Chorioamnionitis as a Risk Factor for Retinopathy of Prematurity: A Systematic Review and Meta-Analysis

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Abstract

Background: The role of chorioamnionitis (CA) in the development of retinopathy of prematurity (ROP) has not been well established. Objective: To conduct a systematic review and meta-analysis of the association between CA and ROP in preterm infants. Data Sources: The authors searched MEDLINE, Embase, CINAHL, Cochrane Central Register of Controlled Trials and PubMed, reviewed reference lists of relevant articles, abstracts and conference proceedings (Society for Pediatric Research, European Society for Paediatric Research 1990–2012), sought results of unpublished trials, and contacted the primary authors of relevant studies. Study Selection: Studies were included if they had a comparison group, examined preterm infants, and reported primary data that could be used to measure the association between exposure to CA and the development of ROP. Data Extraction: Two reviewers independently screened the search results, applied inclusion criteria and assessed methodological quality using the Newcastle-Ottawa Scale. One reviewer extracted data and a second reviewer checked data extraction. Summary relative risks (RRs) were calculated using a random effects model. Data Synthesis: We identified 1,249 potentially relevant studies from the electronic databases. Twenty-seven studies involving 10,590 preterm neonates with 2,562 cases of ROP were included. Taking into account all included studies without adjusting for gestational age (GA), CA was significantly associated with ROP (any stage) [summary RR 1.33 (95% CI 1.14–1.55, I 2 = 77%, p heterogeneity < 0.0001)], and a borderline significant association was observed for severe ROP (stage ≥3) [summary RR 1.27 (95% CI 0.99–1.63, I 2 = 74%, p heterogeneity < 0.0001)]. There was no publication bias with Begg’s test. However, subgroup analysis of studies adjusting for GA showed no significant association on CA with ROP [summary RR 0.98 (95% CI 0.77–1.26, I 2 = 0%, p heterogeneity = 0.89)]. Conclusion: Unadjusted analyses showed that CA was significantly associated with ROP (any stage) as well as with severe ROP (stage ≥3). However, the association disappeared on analysis of studies adjusting for GA. Hence, CA cannot be definitively considered as a risk factor for ROP, and further studies should adjust for potential confounding factors and report results by stage to clarify the association with severe ROP.

Key Words
Chorioamnionitis · Retinopathy of prematurity · Systematic review · Meta-analysis
**Introduction**

Retinopathy of prematurity (ROP) is a retinal vascular disease of preterm infants, first described in 1942 [1]. ROP was initially connected with premature birth in the 1940s and slightly later to oxygen supplementation in these premature infants [2]. It is a major cause of blindness in children in the developing and developed world despite current surgical and laser treatment in the late stage of the disease [3]. Given the high prevalence and sequelae of ROP, effective prevention and timely treatment are essential for the preservation of lifelong vision in these premature neonates. A number of studies over the years have shown that low birth weight, early gestational age (GA) and oxygen use are the major risk factors for ROP [4–8]. However, primary prevention of ROP through elimination of risk factors like prematurity is practically not feasible [7, 9]. Therefore, there is a pressing need to identify contributing factors based on the prenatal origin of ROP, because preventive factors for ROP targeting the mothers and their fetuses may offer new opportunities for successful prevention of ROP [10].

Preterm labor and delivery, the leading identifiable causes of ROP, have been shown to be associated with maternal infections like chorioamnionitis (CA) and systemic infections [11]. Histological CA is defined by maternal inflammatory response with neutrophilic infiltration of the membranes and/or chorionic plate, with or without a fetal inflammatory co-response. On the other hand, clinical CA is defined by the attending physician in presence of maternal fever along with other suggestive clinical features. The incidence of CA has been found to increase with decreasing GA [12, 13]. It has also been shown in observational studies conducted in cohorts of extremely low-birth-weight infants that early-onset sepsis and systemic candidiasis are independently associated with increased severity of ROP and the need for laser treatment [14–16]. These findings suggest that perinatal inflammation and infection may have a deleterious effect on the developing blood vessels in the retina, making them vulnerable to the development of ROP. In a number of recently published studies, histological CA has in fact been shown to be an important risk factor for the development of early-onset sepsis as well as subsequent neonatal disorders of the lung, intestines and brain including white matter injuries and intraventricular hemorrhage [17–25].

