Population Cohort Associating Chorioamnionitis, Cord Inflammatory Cytokines and Neurologic Outcome in Very Preterm, Extremely Low Birth Weight Infants

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ABSTRACT: Intrauterine inflammation may relate to neurologic disability among preterm children. We investigated the relationship between chorioamnionitis, cord serum cytokines, and neurologic outcome. Sixty-one consecutively born very preterm extremely low birth weight (ELBW) infants were prospectively enrolled. Histologic inflammation in placenta and umbilical cord and vascular pathology were evaluated. Cord sera were analyzed for five proinflammatory cytokines. Serial brain ultrasound and magnetic resonance imaging were performed for evaluation of intraventricular hemorrhage (IVH grade I–III) and white matter damage (WMD: cystic periventricular leukomalacia or IVH grade IV). Neurologic and neurocognitive outcomes were assessed at the corrected age of 2 y. The incidences of HCA, WMD, and abnormal neurologic outcome were 48%, 13% and 19%, respectively. HCA or high IL-6 in cord serum predicted spontaneous preterm labor with high accuracy. HCA increased the risk of IVH grade II–III. In HCA, without either clinical chorioamnionitis or histologic placental perfusion defect, the children had a low risk of WMD (0%) and a low risk of abnormal neurologic outcome (6%). In HCA, the concentration of IL-6 in cord serum was lower in children with abnormal neurologic outcome than in children with normal neurologic outcome. In HCA and placental perfusion defect (compound defect) the risk of abnormal neurologic outcome was high. Compound placental defect and WMD additively predicted abnormal neurologic outcome. We propose that HCA together with other insults (placental perfusion defect or maternal systemic infection) increases the risk of poor neurologic outcome in very preterm ELBW infants. (*Pediatr Res* 59: 478–483, 2006)

Five decades ago Eastman and DeLeon (1) found maternal intrapartum fever to be associated with cerebral palsy (CP) in term or near-term children. At present, prenatal factors have been suggested as significant determinants of severe neurologic sequelae among infants born at term (2). In premature infants, prenatal inflammation has been associated with brain damage (3–5), intraventricular hemorrhage (IVH) (3), and defects in long-term neuromotor and neurocognitive outcome

(6,7). However, these observations have not been systematically confirmed (8–10).

Histologic chorioamnionitis (HCA) is indicative of inflammation in tissues of fetomaternal (chorionic plate and decidua) or fetal origin (chorioamniotic membrane, amniotic fluid, and umbilical cord) (11). The cause of HCA may reflect an innate response to microorganisms that gain access into the intrauterine space either *via* the ascending route or hematogenously, starting a complex cascade of inflammatory cell recruitment and induction of proinflammatory cytokines and chemokines. This process may lead to activation of spontaneous premature labor (12).

The cytokine production by inflammatory cells in the fetal chorionic plate is likely to extend to the fetus. The inflammatory mediators may profoundly influence the fetal development. Much of the current evidence suggests that the fetal inflammatory response syndrome contributes to the acute organ injury leading to chronic diseases in various organs, including the CNS (13). It has been proposed that the deleterious process in brain leading to white matter damage (WMD) possibly includes multiple concurrent steps during the vulnerable period of maturation (14). Both infection and ischemia-reperfusion activates inflammatory mediators, which may perturb this complex process (15). Cystic periventricular leukomalacia (cPVL) and intraparenchymal hemorrhage (IVH grade IV) are well defined lesions that share the same contributing factor in their pathogenesis, ischemia-reperfusion, and account for the majority of severe WMD leading to long-term neurologic sequelae among preterm children (14).

The principal aims of the present study were to investigate whether prenatal inflammation defined as HCA leads to elevated levels of proinflammatory cytokines in umbilical cord serum and whether these inflammatory indices serve as a risk factor of WMD, defined as cPVL or IVH grade IV or associate with neurologic and neurocognitive outcome in a homogenous

Submitted February 11, 2005; accepted November 20, 2005.

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This work was supported by grants from The Academy of Finland, Sigrid Jusélius Foundation (M.H.), and Foundation for Pediatric Research, Finland, and Alma and K. A. Snellman Foundation (T.K.)

