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Inflammation at Birth is Associated With Subnormal Development in Very Preterm Infants

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ABSTRACT: Preterm birth carries a risk for impaired developmental outcome. We have previously described an association between increased levels of proinflammatory cytokines during the first 72 postnatal hours and cerebral damage as detected by ultrasound in a cohort of 74 very preterm infants. Sixty-seven of 71 surviving children with a mean gestational age of 27.1 (2.0) wk were examined at 2 y corrected age with a standardized neurologic examination and with Bayley Scales of Infant Development. We hypothesized that proinflammatory cytokine concentrations at or shortly after birth would be associated with an adverse developmental outcome. Increased concentrations of TNF-α in cord blood odds ratio (95% confidence interval) 3.3 (1.1–10.2), $p = 0.013$ and at 6 h 7.8 (0.9–71.8), $p = 0.015$ and of IL-6 in cord blood 1.7 (1.0–2.9), $p = 0.048$ were associated with psychomotor developmental index <85. Increased concentrations of TNF-α in cord blood odds ratio (95% confidence interval) 3.6 (1.002–12.8), $p = 0.044$ and of IL-8 in cord blood 3.5 (1.2–10.6), $p = 0.023$ were associated with cerebral palsy. Associations of TNF-α and IL-8 in cord blood with the respective outcome measures remained significant after adjustment for other clinical variables. Proinflammation at birth is associated with impaired functional outcome at 2 y of corrected age in children with very preterm birth. **(*Pediatr Res* 64: 183–188, 2008)**

Although both survival and developmental outcome in preterm infants have improved during the last years, very preterm birth remains a major risk factor for cerebral injury and for later neurodevelopmental disability (1,2). Recent studies using MRI techniques have shown that very preterm infants have delayed maturation and volume reduction of both cerebral white and gray matter (3). Furthermore, such changes are associated with impaired neuro-developmental outcome (4–6). Intrauterine infection is a common antecedent to preterm birth and has been related to adverse neonatal outcome (7–9). In a recent study, the *combination* of histologic inflammation and placental vascular changes predicted abnormal neurologic outcome at 2 y of age (10).

Increased levels of proinflammatory cytokines in cord blood or in neonatal blood indicating a systemic fetal inflammatory response have been associated with development of white matter brain damage (WMD) and with CP (11,12). We have previously shown that increased levels of proinflammatory cytokines at birth and during the first 72 postnatal hours were associated with arterial hypotension, severe intraventricular hemorrhage (IVH), and WMD in very preterm infants (13). Changes in levels of cytokines occur rapidly within short time intervals (14). In the present cohort of very preterm infants, we observed that circulatory levels of cytokines were generally highest at or shortly after birth and decreased characteristically up until 72 h postnatal age. This suggested that the observed inflammatory response was initiated *in utero* (13).

We hypothesized that circulatory proinflammation is related to impaired developmental long-term outcome in very preterm infants and that this relationship is primarily present at or shortly after birth. Thus, we conducted a follow-up study to evaluate the relationship between proinflammatory and modulatory cytokines at birth and during the first 72 postnatal hours and neuro-developmental outcome at 2 y of age corrected for prematurity.

METHODS

Study population. The study population consisted of a cohort of very preterm infants born at Lund University Hospital between February 2001 and February 2003. Seventy-four infants out of a total of 169 admitted were enrolled before delivery to participate in a study evaluating cytokine patterns during the first 72 postnatal hours (13). Inclusion criteria were GA less than 32 wk, informed parental consent, and absence of major congenital anomalies. All pregnancies were dated by ultrasound at 17–18 gestational weeks. All surviving infants from this cohort ($n = 71$) were asked for participation in a follow-up study at 2 y of corrected age. Sixty-seven children (94%) with a mean (SD) GA at birth of 27.1 (2.0) weeks were assessed at a corrected age of 24.0 (0.53) range (22.5–25) months. The parents of three infants declined participation and one infant was lost to follow-up. None of these infants had cerebral hemorrhage or WMD as detected by neonatal ultrasound. The study was approved by the Regional Committee for Research Ethics.

