk factor for death, neurodevelopmental impairment and CP in VLBW three-year-old preterm infants.

Introduction

Preterm birth is one of the most important causes of perinatal mortality and morbidity, and it is a major public health problem in terms of loss of life, long-term disability, such as cerebral palsy (CP), and healthcare costs [1]. The incidence of preterm birth has increased over the past few decades [1]. Advances in perinatal and neonatal care have resulted in significant improvements in survival of premature infants. In Japan, the overall survival rate for very low birth weight (VLBW) infants was 85% between 2003 and 2008 [2]. In particular, survival rates markedly improved for VLBW infants who were at high risk for neurodevelopmental disability and CP.

The etiology of preterm birth is very complex, but previously published data indicate that infections, such as chorioamnionitis, are important contributors, especially in spontaneous preterm birth at very low gestational age. Histologic chorioamnionitis (HCA) is present in 60–80% of placentas of patients who delivered at <28 weeks and in 40–50% of placentas of patients who delivered at 29–34 weeks [3]. This association seems to increase with decreasing gestational age.

HCA is defined as the infiltration of fetal membranes with polymorphonuclear leukocytes determined by pathological examination of the placenta. Although HCA has an infectious etiology [4], most cases are clinically silent [5].

Intrauterine infection has been associated with several neonatal outcomes: intraventricular hemorrhage (IVH); periventricular leukomalacia (PVL); chronic lung disease (CLD), particularly in VLBW infants (<1500 g) [6] and CP [7].

The use of tocolytic agents to prevent preterm birth at ≥34 weeks is not generally recommended in current treatment...
guidelines, because of possible risks associated with tocolytic and corticosteroids therapy. If a patient shows signs of chorioamnionitis, it is recommended to avoid the administration of tocolytic agents, and expeditious delivery should be considered [8]. However, the scenario may differ if the patient is in the second trimester of pregnancy or the chorioamnionitis is subclinical.

This study aimed to evaluate the impact of HCA on short- and long-term outcomes in VLBW infants, a very high-risk group among preterm infants, by analyzing cases from a large database (the Neonatal Research Network Database) in Japan.

**Methods**

This was a retrospective analysis of 5849 single infants (birth weight <1500 g) born at a gestational age between 22 + 0 and 33 + 6 weeks. Clinical data between 2003 and 2007 were obtained from the Neonatal Research Network database, which was created with a grant from the Ministry of Health, Labor and Welfare of Japan. This database collects data on >50% of VLBW neonates born in Japan. All tertiary neonatal units designated by the government participate in this database. Fifty-four tertiary level perinatal centers of the 66 participating facilities were registered for the Neonatal Research Network Japan in the year 2007. This database contains information on morbidity, mortality and follow-up data of VLBW infants born in participating facilities. Data for infants who were born alive but died in the delivery room were included. The clinician’s perspective on active treatment or withdrawal of care to preterm infants born at 22 and 23 weeks of gestation depended on the clinical status of infants. After 23 weeks of gestation, most clinicians made an effort to save infants [2].

The presence and severity of HCA were examined on the basis of Blanc’s criteria [9]. IVH was reported according to the classification of Papile et al. [10]. PVL was diagnosed by either cranial ultrasonography or cranial magnetic resonance imaging scan, performed ≥2 weeks. Respiratory distress syndrome (RDS) was diagnosed on the basis of clinical presentation and characteristic radiographic appearance. CLD was diagnosed on the basis of dependency on oxygen supplementation at a corrected age of 28 d. Neonatal sepsis was documented by a positive blood culture. Late-onset adrenal insufficiency was clinically diagnosed by systematic hypotension with oliguria, hyperkalemia and myocardial dysfunction [11]. Patent ductus arteriosus (PDA) was defined as persistence of a ductus arteriosus after birth with clinical symptoms. Necrotizing enterocolitis (NEC) was defined according to Bell’s classification stage II or greater [12]. Neonatal mortality was defined as death of an infant before discharge.

The follow-up protocol consisted of routine physical and neurological evaluations and developmental assessments at three years (36–42 months) of chronological age for surviving VLBW infants at each participating center [13].

Neurological evaluation at three years of age included signs and symptom of CP and sensory abnormality. CP was defined as a non-progressive central nervous system disorder characterized by abnormal muscle tone in at least one extremity and abnormal control of movement and posture [14]. Visual impairment was defined as unilateral or bilateral blindness diagnosed by ophthalmologists. Severe hearing impairment included the need for hearing aids. The assessment of cognitive function was performed using the Kyoto Scale of Psychological Development (KSPD) test applied by psychologists in each participating center [15]. When development quotient (DQ) was <70, the infant was judged as ‘‘delayed’’ according to the protocol of the Society for Follow-up Study of High-risk Infants [16]. Infants with CP, visual impairment, severe hearing impairment and DQ of KSPD <70 were designated as having neurodevelopmental impairment (NDI).