Abbreviations: cPVL, cystic periventricular leukomalacia; CRIB Score, Clinical Risk Index for Babies Score; ELBW, extremely low birth weight; HCA, histologic chorioamnionitis; IVH, intraventricular hemorrhage; PPROM, preterm premature rupture of fetal membranes; TNF, tumor necrosis factor; WMD, white matter damage

DOI: 10.1203/01.pdr.0000182596.66175.ee

population of very preterm, extremely low birth weight (ELBW) infants. Low number of infants with poor outcome complicated the analysis. Here, we further study whether the inflammatory activation influences the risk of IVH without parenchymal involvement.

SUBJECTS AND METHODS

Subjects. Altogether, 78 very preterm (gestational age <32 wk), ELBW infants were born alive between November 1998 and November 2002 in Oulu University Hospital. Only four additional very preterm, ELBW infants were born alive in northern Finland; two of them died soon after birth. A written informed consent was signed by the parents. The study was approved by the ethics committee of Oulu University Hospital.

The following exclusion criteria were used: refusal of consent ($n = 1$), unavailability of umbilical cord sample ($n = 5$), death in delivery room ($n = 3$), lethal disease due to extreme immaturity ($n = 7$; one survived), serious congenital disease ($n = 0$) or inability to participate in follow-up ($n = 1$). Altogether 61 infants were eligible. The length of gestation at birth was based on an ultrasound examination at 16–18 wk of gestation.

Seven infants were additionally excluded: five died, one parent refused to continue the study, and one infant was lost to follow-up. Of the remaining 54, 1 infant with normal results on brain imaging at term was excluded from the follow-up, because of a brain tumor diagnosed at 1 y of age.

Prenatal data. The data included maternal age at birth, socioeconomic factors, parity, history of previous preterm births, preexisting maternal medical conditions, administration of antenatal steroid, antibiotic use before delivery, preterm premature rupture of fetal membranes >24 h (PPROM), mode of delivery, and the presence of active labor (regular contractions and cervical changes) (16). The criteria of clinical chorioamnionitis were fever $\geq 37.8^\circ\text{C}$ and at least two of the following: leukocytosis $\geq 15,000$ cells/mm³; maternal pulse rate >100/min or fetal pulse rate ≥ 160 /min; uterine tenderness and foul-smelling amniotic fluid (17).

Placenta. Placentas were fixed in 10% neutral buffered formalin immediately after birth. The rim of membranes was taken from the site of membrane rupture. The umbilical cord specimens were taken from fetal and placental side of the umbilical cord and from the midway point between the both sides of insertion. The full-thickness of placental parenchyma specimen was taken from the midway point between umbilical cord insertion and placental margin. For histologic evaluation, paraffin blocks were made, cut into 5 μm slices, and stained with hematoxylin-eosin. HCA was defined as the presence of polymorphonuclear leukocytes in at least one of the three compartments examined and graded for the degree of severity (mild, moderate, severe) (18). Placental perfusion defect was defined as poorly vascularized villi, multiple capillary lumina in some villi, increased intervillous volume, and reduced total villous capillary bed (18). For the analyses, the placentas were further divided into four groups according to the histologic findings: isolated HCA, isolated perfusion defect, combined HCA and perfusion defect, and placentas without HCA or perfusion defect. The placentas were examined by a single pathologist (R.H.) without knowledge of the clinical data.

Proinflammatory cytokines. For measurements of IL-1 α , IL-1 β , IL-6, IL-8, and tumor necrosis factor (TNF)- α , cord sera were separated and stored frozen at -70°C . The analysis of proinflammatory markers was performed with antibody-based protein microarrays with DNA amplification (19).

Clinical data. Data on umbilical cord pH, 5-min Apgar scores, the Clinical Risk Index for Babies Score (CRIB Score) (20), intra-arterial blood pressures, the use of cardiopulmonary agents, duration of mechanical ventilation, and treatment with systemic corticosteroids were recorded.

Brain imaging. Ultrasound imaging was performed using HDI 5000 (Advanced Technology Laboratories Ultrasound, Botwell, WA) with a curved-array 5–8 MHz transducer. The infants underwent serial brain ultrasound assessments at the following ages: 1 to 3 d, 1, 2, and 4 wk, and thereafter every fourth week until discharge or term. A pediatric radiologist performed the prospective evaluation and filled in a form after each examination. Afterward, the results were reviewed by a single radiologist (M.P.) blinded to the clinical outcome. The final outcome was the consensus of these two evaluations. IVH and PVL were classified using the standard criteria (21,22).