Quantitative analysis of plasma cytokines. Blood sampling was performed from cord blood and from arterial blood at 6, 24, and 72 h postnatal age through an indwelling arterial line. Levels of proinflammatory (TNF-α, IFN-γ, IL-1β, IL-2, IL-6, IL-8, IL-12) and modulatory (IL-4, IL-10) cytokines in plasma were determined by cytometric bead array (Becton Dickinson, San Jose, CA) and flow cytometry. A level of ≤ 0.1 pg/mL was

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Abbreviations: IVH, intraventricular hemorrhage; **MDI,** mental developmental index; **NOS,** neurological optimality score; **PDI,** psychomotor developmental index; **PROM,** premature rupture of membranes; **WMD,** white matter damage

regarded as nondetectable. The method has been described in detail previously (13).

Cerebral ultrasound. Repeated ultrasound examinations of the brain were performed at 1, 3, and 7 postnatal days, at 6 wk and at term age (13). Data from the ultrasound examinations were stored digitally and reviewed by a pediatric radiologist. Images with suspected abnormalities where reassessed by a second pediatric radiologist blinded to the clinical history. Cerebral hemorrhage was classified as subependymal hemorrhage (grade I) or IVH (grade II–III). WMD was defined in the presence of periventricular echodensities persisting for more than 7 d or periventricular cysts. Severe brain damage was defined as IVH grade III and/or WMD. No infants had parenchymal hemorrhage.

Antenatal and neonatal clinical data. Antenatal and neonatal clinical data were prospectively recorded until home discharge. Premature rupture of membranes (PROM) was defined as rupture of membranes before the onset of labor and suspect maternal infection as elevated maternal C-reactive protein (>5 mg/L) and/or fever >38°C. Clinical chorioamnionitis was defined when two of the following criteria were present: maternal fever >38°C, maternal tachycardia, fetal tachycardia, malodorous amniotic fluid and uterine tenderness. The total number of doses of antenatal steroids, preeclampsia, mode of delivery, and multiple pregnancies were registered.

Gender, GA in days, birth weight for GA, Apgar score, treatment with dopamine during the first 72 h, medical treatment or surgical ligation of persistent ductus arteriosus, postnatal septicaemia, ventilator treatment (days), supplemental oxygen at 36 gestational wks and accumulated dose (mg/kg) of hydrocortisone and/or betamethasone administered from birth until discharge were registered. Retinopathy of prematurity was defined according to the international classification (15).

Data at follow-up. CP was defined using the definitions adopted for European classification of CP (16). Children presenting previously not recognized symptoms of CP at follow-up were re-evaluated by a pediatric neurologist. When all children had reached a corrected age of 3 y the regional CP registry was interrogated to identify any additional children being diagnosed after 2 y corrected age. Maternal and paternal educational levels were defined in three categories: completed compulsory school, upper secondary education or university degree.

Neurologic and developmental outcome. Developmental outcome was assessed by a psychologist (AH) using the Bayley Scales of Infant Development (BSID-II) with the two different index scales, Mental Developmental Index (MDI) and Psychomotor Developmental Index (PDI). A subnormal development was defined by an index score of 1 SD below the normative mean (MDI or PDI <85) and a developmental delay was defined as 2 SD below the normative mean (MDI or PDI <70). Infants with MDI or PDI scores of 50 or below were assigned a score of 50 according to the manual (17).

Neurologic assessment was performed by a neonatalist (I.H.-P.), using the Hammersmith Infant Neurologic examination, which is a method previously optimized for assessment of infants and children between 2 and 18 mo of age (18). The method is based on 26 items where each item is scored separately. The item scores can be added, thus achieving a Neurologic Optimality Score (NOS) with a maximum score of 78. A score below the 10th percentile was defined as suboptimal (NOS <74). The developmental and neurologic assessments were performed on the same day and the examiners were blinded to each other's results.

Statistical data analysis. Statistical analysis was performed using SPSS v 15.0 for Microsoft Windows (SPSS Inc., Chicago, IL). Levels of cytokines were logarithmically transformed. Correlations between continuous variables were assessed with the Pearson correlation coefficient. Univariate analyses between clinical variables or cytokines and categorical outcome variables were assessed using χ^2 test, Fischers exact test, Mann-Whitney U-test, or one sample *t* test as appropriate. Logistic regression was used to obtain odds ratios (95% CI) for each of the comparisons. Cytokines exhibiting significant univariate relationships with outcome variables at specific time-points were reevaluated using ANOVA for repeated measures. Cytokines and clinical variables exhibiting significant univariate associations with outcome measures were entered into multivariate analysis using logistic regression analysis (stepwise backward and forward procedures, log-likelihood ratio) to assess the best predictive model for each outcome measure.