A number of studies have tried to evaluate the relationship of CA and ROP. Intrauterine inflammation may be responsible for an adverse ‘first hit’ to the immature fetal organism which is followed by various postnatal events (‘multiple hits’) aggravating the risk of ROP in preterm infants [26, 27]. However, other investigators have found no significant association of CA with ROP [21, 28]. This disagreement may be due to the relatively small sample size, lack of control for known risk factors (GA, birth weight and hyperoxia/hypoxia), wide variation in outcome measures [e.g. any ROP, severe (stage 3 and above) ROP, prethreshold and threshold ROP], and the lack of consensus regarding the definition of CA in previous studies. In an effort to resolve the discrepancy observed across studies, we conducted a systematic review and meta-analysis to synthesize the literature that measures the association of CA with ROP in premature infants.

**Methods**

**Sources**

We searched the Cochrane Central Register of Controlled Trials (*The Cochrane Library* issue 1 of 12, January 2013) using the terms RETINOPATHY OF PREMATURITY AND CHORIOAMNIONITIS. We searched MEDLINE (January 1966 to January 2013) and PubMed (2013) using exploded MeSH terms RETINOPATHY OF PREMATURITY, CHORIOAMNIONITIS; ROP, CHORIOAMNIONITIS; CHORIOAMNIONITIS, NEURODEVELOPMENTAL OUTCOME; CHORIOAMNIONITIS, LONG TERM EFFECTS; PLACENTAL INFLAMMATION; PLACENTA HISTOLOGY, RETINOPATHY OF PREMATURITY and all possible combinations thereof in all languages (or without language restrictions). We searched EMBASE (January 1974 to January 2013) using the terms RETINOPATHY OF PREMATURITY with CHORIOAMNIONITIS. We searched the Science Citation Index (September 1984 to January 2013) for trials which cited the trials identified by our searches as MEDLINE and Embase. We sought registered details of selected trials in the U.S. National Institutes of Health resource Clinicaltrials.gov. We obtained information by personal communication, reviewing the reference lists of relevant articles, abstracts and conference proceedings (Society for Pediatric Research, European Society for Paediatric Research 1990–2012) and seeking results of unpublished trials.

**Study Selection**

Studies were included if they had a comparison group, examined preterm infants, and reported primary data that could be used to measure the association between exposure to CA and the development of ROP. We defined CA cases as clinical, histological or both clinical and histological. Clinical CA was defined as documented clinical signs and symptoms suggestive of CA. Histological CA was defined based on previously reported definitions as an acute inflammatory change in any of the tissue samples (amnion, chorion decidua, umbilical cord and chorionic plate) [29]. ROP was primarily defined according to the International Classification of Retinopathy of Prematurity and severe ROP as stages 3, 4 or 5 [30]. Studies where data on ROP were collected from the neonatal...
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Medical records were also included. To identify relevant studies, 2 reviewers independently screened the results of the searches and applied inclusion criteria using a structured form. Discrepancies were resolved through discussion or in consultation with a third reviewer.

**Quality Assessment**

We assessed the methodological quality using the Newcastle-Ottawa Scale for case-control or cohort studies as appropriate [31, 32]. The scale assesses for potential selection bias, comparability of cohorts or cases and controls and ascertainment of exposure (cohort studies) or outcome (case-control studies). Two reviewers independently assessed the methodological quality of each study and any discrepancy was resolved through consultation with a third reviewer.

**Data Extraction**

One reviewer extracted data from relevant studies using a predetermined data extraction form. A second reviewer checked the data extraction for accuracy and completeness. All data included in the meta-analysis were monitored by the statistician. Discrepancies were resolved by consulting the primary report. Data extracted from each study included citation information, language of publication, country where the research was conducted, objectives, study design, definitions of histological CA and ROP, inclusion/exclusion criteria, patient characteristics and results. Raw data, where available, were extracted from the results to analyze the effect of CA on ROP.

**Statistical Analysis**

We calculated summary relative risks (RRs) comparing risk of ROP among preterm infants born to women with CA with those without CA using a random effects model which accounts for within and between study heterogeneity [33]. The average of the natural logarithm of the RRs was estimated, and the RRs from each study were weighted by the inverse of the variance. A two-tailed p < 0.05 was considered statistically significant. Heterogeneity between studies was evaluated using Q and I² statistics [34]. I² is the amount of total variation that is due to variation between studies. I² values of approximately 25, 50, and 75% indicate low, moderate and high heterogeneity, respectively. Publication bias was assessed using Begg-Mazumdar’s test [35]. We conducted sensitivity analyses excluding one study at a time to test whether the results were robust. Subgroup analyses were done based on the definition of CA, severity of ROP and studies adjusting for GA.