Conventional magnetic resonance imaging (MRI) was performed at term on all but one infant. A 1.5-T system (Signa Horizon Echo Speed; General Electric, Milwaukee, WI) and a head coil were used. The study protocol consisted of T1-weighted sagittal images [TR/TE = 400–460/9, matrix 256 $\times$ 192, 4 mm slices, 1 mm slice gap, and 20 $\times$ 20 cm field of view (FOV)], fast spin echo T2-weighted axial images (TR/TE = 3000–4660/83–168, matrix 256 $\times$ 192 or 512 $\times$ 320, 5 mm slices, 0.5 mm slice gap, and

20 $\times$ 15 cm FOV) and T1-weighted axial images (TR/TE = 460–600/10–20, 256 $\times$ 192 or 256 $\times$ 256 matrix, 4–5 mm slices, 0.5 mm slice gap, and 20 $\times$ 15 cm FOV).

Before and during MRI examination, the infants were fed and swaddled to keep warm, disposable ear plugs were fitted, and they were given a dose of chloral hydrate (25–50 mg/kg). Oxygen saturation and cardiorespiratory status were monitored during the imaging procedure by using a MR-compatible pulse oximeter probe. A pediatrician (T.K.) observed the infant's condition throughout the examination.

WMD was defined as abnormal signal intensity on conventional T1- and T2-weighted MR images, presenting with parenchymal hemorrhage or cystic lesions with or without petechial hemorrhage in the white matter area together with reduction of white matter thickness and compensatory ventricular enlargement (23,24). The MR images were evaluated in consensus by two radiologists (E.P. and M.P.).

Neurologic and neurodevelopmental outcome. At the corrected age of 24 mo (median, range: 24, 18.8–26.7) the 53 infants were evaluated for neurologic status with special attention to the pattern of movements and posture, muscular tone, and reflex status. An abnormal neurologic outcome was defined as neuromotor dysfunction based on the functional ability of the most affected limb. The involvement was severe ($n = 8$) if the child was unable to sit, stand, or walk, and moderate ($n = 2$) if the motor function of the most affected limb was clearly impaired: one child had functional disability of the right side of the body, both arm and leg, another had functional disability of both legs. The Griffiths Mental Developmental Scales (25) were applied by the investigator (T.K.) without knowledge of placental pathology or cord serum analysis. The neurocognitive outcome was assessed using Bayley Scales of Infant Development (26) by a psychologist without knowledge of previous history of the study children.

Statistical analyses. All statistical analyses were made by the SPSS program (SPSS Inc., Chicago, IL). For the differences in categorical variables between the groups, χ^2 test or, when appropriate, Fisher's exact test was used. The differences in continuous variables were studied using independent-samples t test (normal distribution) or Mann-Whitney U test. In comparing the four placental groups, gestational age was adjusted by using univariate analysis of variance and t test was used as a *post hoc*. Cytokines were correlated with the length of gestation by using Pearson's correlation coefficient after logarithmic transformation. A receiver-operator characteristic curve was used to evaluate the relationship between sensitivity and specificity for values of IL-6.

The independent risk factors were further considered in multiple logistic regression analysis. The confounding variables, gestational age and birth weight, were dichotomized into two categories: gestational age <27.0 wk and/or birth weight <770 g and gestational age ≥ 27.0 wk and/or birth weight ≥ 770 g, using mean values as cut-off points. All tests were two-tailed. The level of significance was set at $p < 0.05$.

RESULTS

The birth characteristics are shown in Table 1. Maternal age at birth, socioeconomic factors, parity, and history of previous preterm births did not influence the risk of neurologic, neurodevelopmental, or cognitive outcome at the corrected age of two years (data not shown).

Placental pathology. Twenty-five infants were born to 24 mothers with HCA. Inflammation involving the fetal membranes, chorionic plate, and umbilical cord was found in 13 infants. Both the fetal membranes and the chorionic plate were affected in four cases, whereas in eight cases only the fetal membranes were inflamed. Twenty-two infants were born to 22 mothers with lesions indicative of a vascular perfusion defect in placenta. PPROM was evident in 16 (67%) mothers with HCA compared with 4 (14%) mothers without HCA ($p < 0.001$). Eighteen (75%) mothers with HCA had antibiotic treatment before the delivery. Two mothers had clinical chorioamnionitis and they both had HCA as well.

Active labor was evident in 27 mothers. HCA predicted the active labor with a sensitivity and specificity of 79% and 87% (OR: 24.4; 95% CI 5.4–111, $p < 0.001$).