RESULTS

Outcome measures at 2 y of age. The median (range) scores of NOS, MDI and PDI at assessment were 71 (24–78), 86 (50–118) and 96 (50–117), respectively. The NOS correlated significantly with both the MDI and PDI, r = 0.61 ($p < 0.001$) and r = 0.72 ($p < 0.001$) respectively.

Altogether 47 children (70%) had a suboptimal NOS <74. Thirty-one children (46%) had a MDI <85 and eight children (12%) had a MDI <70. In twenty children (30%) the PDI was <85 and in seven children (10%) it was <70. All 20 children with PDI <85 also had a NOS <74.

Five children (7%) had developed CP. Two children had spastic bilateral CP (diplegia), two had spastic unilateral CP (hemiplegia), and one had dystonic CP.

Ante- and neonatal variables, parental education, and outcome at 2 y of age. Univariate analysis identified antenatal and neonatal clinical variables associated with suboptimal NOS (<74), subnormal MDI or PDI (<85) and CP respectively (Table 1). A suboptimal NOS was associated with maternal infection, lower GA at birth and any cerebral hemorrhage. A subnormal MDI was associated with Apgar score

Table 1. *Univariate associations between antenatal and neonatal variables and Neurological Optimality Score (NOS) <74, Mental Developmental Index (MDI) <85, Psychomotor Developmental Index (PDI) <85 and cerebral palsy at 2 y of age*

Variable	All infants *N* (%)	NOS <74 *N* = 47		MDI <85 *N* = 31		PDI <85 *N* = 20		Cerebral palsy *N* = 5	
		OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
Premature rupture of membranes	26 (39)	1.7	0.6–5.3	0.3	0.1–0.8*	0.6	0.2–1.8	1.1	0.2–6.8
Maternal infection	20 (30)	5.6	1.2–27.0*	1.6	0.6–4.7	5.2	1.6–16.2†	11.5	1.2–111*
Gestational age (d)	—	0.9	0.9–0.98†	0.97	0.9–1.01	0.9	0.9–0.98†	0.9	0.9–1.03
Apgar score <7 (5 min)	22 (33)	2.5	0.7–8.6	5.3	1.7–16.4†	4.0	1.3–12.1*	9.8	1.02–93.6*
Any cerebral hemorrhage	16 (24)	8.4	1.02–69.2*	2.3	0.7–7.3	3.9	1.2–12.5*	14.8	1.5–144*
Severe brain damage§	10 (15)	1.8	0.4–9.6	3.2	0.8–13.7	4.6	1.1–18.7*	37.3	3.6–390†
Mean arterial blood pressure (0–72 h)	—	0.9	0.8–1.01	0.9	0.8–0.99*	0.9	0.8–1.06	0.9	0.8–1.2
Persistent ductus arteriosus	21 (31)	2.3	0.7–7.9	4.7	1.5–14†	2.4	0.8–7.2	0.5	0.06–5.0
Retinopathy of prematurity	14 (21)	1.1	0.3–4.0	3.8	1.1–13.8*	2.1	0.6–7.1	2.8	0.4–18.5
Ventilator treatment (d)	—	1.1	0.98–1.1	1.1	1.02–1.2*	1.1	1.004–1.1*	1.0	0.96–1.1
Maternal educational level‖	16 (24)	0.6	0.2–2.1	0.2	0.05–0.7*	0.5	0.1–1.8	2.3	0.3–15.1
Paternal educational level‖	11 (16)	0.4	0.1–1.6	0.2	0.04–1.0*	0.2	0.02–1.6	—‡	—‡

* $p < 0.05$.

† $p < 0.01$.

‡ OR was not calculated due to zero cells.

§ Intraventicular hemorraghe grade III and/or white matter brain damage.

‖ University degree *vs*. no university degree.