We analyzed the effect of HCA on long-term mortality and major adverse outcomes (CP, visual impairment, severe hearing impairment and DQ <70) as main outcomes. As secondary outcomes, we studied short-term outcomes (neonatal seizure, IVH, PVL, RDS, CLD, sepsis, late onset adrenal insufficiency, PDA and NEC). The protocol of this study was approved by the central internal review board at Tokyo Women’s Medical University, where all data were collected and stored.

**Statistical analysis**

Continuous data are presented as means ± standard deviation (SD) and ordinal data as median and range. Differences between the groups with and without HCA were tested using a chi-square test and $t$-test, as appropriate. Multivariable logistic regression analyses were performed to assess the effect of HCA on mortality and morbidity. Odds ratios (ORs) or coefficients adjusted for confounding variables and 95% confidence intervals (CIs) were calculated. Multivariate logistic regression analysis was performed after adjusting for maternal age, parity, diabetes, premature rupture of the membranes (PROM), preeclampsia, non-reassuring fetal status (NRFS), mode of delivery, administration of antenatal corticosteroids, gestational age at delivery, birth weight at delivery, small for gestational age (SGA) and sex of the infant.

Statistical analyses were performed using SPSS version 17.0 (SPSS Inc., Chicago, IL). Statistical tests were considered significant at a $p$ value of <0.05.

**Results**

The study group is illustrated in Figure 1. A total of 10 394 infants were registered in the database between 2003 and 2007. In total, 2808 infants were excluded because of multiple pregnancies, 632 infants were excluded because of major congenital malformation and 1071 infants were excluded because they were born outside participant centers. Data of 5849 single infants born at gestational ages between 22 + 0 and 33 + 6 weeks were available. Of these, 4078 (69.7%) had data on pathological examination of placenta. Of the 4078 subjects, 370 died (9.1%) before three years of age; follow-up data were obtained from 2201.

Maternal and delivery characteristics for the groups are listed in Table 1. The HCA group had significantly higher rates of PROM ($p < 0.001$) and administration of antenatal corticosteroids ($p < 0.001$), lower rates of preeclampsia ($p < 0.001$), NRFS ($p = 0.02$), cesarean delivery ($p < 0.001$), SGA ($p < 0.001$), lower gestational age at delivery ($p < 0.001$) and lower birth weight ($p < 0.001$).

As the main outcome, the effect of HCA on long-term outcomes is listed in Table 2. The rate of death before three
Table 1. Demographics and baseline characteristics (total n=4078).

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>HCA (n = 1235)</th>
<th>no-HCA (n = 2843)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age</td>
<td>30.8 ± 5.3</td>
<td>31.2 ± 5.2</td>
<td>0.02</td>
</tr>
<tr>
<td>Parity</td>
<td>0.7 ± 0.8</td>
<td>0.6 ± 0.8</td>
<td>0.001</td>
</tr>
<tr>
<td>Diabetes</td>
<td>21/1234 (1.7)</td>
<td>48/2843 (1.7)</td>
<td>0.98</td>
</tr>
<tr>
<td>Preeclampsia</td>
<td>76/1235 (6.2)</td>
<td>741/2843 (26.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PROM</td>
<td>656/1235 (53.1)</td>
<td>706/2843 (24.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>NRFS</td>
<td>308/1234 (25.0)</td>
<td>835/2841 (29.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Antenatal corticosteroid</td>
<td>634/1235 (51.3)</td>
<td>1055/2842 (37.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mode of delivery</td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Vaginal</td>
<td>507 (41.1)</td>
<td>604 (21.2)</td>
<td></td>
</tr>
<tr>
<td>With manipulation</td>
<td>8 (0.6)</td>
<td>43 (1.5)</td>
<td></td>
</tr>
<tr>
<td>Cesarean section</td>
<td>720/1235 (58.3)</td>
<td>2196/2843 (77.2)</td>
<td></td>
</tr>
<tr>
<td>GA at delivery</td>
<td>26.5 ± 2.6</td>
<td>28.1 ± 2.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Birth weight</td>
<td>921 ± 295</td>
<td>995 ± 302</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SGA</td>
<td>99/1235 (8.0)</td>
<td>782/2843 (27.5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Male sex</td>
<td>643/1235 (52.1)</td>
<td>1355/2843 (47.7)</td>
<td>0.83</td>
</tr>
</tbody>
</table>