Table 1. Pregnancy and birth characteristics of the 52 mothers and 54 infants

Characteristic	Quantitation
Multiple pregnancy, <i>n</i> (%) [*]	5 (10)
Antenatal steroid	47 (90)
Preeclampsia	17 (33)
PPROM	20 (39)
Histologic chorioamnionitis	25 (48)
Clinical chorioamnionitis	2 (4)
Active labor	27 (52)
Vaginal delivery	14 (26)
Gender	
Male	22 (41)
Female	32 (59)
Gestational age, wk (mean $\pm$ SD)	27.0 $\pm$ 1.8
Birth weight, g	772 $\pm$ 155
SGA, <i>n</i> (%)	19 (35)
Umbilical cord pH, mean $\pm$ SD	7.27 $\pm$ 0.08
Apgar score at 5 min $\leq$ 6, <i>n</i> (%)	33 (61)

SGA, small for gestational age. Infants with birth weight below 2 SD from the mean of gestation-adjusted birth weight according to Finnish growth standards were classified as SGA.

^{*} Two twin pairs and three isolated cases (two from twin pregnancies, one from a triplet pregnancy).

Of the 17 mothers with preeclampsia, 12 had isolated perfusion defect in placenta and 3 had HCA and perfusion defect (compound pathology). None of the mothers with preeclampsia had active labor before delivery. The six cases with compound placental pathology included only singleton pregnancies; none of the mothers had clinical signs of infection, and the prevalence of intrauterine growth retardation was not different from the cases of isolated perfusion defect (4/6 versus 11/16).

Cord serum cytokines. Cord serum IL-6 was higher in the isolated HCA group than in the group with no HCA or perfusion defect ($p = 0.036$), and IL-8 was higher in the isolated HCA group than in the perfusion defect group ($p = 0.008$) (Table 2). For IL-1 α , IL-1 β , or TNF- α , no detectable differences were found between the groups. In the whole study population, the levels of IL-1 α , IL-1 β , IL-6, IL-8, and TNF- α declined with increasing of gestational age ($p \leq 0.005$). The infants born to mothers with isolated HCA had lower gestational age (mean 26.1 wk, SD $\pm$ 1.5) than the other infants (27.6 $\pm$ 1.7 wk) ($p = 0.002$). In HCA, the proinflammatory cytokines had no association with the length of gestation.

High IL-6 in cord blood (cut off value >800 using receiver-operator characteristic curve analysis) predicted the active labor with a sensitivity of 85% and specificity of 57% (OR 7.7; 95% CI 2.0–30, $p = 0.005$).

Intraventricular hemorrhage without parenchymal involvement. Forty infants had no IVH or had IVH grade I. IVH grade II–III was diagnosed in 11 out of 54 infants and was evident within the first 7 d after birth in all cases. Five (45.5%) of these infants had early IVH, diagnosed during the first 3 d after birth, and each of them had HCA. A comparison of clinical characteristics between the infants with IVH grade II–III and those with IVH grade I or no IVH is shown in Table 3.

HCA associated with IVH grade II–III ($p = 0.019$, all cases with HCA; $p = 0.075$, isolated HCA cases). The association of HCA remained significant in multiple logistic regression analysis after controlling for gestational age and adjustment for the confounding factors (vaginal delivery, CRIB score, and lowest mean intra-arterial blood pressure during the first 24 h) (OR: 8.1; 95% CI 1.6–43, $p = 0.013$). No association was found between the umbilical cord serum cytokines and IVH grade II–III. The infants with isolated perfusion defect in placenta were protected from IVH grade II–III (OR: 0.71; 95% CI 0.58–0.87, $p = 0.023$).

White matter damage. Seven infants had detectable WMD, defined by MRI and ultrasound: four had cPVL and three revealed IVH grade IV. Due to low numbers, neither HCA of any degree of severity or in any combination, nor the cord serum cytokines could be associated with WMD. One infant born from a pregnancy complicated by clinical chorioamnionitis had WMD. Antenatal glucocorticoid treatment was less frequent and postnatal dexamethasone more frequent among the infants with WMD than those without WMD. Several other characteristics of severe postnatal cardiopulmonary compromise associated with WMD (Table 4).