**Description of the Studies**

We identified 1,249 potentially relevant studies from the electronic databases. Twenty-seven studies including 10,590 preterm infants met our study requirements (fig. 1). The clinical profile of the infants and the methodological characteristics of the studies have been summarized in online supplementary appendix A (see www.karger.com/doi/10.1159/000357556 for all online suppl. material) [36–69].

Fig. 1. Study flow diagram.
tives of the studies varied. The risk factors for developing ROP in pre-term infants were studied in 4 studies [26, 39, 51, 68]. The rest of the studies evaluated the occurrence of ROP as one of the outcomes of clinical or histological CA. Additional information was collected by personal communication with the authors from 3 studies [39, 51, 52].

Eight studies defined both clinical as well as histological CA [42–44, 50, 56, 61, 68]. Two studies dealt with only clinical CA [26, 28], while the rest of the studies only defined histological CA. In the studies that defined clinical CA, the definition was not specified in one of the studies [26]. In the rest of the studies defining clinical CA, the definitions were by and large similar in accordance with the criteria suggested by Gibbs et al. [69]. Histological CA was not defined by 2 studies [49, 50]. The criteria used for histological CA definition varied in the studies where it was mentioned [29, 37, 38, 48, 55, 59, 67]. Redline’s criteria were the most commonly used criteria to define histological CA [38]. In all except 2 studies, the mean or median GA was less than 30 weeks [21, 58]. In 19 of 27 studies, the patient characteristics were clearly differentiated between the CA and non-CA groups. In 15 out of these 19 studies, there was a significantly higher GA at birth among the non-CA group compared to the CA group.
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There was a significant positive association between CA and ROP (any stage) [summary RR 1.33 (95% CI 1.14–1.55, \(I^2 = 77\%\), \(p_{\text{heterogeneity}} < 0.0001\); fig. 2]. In sensitivity analyses excluding the most influential studies, the summary RR ranged from 1.30 (95% CI 1.12–1.51) when excluding the

![Funnel plot with pseudo-95% confidence limits for publication bias assessment for studies analyzing the effect of CA on severe ROP (stage ≥ 3).](image)

**Fig. 5.** Funnel plot with pseudo-95% confidence limits for publication bias assessment for studies analyzing the effect of CA on severe ROP (stage ≥ 3).

![Forest plot for 16 studies examining the effect of CA (any type) on severe ROP (stage ≥ 3). RR >1 indicates increased risk of ROP in infants born to mothers with CA.](image)

**Fig. 4.** Forest plot for 16 studies examining the effect of CA (any type) on severe ROP (stage ≥ 3). RR >1 indicates increased risk of ROP in infants born to mothers with CA.
Subgroup Analyses

Analysis Based on Severity of ROP

There was a marginally significant association between CA and severe ROP [summary RR 1.27 (95% CI 0.99–1.63, I² = 74%, p_heterogeneity < 0.0001); fig. 4]. In sensitivity analyses excluding the most influential studies, the summary RR ranged from 1.20 (95% CI 0.95–1.53) when excluding the study by Dammann et al. [26] to 1.40 (95% CI 1.16–1.70) when excluding the study by Lau et al. [21]. There was no evidence of publication bias for severe ROP with Begg’s test (p = 0.50; fig. 5).
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Analysis Based on Definition of CA

The studies were stratified according to the definition of CA into: clinical CA, histological CA as well as clinical and histological CA. We did a stratified analysis on each subgroup (fig. 6). Clinical CA was significantly associated with ROP (any stage) [RR 1.60 (95% CI 1.31–1.96, $I^2 = 0\%$)]. Histological CA was also found to be significantly associated with ROP (any stage) [RR 1.38 (95% CI 1.12–1.71, $I^2 = 81.7\%$)]. However, in the subgroup dealing with both clinical and histological CA, no association with ROP (any stage) [RR 1.17 (95% CI 0.93–1.48, $I^2 = 39.8\%$)] was found, although there was a nonsignificant increase in ROP with CA in this subgroup. On meta-regression analysis, there was no significant heterogeneity between the subgroups ($p = 0.90$).

Analysis Based on Studies Adjusted for GA

To eliminate the effect of prematurity as a confounder, we did a subgroup analysis to include the studies that showed no significant difference in GA between the CA group and the non-CA group. Eight studies were included in the analysis. A meta-analysis of these 8 studies showed no significant association of CA with ROP [summary RR 0.98 (95% CI 0.77–1.26); fig. 7]. No significant heterogeneity was observed ($I^2 = 0\%, p_{\text{heterogeneity}} = 0.89$).