<7 at 5 min, persistent ductus arterious, retinopathy of prematurity and increased number of ventilator days respectively whereas an increase in MABP (0–72 h), PROM and either a maternal or a paternal educational level corresponding to university degree were associated with a decreased risk for a subnormal MDI. A subnormal PDI was associated with maternal infection, lower GA at birth, Apgar score <7 at 5 min, any cerebral hemorrhage, severe brain damage and increased number of ventilator days. CP was associated with maternal infection, Apgar score <7 at 5 min, any cerebral hemorrhage and severe brain damage. No significant associations were observed between the variables preeclampsia, clinical chorioamnionitis, birth weight for GA, gender, postnatal septicemia, supplemental oxygen at 36 gestational wks and accumulated dose of hydrocortisone and/or betamethasone and any of the respective outcome measures.

Levels of cytokines at birth and outcome at 2 y of age. Significant associations between concentrations of cytokines and outcome measures were only present in cord blood and in blood samples taken at 6 h and were restricted to concentrations of TNF-α, IL-6 and IL-8 and to the outcome measures PDI <85 and CP. Increased concentrations of TNF-α in cord blood OR (95% CI) 3.3 (1.1–10.2), $p = 0.013$ and at 6 h 7.8 (0.9–71.8), $p = 0.015$ and of IL-6 in cord blood 1.7 (1.0–2.9), $p = 0.048$ were associated with PDI <85. Increased concentrations of TNF-α in cord blood OR (95% CI) 3.6 (1.002–12.8), $p = 0.044$ and of IL-8 in cord blood 3.5 (1.2–10.6), $p = 0.023$ were associated with CP. These univariate relationships at specified time-points remained significant after adjustment for repeated measurements. Concentrations of TNF-α and IL-8 in cord blood and at 6, 24, and 72 h postnatal age in relation to PDI and CP are shown in Figs. 1 and 2.

No significant associations were observed between concentrations of IFN-γ, IL-1β, IL-2, IL-4, IL-6, IL-10, IL-12 and any of the outcome measures.

Multivariate analysis. Levels of cytokines were assessed together with clinical variables in relation to outcome using logistic regression analysis. A prerequisite for inclusion of a variable into multivariate analysis was a significant *univariate* association with the respective outcome measures. Models with the highest combined predictive capacity for the respective outcome measures are given in Tables 2–5.

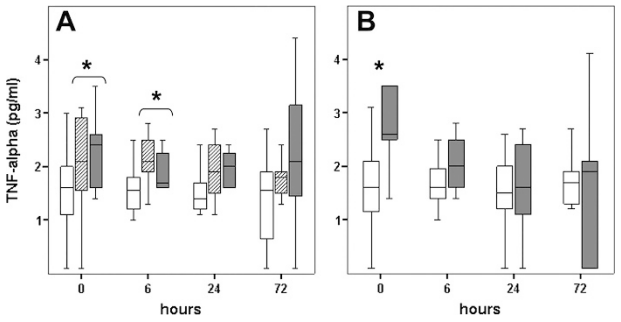

Figure 1. Levels of TNF-α in cord blood and at 6, 24 and 72 h of postnatal age in relation to psychomotor developmental index (PDI) (*A*) and cerebral palsy (CP) (*B*) at 2 y of age. In panel A, PDI ≥85 (□: $n = 47$); PDI 70–84 (▨: $n = 13$); PDI <70 (■: $n = 7$). In panel B, presence of CP (■: $n = 5$); no CP (□: $n = 62$). Medians and interquartile ranges are indicated. * = $p < 0.05$.

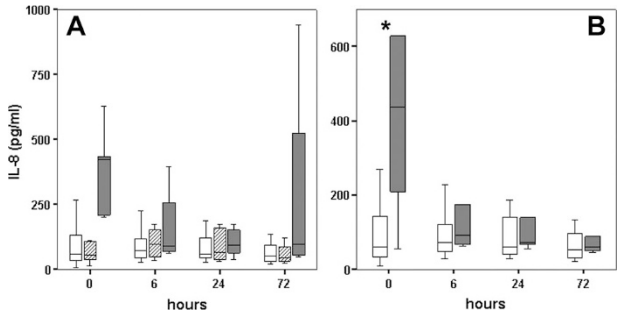

Figure 2. Levels of IL-8 in cord blood and at 6, 24 and 72 h of postnatal age in relation to psychomotor developmental index (PDI) (*A*) and cerebral palsy (CP) (*B*) at 2 y of age. In panel A, PDI ≥ 85 (□: $n = 47$); PDI 70–84 (▨: $n = 13$); PDI <70 (■: $n = 7$). In panel B, presence of CP (■: $n = 5$); no CP (□: $n = 62$). Medians and interquartile ranges are indicated. * = $p < 0.05$.