Neurologic and neurodevelopmental outcome. Ten out of 53 children had an abnormal neurologic outcome (Table 5). The children with abnormal neurologic outcome had higher CRIB score ($p = 0.015$), longer duration of assisted ventilation ($p = 0.003$), and they received dexamethasone treatment more frequently ($p = 0.001$) than the other ELBW children. Five of the 24 children born with HCA had abnormal neurologic outcome. In HCA, cord serum IL-6 was higher in children with normal outcome than in those children with abnormal neurologic outcome (median 13950, range 631–

Table 2. Cytokine levels according to the histological findings in placenta

Variable	Isolated HCA (<i>n</i> = 19)	No HCA, no perfusion defect (<i>n</i> = 13)	Isolated perfusion defect (<i>n</i> = 16)	HCA + perfusion defect (<i>n</i> = 6)
Gestational age (wk, mean $\pm$ SD)	26.1 $\pm$ 1.5	26.5 $\pm$ 1.4	28.3 $\pm$ 1.7	28.1 $\pm$ 1.6
IL-1 α	737 (2 636; 373)	499 (971; 419)	382 (649; 328)	457 (1 167; 339)
IL-1 β	1 928 (12 124; 728)	579 (1 055; 365)	392 (557; 297)	445 (2 884; 383)
IL-6	11 781* (64 209; 2 215)	921 (2 649; 663)	763 (1 038; 657)	780 (14 662; 566)
IL-8	13 838† (46 262; 5 947)	6 003 (7 442; 2 675)	3 769 (5 263; 1 951)	4 016 (18 160; 2 363)
TNF- α	544 (802; 316)	325 (710; 242)	483 (784; 247)	369 (786; 312)

The cytokine data are reported as arbitrary fluorescence units and median (upper quartile; lower quartile). For comparison, the results were adjusted to gestational age using univariate analysis of variance and *t* test was used *post hoc*.

* Isolated HCA different from the placentas with no HCA or perfusion defect ($p = 0.036$).

† Isolated HCA different from the placentas with isolated perfusion defect ($p = 0.008$).

Table 3. Comparison between infants with IVH grade II to III and IVH grade 0 to I

Factor	IVH grade II to III n = 11	IVH grade 0 to I n = 40
Placenta		
HCA, all cases, n (%)	9 (82)	16 (40)
Isolated HCA	7 (64)	12 (30)
Isolated perfusion defect*	0 (0)	14 (35)
HCA + perfusion defect	2 (18)	4 (10)
No HCA, no perfusion defect†	2 (18)	10 (25)
Gestational age, wk‡	25.4 (27.4; 24.6)	27.4 (28.3; 26.3)
Vaginal delivery, n (%)	6 (55)	8 (20)

The following factors associated with IVH grade II to III: high CRIB score, $p = 0.023$; low mean arterial pressure in the 1st day, $p = 0.015$; long duration of assisted ventilation, $p = 0.032$; and postnatal dexamethasone treatment, $p = 0.037$.

* Two infants with IVH grade IV were not included in the analysis.

† One infant with IVH grade IV was not included in the analysis.

‡ Gestational age is shown as median (upper quartile; lower quartile).

Table 4. Comparison of infants with and without white matter damage

Factor	WMD n = 7	No WMD n = 47
Placenta		
HCA, all cases, n (%)	2 (29)	23 (49)
Isolated HCA*	1 (14)	18 (38)
Isolated perfusion defect	2 (29)	14 (30)
HCA + perfusion defect	1 (14)	5 (11)
No HCA or perfusion defect	3 (43)	10 (21)
Antenatal corticosteroid, n (%)	4 (57)	43 (92)
Gestational age, wk†	26.1 (28.9; 24.1)	27.4 (28.1; 25.9)
Birth weight, g†	670 (815; 575)	820 (920; 670)

The following factors associated with WMD: high CRIB score, $p = 0.008$; long duration of assisted ventilation, $p = 0.001$; and postnatal dexamethasone treatment $p = 0.011$.

* Includes two cases of clinical chorioamnionitis, one with WMD.

† Continuous variables are shown as median (upper quartile; lower quartile).

65536 versus 1346, 469–11240; $p = 0.037$, Mann-Whitney U test). Two infants with isolated HCA had abnormal neurologic outcome; one of them had clinical chorioamnionitis. Interestingly, HCA and concomitant placental perfusion defect (compound placental defect) associated with abnormal neurologic outcome after the adjustment for confounding factors ($p = 0.031$).