Discussion

A total of 10,590 preterm infants across 27 studies were evaluated to assess the relation between CA and ROP. The meta-analysis of unadjusted data extracted from all studies showed that the RR of developing ROP was significantly (33%) higher in infants exposed to any form of CA, either clinical, histological or both. There was also a borderline significant (27%) increase in the RR of developing severe ROP (stage ≥ 3). However, when adjusted for GA, the meta-analysis of data extracted from 8 such studies showed no significant association between CA and ROP. The included studies were of good quality according to the Newcastle-Ottawa Scale with 22 out of the 27 included studies having a high score of 7–9 on methodological quality assessment. To our knowledge, this meta-analysis is the first of its kind that analyzes the effect of prenatal inflammation in the form of CA on the development of ROP in preterm infants.

Perinatal inflammation has been associated with a number of adverse neurological outcomes in preterm infants such as white matter injury, intraventricular hemorrhage, cystic periventricular leukomalacia and cerebral palsy [23, 70–72]. Maternal intrauterine infection and inflammation can induce a production of proinflammatory cytokines in the fetal brain such as tumor necrosis factor-α (TNF-α) and interleukins (IL)-1, IL-6, and IL-8. These cytokines may disrupt the blood-brain barrier, thereby leading to increased plasma protein influx and oligodendrogial damage [73, 74]. On the other hand, increased cytokine levels in the first 72 h of life (IL-6 >357 pg/ml, IL-8 >216 pg/ml, and TNF-α >245 pg/ml) have been linked with development of severe ROP [75]. It has also been observed that maternal systemic inflammation leads to decreased levels of insulin-like growth factor-1, which in turn is associated with an increased risk of developing ROP [68, 76]. Cytokine levels in the vitreous of 19 patients with stage 4 ROP were recently analyzed by Sato et al. [77], who demonstrated that increased levels of vascu-

<table>
<thead>
<tr>
<th>Study</th>
<th>RR (95% CI)</th>
<th>Weight, %</th>
</tr>
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<tbody>
<tr>
<td>Nasef, 2012</td>
<td>0.96 (0.61–1.50)</td>
<td>30.5</td>
</tr>
<tr>
<td>Lee, 2011</td>
<td>1.12 (0.68–1.85)</td>
<td>24.6</td>
</tr>
<tr>
<td>Wirbelauer, 2011</td>
<td>2.12 (0.39–11.64)</td>
<td>2.1</td>
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<tr>
<td>Schlapbach, 2010</td>
<td>0.50 (0.10–2.55)</td>
<td>2.3</td>
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<tr>
<td>Rocha, 2006</td>
<td>1.22 (0.51–2.92)</td>
<td>8.2</td>
</tr>
<tr>
<td>Fung, 2003$^a$</td>
<td>0.67 (0.19–2.30)</td>
<td>4.1</td>
</tr>
<tr>
<td>Fung, 2003$^b$</td>
<td>1.26 (0.40–3.99)</td>
<td>4.7</td>
</tr>
<tr>
<td>Morales, 1987</td>
<td>0.84 (0.50–1.41)</td>
<td>23.6</td>
</tr>
<tr>
<td><strong>Subtotal</strong></td>
<td><strong>0.98 (0.77–1.26)</strong></td>
<td><strong>100.0</strong></td>
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Fig. 7. Forest plot for stratified analysis of 8 studies (adjusted for GA) examining the effect of CA on ROP. RR >1 indicates increased risk of ROP in infants born to mothers with CA. $^a$[42], $^b$[43].
lar endothelial growth factor had the highest correlation with vascular activity in ROP eyes compared to other cytokines. Sood et al. [78] studied cytokine levels in extremely low-birth-weight neonates on days 1, 3, 7, 14, and 21 and showed that some cytokines were significantly different across ROP groups, suggesting that perinatal inflammation may be involved in the pathogenesis of ROP. Thus perinatal inflammation in the form of CA does seem to play an important role in the development of ROP in preterm infants. However, establishing this relation on statistical terms becomes a major challenge due to the simultaneous interplay of a number of confounding factors.

