# Chorioamnionitis as a Risk Factor for Necrotizing Enterocolitis: A Systematic Review and Meta-Analysis

Jasper V. Been, MD, MPH, PhD[1,2], Sanne Lievense, MD[1], Luc J. I. Zimmermann, MD, PhD[1,3], Boris W. Kramer, MD, PhD[1,3], and Tim G. A. M. Wolfs, PhD[1,3]

**Objective** To accumulate available evidence regarding the association between antenatal inflammation and necrotizing enterocolitis (NEC).

**Study design** A systematic literature search was performed using Medline, Embase, Cochrane Library, ISI Web of Knowledge, and reference hand searches. Human studies published in English that reported associations between chorioamnionitis or other indicators of antenatal inflammation and NEC were eligible. Relevant associations were extracted and reported. Studies reporting associations between histological chorioamnionitis (HC) and NEC, HC with fetal involvement and NEC, and clinical chorioamnionitis and NEC were pooled in separate meta-analyses.

**Results** A total of 33 relevant studies were identified. Clinical chorioamnionitis was significantly associated with NEC (12 studies; n = 22 601; OR, 1.24; 95% CI, 1.01-1.52; $P$ = .04; $I^2$ = 12%), but the association between HC and NEC was not statistically significant (13 studies; n = 5889; OR, 1.39; 95% CI, 0.95-2.04; $P$ = .09; $I^2$ = 49%). However, HC with fetal involvement was highly associated with NEC (3 studies; n = 1640; OR, 3.29; 95% CI, 1.87-5.78; $P \leq .0001$; $I^2$ = 10%). Selection based on study quality did not affect the results. No indications of publication bias were apparent. Multivariate analyses in single studies generally attenuated the reported associations. Several associations between other markers of antenatal inflammation and NEC are reported.

**Conclusion** Currently available evidence supports a role for antenatal inflammation in NEC pathophysiology. This finding emphasizes the need to further study the underlying mechanisms and evaluate potential interventions to improve postnatal intestinal outcomes. *(J Pediatr 2013;162:236-42)*.

Preterm birth is the leading cause of neonatal morbidity and mortality. The majority of extremely preterm births are associated with chorioamnionitis, an inflammation of the placenta and fetal membranes.[1] This antenatal inflammatory process is often clinically silent and is detected through histological examination of the placenta after birth. Histological signs of a fetal inflammatory response, as well as clinically apparent chorioamnionitis, are thought to reflect the more serious side of the continuum.

Chorioamnionitis is classically recognized for its modulating effects on lung development.[2] However, recent studies provide evidence that chorioamnionitis results in developmental changes in a whole range of organs including the gut, and thus may be considered a multiorgan disease of the fetus.[3]

Necrotizing enterocolitis (NEC) is the most serious gut-related complication of preterm birth. It affects between 5% and 10% of very low birth weight infants and is associated with a mortality rate of 20%-30% and considerable morbidity among survivors.[4] Gastrointestinal (GI) inflammation is one of the hallmarks of NEC, and postnatal inflammatory processes, including sepsis, are highly associated with its development.[4] The contribution of chorioamnionitis, a proinflammatory process starting antenatally, is less clear. Several studies have reported an association between chorioamnionitis and NEC,[5-7] while others have failed to reproduce this.[8-17] In an attempt to clarify this issue, we aimed to accumulate the available evidence regarding the association between chorioamnionitis or other indicators of antenatal inflammation and NEC.

## Methods

We performed a systematic literature search in accordance with the Meta-Analysis of Observational Studies in Epidemiology criteria for a meta-analysis of observational studies.[18] Databases for medical literature (Medline, Embase, and Cochrane Database of Systematic Reviews) were searched independently by 2 investigators

| | |
|---|---|
| BPD | Bronchopulmonary dysplasia |
| CC | Clinical chorioamnionitis |
| GI | Gastrointestinal |
| HC | Histological chorioamnionitis |
| IL | Interleukin |
| NEC | Necrotizing enterocolitis |

From the [1]Department of Pediatrics, [2]School for Public Health and Primary Care (CAPHRI), and [3]School for Oncology and Developmental Biology (GROW), Maastricht University Medical Center, Maastricht, The Netherlands

J.B. is funded by a Maastricht University MC Kootstra Talent Fellowship. The authors declare no conflicts of interest.


http://dx.doi.org/10.1016/j.jpeds.2012.07.012

(J.B. and S.L.) in April 2012. We used broad search terms to identify potentially relevant articles: (chorioamnionitis OR intrauterine infection OR intrauterine inflammation OR prenatal infection OR prenatal inflammation OR antenatal infection OR antenatal inflammation) AND (gut OR necrotizing entercolitis OR NEC OR gastrointestinal OR intestine OR ileum OR jejunum OR stomach OR colon). We performed additional searches by screening reference lists from articles of interest as well as citations to articles of interest, using the ISI Web of Knowledge.