Mean ± SD. HCA, histologic chorioamnionitis; PROM, preterm rupture of membranes; NRFS, non-reassuring fetal status; and SGA, small for gestational age.
years of age was 11.1% and 8.2% in the HCA and no-HCA groups, respectively. In the logistic regression analysis, HCA had no association with death before three years of age (adjusted OR, 0.98; 95% CI, 0.75–1.30; p = 0.91), CP (adjusted OR, 0.91; 95% CI, 0.63–1.32; p = 0.63), visual impairment (adjusted OR, 1.08; 95% CI, 0.65–1.78; p = 0.77), severe hearing impairment (adjusted OR, 0.98; 95% CI, 0.75–1.30; p = 0.91), DQ of KSPD <70 (adjusted OR, 1.27; 95% CI, 0.90–1.79; p = 0.17) and NDI (adjusted OR, 0.98; 95% CI, 0.74–1.31; p = 0.91) at three years of age. Adjusted OR and 95% CI of short-term outcomes of neonates that correlated with HCA are listed in Table 3. HCA resulted in a significant decreased risk for RDS (adjusted OR, 0.74–1.31; p < 0.001) and PDA (adjusted OR, 0.83; 95% CI, 0.77–0.99; p = 0.03) and in a significant increased risk for CLD (adjusted OR, 1.68; 95% CI, 1.41–2.01; p < 0.001) and sepsis (adjusted OR, 1.71; 95% CI, 1.33–2.20; p < 0.001) in logistic regression models adjusting for potential confounders. There was no association between HCA and neurological morbidities such as IVH and PVL. Regarding respiratory outcomes at follow-up examination, the incidence of asthma was similar in both groups (adjusted OR, 1.22; 95% CI, 0.83–1.73; p = 0.28). HOT was reported less frequently in the no-HCA group (adjusted OR, 3.09; 95% CI, 1.88–5.07; p < 0.001).

### Discussion

This extremely large population-based cohort study sought to determine the effects of HCA during pregnancy on short- and long-term outcomes. To our knowledge, this was the first multicenter study that analyzed the outcomes at three years of age in VLBW infants after chorioamnionitis in Japan. AC therapy was made among only 41.4% women delivered before age in VLBW infants after chorioamnionitis in Japan. AC was used off-label in Japan. In 2009, AC therapies were labeled for use by the national government. Published literature indicated that in utero exposure to infection/inflammation was a risk factor for CP and neurodevelopmental disability [17]. Previous reports showed that elevated proinflammatory cytokines such as tumor necrosis factor (TNF)-alpha, interleukin (IL)-1 beta and IL-6 in both amniotic fluid and fetal cord blood have been associated with white brain matter lesions such as PVL or IVH [18]. It is thought that these cytokines are capable of inducing damage to the oligodendrocyte, which is responsible for the deposition of myelin [19]. Furthermore, Leviton proposed that TNF induced hypotension and brain ischemia [20]. In addition, it is estimated that more than 60% of patients with PVL will eventually develop CP [20].
In contrast to our findings, Yoon et al. reported that elevations of IL-6 and IL-8 in amniotic fluid were associated with increased risk of CP at three years of age [21]. It is important to note that the mean gestational age at birth in the study by Yoon et al. [21] was higher than that in our study, as well as that of Grether et al. [17], Nelson et al. [22] and Andrews et al. [23]. Jacobsson et al. reported that HCA was associated with increased risk of CP in preterm infants (OR, 3.6; 95% CI, 1.16–12.1) in a population-based study from Sweden [24]. This study included the population born during the period of 1983–1990, before routine antenatal steroid therapy was implemented, and the results were not adjusted for confounding factors such as gestational age. A recent meta-analysis of studies after the year 2000 showed that HCA was associated with CP (relative risk, 1.83; 95% CI, 1.17–2.89) [25]. However, this meta-analysis did not examine term and preterm infants separately. The etiology of CP was found to be multifactorial. The commonly identified prenatal risk factors of CP were prematurity, SGA and chorioamnionitis. Contributing factors such as gestational age should be considered as potential confounders.