The major confounding factor in such an analysis is prematurity. Since CA is often associated with preterm birth, the inflammatory response commonly develops in a population already at high risk for brain damage and poor neurologic outcome [79]. It has been shown in a number of studies that extreme prematurity plus multiple hits of perinatal inflammation appear to be involved in ROP etiology and progression [26, 39]. Again, lower GA and birth weight are associated with increased oxygen therapy, which is an independent risk factor for development of ROP [80]. In a recent meta-analysis by Saugstad and Aune [81], it was found that a lower oxygen saturation targeting by pulse oximetry reduced severe ROP by 50%, i.e. from 20.9 to 9.5%. In our analysis, infants in the CA group had lower GAs compared to the non-CA group in 15 out of the 27 studies. This underlies the importance of a subgroup analysis adjusting for the major confounding factor GA. In our study, we found only 8 studies that have adjusted for GA. The meta-analysis of these studies incidentally showed no association between CA and ROP. Hence, prematurity itself along with other factors associated with prematurity, like oxygen therapy, could possibly confound the unadjusted results. This also brings to light the dearth of studies on this issue that adjust for potential confounders, which prevents us from drawing a definitive conclusion.

Another factor that was not accounted for in the studies is the influence of genetic variation. It has been mentioned in the study by Dammann et al. [26] that single nucleotide polymorphisms in the genes encoding for increased production of IL-1β, TNF-α and reduced Toll-like receptor 4 signal transduction were associated with a tendency towards reduced likelihood for ROP occurrence. In a recent study comparing whole-genome expression in the first month of life in groups of infants with and without ROP, it was found that more genes were underexpressed than overexpressed in the ROP group [82]. This may in part account for the complex pathophysiology of ROP and may explain why ROP progresses to a severe stage in some infants, while in others, despite similar clinical conditions, regresses spontaneously, thus leading to variable results on comparative analysis with a single factor like CA.

Substantial heterogeneity was observed across the studies in the magnitude and direction of associations. We explored heterogeneity by grouping the studies by type of CA, but heterogeneity remained substantial among the subgroups, except in the clinical CA group. This may be explained by the very small number of studies in the clinical CA group compared to a much larger number of studies in each of the other subgroups [26, 28]. One important source of heterogeneity may be the substantial variation in the definition of histological CA. While few of the studies mentioned diagnostic criteria for defining histological CA such as Redline’s Criteria, College of American Pathologists Guidelines, Salafia staging system, Blanc’s criteria, criteria from Amniotic Fluid Infection Nosology Committee, Roger’s protocol and Yoon’s criteria, most of them used non-specified diagnostic methods [36, 39, 58, 62, 67, 69]. Placental microbiology was not taken into account in our analysis due to the insufficient number of studies. Interestingly, it had been shown in one of the studies by Chen et al. [39] that although presence of placental organism or placental inflammation individually did not appear to be associated with an increased risk of ROP, the two risk factors, when co-occurring, were associated with a significantly increased risk of ROP in zone I. This may suggest that underappreciation of the etiologic complexity of ROP pathogenesis in many of the other studies may have led to a lack of association between mere placental inflammation and ROP. There was less discrepancy over the defining criteria for ROP, with almost all the included studies following the International Classification of Retinopathy of Prematurity [30]. ROP stage 3 and above being a distinctive marker of proliferative retinopathy was unanimously classified as severe ROP in most of the studies. Apart from gross variation in the definition of CA, lack of adjustment for probable confounders like GA, birth weight and duration of oxygen therapy most likely contributed to the high degree of heterogeneity observed across studies which again prevented us from drawing any definitive conclusion. Interestingly, we noted no heterogeneity in the subgroup analysis across the 8 studies which adjusted for GA, and these studies showed no association between CA and ROP. This underlies the importance of
taking into account all possible sources of heterogeneity across studies including major confounding factors while interpreting the results of a meta-analysis. This study is a perfect example where exclusion of a major confounder in the form of GA and consequent elimination of heterogeneity vastly changed the results and interpretation of the statistical analysis.

An interesting observation to note was that our study did not show any publication bias. In our review, 23 out of the 27 included studies (85.2%) were not primarily designed to assess the effect of CA on ROP. They were designed to assess the effect of placental inflammation on neonatal morbidity and neurodevelopmental outcome, and ROP was included as one of the effects. This might have negated the tendency to avoid publication of studies not showing any relation of CA with ROP, or in other words, the ‘file-drawer effect’ was not present in our analysis.

Conclusion

We conclude that though unadjusted analyses showed significant association of CA with ROP (any stage) as well as with severe ROP (stage ≥ 3), no such association was found on subanalysis of the studies adjusting for GA. Hence, CA cannot be definitively considered as a risk factor for ROP based on current evidence. However, in view of dearth of studies specifically focusing on the relation between CA and ROP, there is a need to conduct further studies on this topic adjusting for potential confounding factors and report results by stage to clarify the association with severe ROP.

Disclosure Statement

The authors have no conflict of interest to disclose.

References


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