Neurodevelopmental and neurocognitive outcome was further assessed at the corrected age of 24 mo using the total scores on Griffiths Developmental Scales and the mental developmental index on Bayley Scales in children with and without HCA. The total scores on Griffiths Scales in children with HCA were mean 92 ± 23 . The total scores on Griffiths Scales in children without HCA were 95 ± 23 . The scores on Bayley Scales in children with HCA were 92 ± 23 . The scores on Bayley Scales in children without HCA were 92 ± 23 .

To assess the independent prenatal and neonatal risk factors, logistic regression analyses were performed. Several clinical factors in the neonatal period independently and con-

Table 5. Birth characteristics of children with the abnormal neurologic outcome and those with not abnormal neurologic outcome at corrected age of 24 months

Factor	Abnormal outcome n = 10	Not abnormal outcome n = 43
Placenta		
HCA, all cases, n (%)	5 (50)	19 (44)
Isolated HCA*	2 (20)	16 (37)
Isolated perfusion defect	2 (20)	14 (33)
HCA + perfusion defect	3 (30)	3 (7)
No HCA or perfusion defect	3 (30)	10 (23)
Gestational age, wk†	26.4 (28.9; 24.8)	27.4 (28.1; 26.1)
Birth weight, g†	663 (846; 613)	815 (890; 670)

The following factors associated with abnormal neurologic outcome: high CRIB score, $p = 0.015$; long duration of assisted ventilation, $p = 0.003$; and postnatal dexamethasone treatment, $p = 0.001$.

* Includes two cases of clinical chorioamnionitis, one with abnormal outcome. One child was excluded from the analysis because of a brain tumor diagnosed at 1 year of age.

† Continuous variables are shown as median (upper quartile; lower quartile).

currently predicted abnormal neurologic outcome (Table 6). WMD, when presenting together with compound placental defect, additively predicted abnormal neurologic outcome (OR: 20.7, 95% CI: 2.6–163; OR: 14.9, 95% CI: 1.2–192, respectively) (Table 6). Compound placental defect and/or WMD predicted the abnormal neurologic outcome with a specificity of 88%, sensitivity of 70%, positive predictive value of 58% and negative predictive value of 93% ($p < 0.001$).

DISCUSSION

Our study demonstrated that histologic chorioamnionitis, when compounded with placental perfusion defect, enhanced the risk of abnormal neurologic outcome in very preterm ELBW infants after adjustment for confounders. This placental abnormality and WMD were independent risk factors that jointly predicted the abnormal outcome.

Proinflammatory cytokines have been related to acute HCA (27) as well as induction of preterm labor (28). Fetal inflammatory response, *i.e.*, funisitis and/or chorionic plate vasculitis, have been associated with brain damage and neuromotor disability (5,6). According to the previous (29,30) and present findings, umbilical cord serum IL-6 and IL-8 closely associated with HCA. Both HCA and IL-6 also accurately predicted the spontaneous preterm labor. However, for the present population neither HCA nor cord serum proinflammatory cytokines could be used as risk factors of WMD or neurologic outcome. Only two of the 25 HCA cases also had clinical chorioamnionitis and both of these infants had either WMD or poor neurologic outcome. This is in line with recent meta-analysis of preterm children (31) suggesting lack of association between histologic chorioamnionitis and cystic periventricular leukomalacia or cerebral palsy but underlining the importance of clinical chorioamnionitis as a risk factor.

The low risk of brain damage in infants with HCA in our series may be explained by confounding variables, such as

Table 6. Logistic regression models describing factors of abnormal neurologic outcome at the corrected age of 24 mo

Risk factor	Single risk factor OR (95% CI)	Two risk factors		
		Model 1 OR (95% CI)	Model 2 OR (95% CI)	Model 3 OR (95% CI)
HCA + perfusion defect	15.2 (1.3–181)	14.9 (1.2–192)		
CRIB score	1.3 (1.05–1.7)			
Assisted ventilation (d)	1.08 (1.02–1.1)		1.06 (1.01–1.12)	
WMD	21.1 (3.0–150)	20.7 (2.6–163)		12.6 (1.3–121)
Dexamethasone after birth	31.7 (2.9–345)		15.1 (1.3–177)	21.8 (1.9–253)

Each putative risk factor was tested after adjustment for confounding factors (gestational age, birth weight). All five single risk factors and all combinations of two risk factors together were tested. Of the two-risk-factor models, only the three models that revealed an additive effect are shown.

longer duration of gestation or higher birth weight. However, none of these confounders were prevalent among the HCA cases. In fact, the infants with isolated HCA and high proinflammatory cytokines in cord blood had shorter gestation than the rest of the population. The negative result was therefore not due to lack of common risk factors. Cytokines have several protective effects and their adverse effects may depend on interactions with constitutional or environmental factors, such as the degree of brain maturity (19,32). In present study of very preterm ELBW infants, we show evidence that in pregnancies complicated by HCA, cord serum IL-6 was lower in children with abnormal neurologic outcome compared with cord IL-6 in children with normal outcome.