Table 2. *Best model of variables contributing to Neurologic Optimality Score (NOS) <74; NOS <74 (N = 47) vs. ≥74 (N = 20). Independent variables entered in the logistic regression model were gestational age (d), maternal infection (yes/no), and cerebral hemorrhage (yes/no)*

Risk factor (stepwise backward and forward)	OR (95% CI)
Gestational age (d)	0.9 (0.9–0.99)*
Cerebral hemorrhage (no = 0; yes = 1)	7.8 (0.9–65.5)*

* $p < 0.05$.

Table 3. *Best model of variables contributing to Mental Developmental Index (MDI) <85; MDI <85 (N = 31) vs. MDI ≥85 (N = 36). Independent variables entered in the logistic regression model were premature rupture of membranes (yes/no), Apgar score <7 at 5 min (yes/no), persistent ductus arteriosus (yes/no), mean arterial blood pressure (0–72 h) (mm Hg), total no of ventilator days, retinopathy of prematurity (yes/no) and maternal/paternal educational level (university degree yes/no)*

Variable (stepwise backward and forward)	OR (95% CI)
Premature rupture of membranes (no = 0; yes = 1)	0.05 (0.006–0.4)*
Apgar score <7 at 5 min (no = 0; yes = 1)	25.5 (3.1–212)*

* $p < 0.01$.

Table 4. *Best models of variables contributing to Psychomotor Developmental Index (PDI) <85; PDI <85 (N = 20) vs. PDI ≥85 (N = 47). Independent variables entered in the logistic regression model were TNF-α (pg/ml, cord blood and 6 h), IL-6 (pg/ml cord blood) gestational age (d), maternal infection (yes/no), Apgar score <7 at 5 min (yes/no), severe brain damage (yes/no) and total number of ventilator days*

Variable (stepwise backward and forward)	OR (95% CI)
TNF-α (pg/ml, cord blood)	4.5 (1.3–16.1)†
Gestational age (d)	0.9 (0.9–0.99)*

* $p < 0.05$.
† $p < 0.01$.

A PDI <85 was best predicted by the combination of increased concentration of TNF-α in cord blood and decreasing GA. CP was best predicted by the combination of increased concentration of IL-8 in cord blood, Apgar score <7 at 5 min and severe brain damage.

Table 5. *Best model of variables contributing to cerebral palsy; yes (N = 5) vs. no (N = 62). Independent variables entered in the logistic regression model were TNF-α (pg/ml, cord blood), IL-8 (pg/ml, cord blood), maternal infection (yes/no), Apgar score <7 at 5 min (yes/no) and severe brain damage (yes/no)*

Variable (stepwise backward and forward)	OR (95% CI)
IL-8 (pg/ml, cord blood)	4.5 (0.9–22.7)*
Apgar score <7 at 5 min (no = 0; yes = 1)	45.8 (0.8–2501)*
Severe brain damage (no = 0; yes = 1)	50.9 (2.1–1248)†

* $p < 0.05$.
† $p < 0.01$.

DISCUSSION

The main findings of this study were that increased levels of the proinflammatory cytokines TNF-α and IL-8 in cord blood and at 6 h were associated with subnormal PDI and with CP at 2 y of corrected age in very preterm infants. These findings support that proinflammation initiated *in utero* is a risk factor for impaired development in preterm infants.

Serial sampling of cytokines performed at predefined time points and a high rate of participation at follow-up at 2 y of age enabled an assessment of which time-point carries the best predictive value of subsequent impairment. Concentrations of proinflammatory mediators, namely TNF-α and IL-8 in cord blood, were associated with subnormal development at 2 y. This finding may explain why previous investigators sampling inflammatory mediators at a later time point have failed to observe an association between proinflammation and subsequent neurodevelopmental impairment in preterm infants (19).