Articles written in English that reported associations between chorioamnionitis or other indicators of antenatal inflammation and NEC in humans were eligible for inclusion. Study selection and data extraction were performed independently by 2 investigators (J.B. and S.L.), and any disagreements were resolved by consensus.

We performed meta-analyses for the association between chorioamnionitis and NEC. We used Mantel-Haenszel analysis to calculate aggregate ORs and 95% CIs using a random-effects model. Study quality was assessed independently by 2 investigators (J.B. and S.L.) using the Newcastle-Ottawa Scale for observational studies, and disagreement was resolved by consensus. We used the Eggers regression test and funnel plots to assess publication bias. We applied an $\alpha$ level of 0.05 in all analyses, and performed all calculations using RevMan 5.1 software (Cochrane Library; ims.cochrane.org/revman).

## Results

Our search identified a total of 33 relevant studies (**Table I**). The selection process is summarized in **Figure 1** (available at www.jpeds.com). Studies reporting associations between histological chorioamnionitis (HC) and NEC (13 cohort studies),[5-14,19-21] between HC with fetal involvement and NEC (3 cohort studies),[5,7,11] and between clinical chorioamnionitis (CC) and NEC (9 cohort and 3 case-control studies)[5,9,10,15-17,21-26] were pooled in separate meta-analyses. Many of these studies, as well as other studies, reported associations between indicators of antenatal inflammation other than chorioamnionitis and NEC, which are reported separately.

### HC and NEC

Pooling of the studies on HC revealed no significant overall association with NEC (**Figure 2**, A). Considerable heterogeneity was present among the studies (**Figure 2**, A). Findings were similar in retrospective studies and prospective studies (OR, 1.38; 95% CI, 0.81-2.36 vs OR, 1.39; 95% CI, 0.76-2.54). Likewise, the point estimate was not greatly altered by the exclusion of one study that pooled HC and CC (OR, 1.43; 95% CI, 0.96-2.13).[9]

Severe cases of chorioamnionitis may exhibit signs of fetal involvement. Three of the studies of HC compared the incidence of NEC between infants with chorioamnionitis plus fetal involvement and infants without chorioamnionitis.[5,7,11] All demonstrated a significant association between HC with fetal involvement and NEC, resulting in a prominent pooled OR (**Figure 2**, B).

### CC and NEC

Pooling of the studies on CC revealed a significant association with NEC (**Figure 2**, C). Exclusion of a study that contributed 39.4% of the total weight in the meta-analysis slightly augmented the association (OR, 1.29; 95% CI, 1.04-1.59). The point estimate was lower for case-control studies compared with cohort studies (OR, 1.13; 95% CI, 0.60-2.13 vs OR, 1.29; 95% CI, 1.02-1.63), and for retrospective studies compared with prospective studies (OR, 1.21; 95% CI, 0.91-1.61 vs OR, 1.38; 95% CI, 0.99-1.93). Omission of a study that pooled HC and CC[9] slightly augmented the association between CC and NEC (OR, 1.27; 95% CI, 1.02-1.58). Studies including only patients with preterm premature rupture of the membranes[22,26] had a higher point estimate than those without this restriction (OR, 1.91; 95% CI, 1.13-3.29 vs OR, 1.12; 95% CI, 0.95-1.32).

We found no statistical indications for publication bias in any comparison. Overall study quality was moderate to high (**Table II**; available at www.jpeds.com). Restriction of the analyses to high-quality studies (Newcastle-Ottawa Scale score $\geq$7) did not greatly alter point estimates of the association between HC or CC and NEC (OR, 1.48; 95% CI, 0.96-2.30 and OR, 1.22; 95% CI, 0.95-1.57, respectively).

A minority of studies performed multivariate analyses to adjust for potential confounders affecting the association between chorioamnionitis and NEC.[5,6,8,15,22] This resulted in somewhat attenuated ORs in most studies,[5,6,15,22] and a loss of statistical significance of the association in 2 studies.[5,6]

### Other Indicators of Antenatal Inflammation and NEC

Fifteen studies associated one or more indicators of antenatal inflammation other than chorioamnionitis with NEC (**Tables I** and **III**). Significant positive associations have been reported between NEC and umbilical cord polymorphonuclear cell infiltration,[27] NEC and positive amniotic fluid microbial polymerase chain reaction,[28,29] NEC and *Ureaplasma urealyticum* colonization,[21] and NEC and increased cord blood interleukin (IL)-6[30,31] and IL-8 levels.[32] Several of these associations remained significant after multivariate analysis.[7,21,29,31] However, other studies have been unable to detect similar associations with chorioamnionitis severity,[14,33] fetal vasculitis,[7,21] *U urealyticum* colonization,[30,34,35] or cord blood IL-6 levels.[21,32,36]

### Additional Studies

Interpretation of results from 2 additional small studies is complex because "normal" controls are missing. One of the studies reported no significant difference in NEC incidence between selected infants with necrotizing funisitis versus those with acute funisitis (22% vs 7%).[37] A second study