Eighteen out of the 24 mothers with HCA had had antibiotic treatment before delivery. Recently, maternal antibiotic administration before delivery has been associated with a decreased risk of cPVL (33) suggesting that changes in management have diminished the adverse consequences of HCA. Besides the obstetric treatment of the high-risk pregnancies, the management practices of children born very preterm with cardiorespiratory failure are likely to influence the outcome (34).

Some recently published reports underline the importance of noninflammatory placental pathology in predicting brain damage and later neurologic sequelae (8,35). Kumazaki *et al.* (8) found disturbed placental circulation to predict the risk of PVL but they did not find chorioamnionitis to be an independent risk factor to PVL. Redline *et al.* (35) reported combined placental lesions in very preterm infants to associate with neurologic impairment at a corrected age of 20 mo. According to Mallard *et al.* (36) impaired placental circulation together with hypoxemia resulted in neuronal damage, reduced myelination, and focal white matter lesions in fetal sheep. Interestingly, intra-amniotic administration of endotoxin in pregnant mice has been shown to decrease the blood flow and to increase the pulsatility index values in umbilical artery and ductus venosus, as seen in pregnancies complicated by severe placental insufficiency (37,38). The fetal cardiac failure may affect the cerebral blood flow, decreasing the perfusion in the distal fields of the watershed area in white matter (14).

Whether the inflammation caused the placental perfusion defect, or whether the two findings co-occurred by chance remains unsolved. Endotoxin administration has triggered pre-eclamptic symptoms in pregnant rats (39). To that end, inflammatory cells and mediators accompanied by chorioamnionitis could induce vascular endothelial dysfunction leading to

severe perfusion defect in placenta. Three of the cases with combined placental defects had a clinical diagnosis of pre-eclampsia. In the present population, neither the two histologic placental defects nor the clinical syndromes (clinical chorioamnionitis, preeclampsia) concurred more frequently than could be anticipated by chance.

Although the placental inflammation was associated with low incidence of WMD, univariate and multiple regression analyses demonstrated the association with IVH grade II–III. This is consistent with the proposal that prenatal inflammatory mediators affect endothelial cells of the germinal matrix, leading to up-regulation of chemokines and adhesion molecules and recruitment of white blood cells, resulting in damage of the blood-brain barrier (40). Disruption of the endothelial lining in fragile capillaries contributes toward intraventricular bleeding.

Despite the fact that histologic chorioamnionitis and intraventricular hemorrhage relate with the degree of prematurity, gestational age at birth was not an independent risk factor of IVH in this population of very preterm infants. The incidence of HCA (48%) was lower than previously reported for births of very preterm infants (52%–84%) (9,10). Interestingly, none of the infants with isolated placental perfusion defect (30% of all cases) had IVH grade II–III. This is complementary to the previous findings of the investigators of the Developmental Epidemiology Network (41) and relates to the protective effect of preeclampsia against IVH.

Current results confirm the previous studies (8–10,31,42–43), suggesting that placental inflammation or high proinflammatory cytokine levels in cord serum alone are not risk factors of WMD, poor neurologic outcome, or poor cognitive outcome among the very preterm ELBW infants. However, HCA together with other insults may increase the risk of poor neurologic outcome. These adverse factors include placental perfusion defect and systemic infection in the mother. Extending the detailed studies on placental and fetal circulation to all cases of threatened very preterm birth may help to further define or prevent the risk of neurologic sequelae.

Acknowledgments. We are grateful to Ebenezer Satyaraj, Ph.D. and Steven Piccoli, Ph.D. from Molecular Staging Inc., New Haven, CT and Dhavalkumar Patel, M.D., Ph.D. from Department of Medicine, Duke University Medical Center, Durham, NC, for the analysis of the proinflammatory cytokines. We thank R. Bloigu M. Sc. for consultations concerning statistical analyses.

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