The number of infants with CP or developmental delay (MDI/PDI <70) was low thus limiting the possibility of evaluating significant associations between cytokine concentrations and severe developmental impairment at 2 y of age. The twenty children with a subnormal PDI (<85) included all who had severe IVH grade III and a large proportion of those with low Apgar score at 5 min. In addition, all infants who developed CP had a subnormal PDI and all twenty children with subnormal PDI had suboptimal NOS (<74). This infers that the applied cut-off of PDI below 85 at 2 y of age defines an outcome, which is associated with perinatal morbidity and with a risk of future developmental impairment. On the contrary, a considerably higher proportion of children ($n = 47$) had a suboptimal NOS (<74) which suggests that suboptimal NOS included a higher proportion of healthy infants than subnormal PDI. Nevertheless all infants with IVH grade III or CP had suboptimal NOS. Assessment of NOS was performed at a higher age than in previous study which may have affected the proportion of infants with a score defined as subnormal (18).

A limitation in the present study was the absence of placental histology. We therefore used clinical definitions such as clinical chorioamnionitis, PROM, or maternal infection as markers of antenatal inflammation. Clinical chorioamnionitis was infrequent and we did not find any significant associations with developmental outcome. Maternal infection before delivery was associated with suboptimal NOS, subnormal PDI and with CP in univariate analysis. Maternal leukocytes have the capacity to invade fetal membranes suggesting that a maternal inflammatory response may influence detected concentrations of cytokines in cord blood (20). We observed an association between maternal infection and increased levels of IL-6 in cord blood (data not shown). In an experimental model of preterm placentas, infusion of lipopolysaccharide into the maternal compartment was followed by increased TNF-α secretion on the fetal but not the maternal side (21).

In the current evaluation, PROM was protective against subnormal MDI. This is contradictory to another study of extremely preterm infants where PROM was a risk factor for low MDI whereas clinical chorioamnionitis was protective for later development of CP (22). MDI performed at 20 mo of age has poor predictive value for subsequent neurodevelopmental outcome and is also influenced by parental interaction especially in the range between 70 and 84 (23). This influence was also observed in the present study. Further, none of the variables representing cerebral damage such as severe brain damage as defined by ultrasound was associated with subnormal MDI. Cytokines were not related to MDI at any time point.

The discrepancy in associations between different antenatal risk variables associated with fetal inflammation and later developmental outcome may be explained by the timing, duration, and extent of intrauterine inflammation. The interaction between a sub-threshold insult (*i.e.*, proinflammatory cytokines) followed by another later severe insult (*i.e.*, hypoxia) has been described where a severe insult may result either in decreased brain injury (tolerance) or in aggravated brain injury (sensitization). The interval between the subthreshold insult and the severe insult determines whether tolerance or sensitization occurs (24–26).

TNF-α was the cytokine most consistently showing associations with outcome measures. TNF-α plays a central role in experimental settings of brain injury and is involved in mechanisms inducing both sensitization and tolerance after lipopolysaccharide stimulation (25). TNF-α promotes brain injury through several pathways such as inhibition of migration and proliferation of neuronal precursor cells, prevention of differentiation of oligodendrocyte progenitors, induction of apoptosis and stimulation of reactive astrogliosis, all common findings in brains with white matter injury (27–29).

In a recent study, preterm infants with early WMD as detected by ultrasound and confirmed by MRI had higher cord blood concentrations of TNF-α and IL-1β (30). Both white and gray matter abnormalities detected by MRI in preterm infants have been associated with abnormal functional outcome (5). TNF-α modulates synaptic connectivity and facilitates glutamate dependent neuron death *in vitro* suggesting that increased concentrations of this cytokine may have a damaging effect on gray matter structures (31,32).