In contrast, Gather et al. reported that neither clinical (clinical chorioamnionitis or maternal fever) nor placental histopathological indicators of intrauterine infection/inflammation were associated with CP in children born before 32 weeks [17]. In addition, Nelson et al. recently reported that there was no association between cytokine concentrations in neonatal blood of infants born prior to 32 weeks of gestation and a later diagnosis of CP [22]. Andrews et al. reported no relationship between intrauterine infection/inflammation and neurodevelopmental disability in a prospective cohort study [23]. A meta-analysis of observational studies found that HCA was not associated with CP (relative risk, 1.6; 95% CI, 0.9–2.7) in preterm infants [25].

Our study period allowed a detailed, long-term neurodevelopmental follow-up that could be expanded to include CP as well as other neurodevelopmental outcomes. It is thought that NDIs are caused by PVL or white brain matter injury; the risks of both of these pathologies increased with intrauterine infection/inflammation [21]. Suppiej et al. showed the HCA increased the risk of NDIs at 18 months of ages in preterm infants. However, the results were not adjusted for confounding factor such as gestational age [26]. Our study showed that HCA had a tendency to increase the risk of IVH, PVL and neurodevelopmental disability such as DQ < 70. After adjusting for potential confounders, HCA was not associated with increased risk of PVL and IVH, as Elvira et al. reported [27], and we assumed that these led to the lack of association between HCA and neurodevelopmental disability. A previous study reported severe chorioamnionitis with funisitis or elevated amniotic fluid cytokines to be associated with neurological morbidity [28]. If only the infants with severe chorioamnionitis were included in their analysis, it had potential for significant associations.

Similar to previous studies, we did not find a significant association between HCA and NDIs including DQ < 70, visual impairment and severe hearing impairment [23]. In a recent report on extremely low birth weight (ELBW) and VLBW infants, the proportions of Bayley developmental index < 70 (< 2 SD below the mean) were 21% and 15%, respectively [29]. The incidence of developmental delay measured by DQ of KSPD in this study (22.5% in ELBW and 15.4% in VLBW) was very similar to recent studies. However, we are thoroughly aware that the tests used differ.

The mortality and major morbidity rate was significant lower in patients registered in the Japanese database compared with other large databases [30] in which the survival rate dramatically increased, especially in infants with a low gestational age or a low birth weight, and the overall incidence of severe IVH decreased. One of the issues was the fact that the proportion of some adverse outcomes in survivors of less-premature infants might be increasing. A study in Japan described that the rate of CLD in the year 2000 had increased, compared with that in 1995. However, a Japanese study in the year 2012 clearly showed a decreased incidence of severe IVH in contrast to the rate in other countries [31]. This result could represent an improvement in developmental outcomes among the survivors. Although it is not clear which intervention might contribute to the decreased incidence of severe IVH, low incidence of a major morbidity as a result from some sort of intervention could contribute to the resulting lack of difference between HCA and no-HCA groups in Japan, observed in our study.

With respect to short-term outcomes, we observed a significant decrease of RDS in the HCA group. Fetuses were reportedly stressed by the presence of intrauterine infection/inflammation such as chorioamnionitis, which thereby accelerated lung maturity by encouraging the secretion of endogenous corticosteroids resulting in the production of surfactant [32]. CLD appeared to significantly increase in infants with HCA. This result could be attributed not only to the effect of HCA but also to a larger number of infants in serious condition that required long-term ventilation management, oxygen administration and artificial nutrition. Significant increased risk of HOT at three years of age indicated that CLDs in the HCA group were more severe than in infants without HCA. Furthermore, the increasing risk of sepsis that did not lead to an increase in mortality is correlated to the infant's condition severity.

The strength of this study was the very large sample size. Nevertheless, the multidisciplinary follow-up rate reaching 63.0% was rather high. Furthermore, the follow-up dates were collected under unified criteria.

This study had limitations. HCA is usually silent. The diagnosis becomes apparent after delivery when the histological examination of the placenta and umbilical cord is performed. In 30% of the included cases, a pathological examination of placenta was not done. Timing of termination of pregnancy with chorioamnionitis may potentially have differed depending on the physicians or facilities. The antibiotic therapy applied was also dependent on each physician. In addition, the retrospective study design has inherent potential biases as stated above.

Our study design did not account for infants in the different stages of HCA or funisitis. In this study population, proportions of patients with HCA Grades 1, 2 and 3 were 25.1%, 34.3%, 40.6%, respectively. Thus, the majority of our patients had severe grades of HCA. However, further study with a staging evaluation of the placenta and funisitis may aid in
recognizing that severe stage of HCA or funisitis are associated with CP and NDIs.

Conclusion

On the basis of the evidence provided in this study, HCA was not a risk factor for death, NDI and CP at three years of age for preterm infants with very low gestational age.

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Declaration of interest

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