We have previously described an association between increased circulatory levels of the cytokine IFN-γ during the first 72 postnatal hours and WMD as detected by ultrasound in preterm infants (13). This is in line with reported increased levels of IFN-γ in cerebrospinal fluid of preterm infants with posthemorragic hydrocephalus and cystic WMD (33). Of note, no significant associations between IFN-γ and any of the outcome measures at 2 y was found although three of seven

children with WMD developed CP, all with cystic periventricular leukomalacia. WMD as defined by ultrasound was neither associated with MDI (<85) nor with PDI (<85) and *diffuse* WMD did not relate to CP (data not shown). The weak relationship between WMD and outcome was not unexpectedly accompanied by a lack of association between IFN-γ and outcome measures. Cerebral ultrasound has been described as less reliable than MRI in defining diffuse WMD and continued evaluation of associations between components of inflammation like IFN-γ and WMD must rely on the use of MRI (34).

Induced inflammation within a short time interval before or at the time of ischemia has a sensitizing effect on the brain (24). We observed that both TNF-α in cord blood and low Apgar score at birth were univariately associated with subnormal PDI and with CP. Aggravated brain injury after intracerebral administration of TNF-α at the time of an ischemic insult has recently been shown in adult mice (25). Interestingly, we did not observe any association between decreased Apgar score and cytokine levels in cord blood (data not shown). This suggests that the two phenomena have separate etiologies but a synergistic aggravating effect on outcome. One may speculate that the combination of fetal proinflammation with increased levels of TNF-α in combination with decreased Apgar score may result in neuronal damage and thus gray matter impairment.

The associations between TNF-α and developmental outcome measures were observed although cord blood concentrations of TNF-α were generally low and frequently undetectable. We suspect that TNF-α was released and peaked in many individuals already *in utero*, higher levels thereby escaping detection in cord or postnatal blood (13). The adenosine system has been shown to selectively inhibit toll-like receptor mediated TNF-α production in newborn infants and to enhance the production of the anti-inflammatory cytokine IL-10 (35,36). Metabolically stressful events such as birth increase extracellular levels of adenosine (35) and we have previously shown a characteristic increase in IL-10 peaking at 6 h after preterm birth in most infants (13). Thus, the process of birth may initiate a mechanism, which obscures the detection of increased levels of TNF-α.

Increased concentration of the chemokine IL-8 in cord blood was together with low Apgar score and cerebral damage the most predictive combination of variables for development of CP whereas TNF-α was only related to CP in univariate analysis. An association between increase in IL-8 in cord blood and CP in preterm infants has been described previously (37). Further, IL-6 in cord blood did show an association with PDI <85 but this association disappeared after adjustment for severe IVH/WMD. In line with this is our previous finding of an association between increased levels of IL-6 and IL-8 and arterial hypotension as well as development of severe IVH (13). In contrast to WMD, IVH grade III was associated with both subnormal PDI and CP (data not shown) with a majority developing CP and was thus the morphologic variable most predictive of functional impairment. Increased levels of TNF-α in cord blood or at 6 h were neither associated with development of IVH nor with WMD. Correspondingly, multivariate analyses showed that the association of TNF-α with outcome measures was not established *via* IVH or through WMD.

Levels of IL-8 and TNF-α in infants *with impaired development* (*i.e.*, PDI <85 or CP) were highest in cord blood samples. Although, presence or location of antenatal inflammation was not confirmed by placental histology or amniotic fluid sampling, the results suggest that prenatal initiation of inflammation is of importance for later impairment. In comparison to TNF-α, there is scarce support for the *direct* contribution of IL-8 in development of brain damage. IL-8 is relatively easy to detect and peaks later than TNF-α. After the initiation of the inflammatory stimulus, IL-8 still shows a steady increase after 48 h *in vitro* (14). However, in this study, significant relationships between IL-8 and outcome measures were restricted to concentrations in cord blood. Decline in levels of IL-8 after birth was varied with some infants exhibiting a very short half-life for IL-8 (data not shown). Half-life *in vivo* after preterm birth would thus appear considerably shorter than that described *in vitro*.

In conclusion, these findings support the theories of TNF-α being a key cytokine for cerebral damage in combination with hypoxic events and that proinflammation at birth in very preterm infants has implications for functional outcome. Future noninvasive methods of diagnosing fetal inflammation *in utero* might be of help to predict an optimal time point for delivery and open the possibility of targeting anti-inflammatory therapies with the purpose to reduce brain injury in preterm infants.

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