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Cytokines and Post-hemorrhagic Ventricular Dilation in Premature Infants

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Abstract

Objective—To determine in extremely low birth weight (ELBW) infants if elevated blood interferon- γ (IFN- γ), interleukin-1 β (IL-1 β), IL-18, tumor necrosis factor- α (TNF- α), and transforming growth factor- β (TGF β) are associated with need for shunt following severe intraventricular hemorrhage (IVH), or with ventricular dilation following milder grades/no IVH.

Study design—Whole blood cytokines were measured on postnatal days 1, 3, 7, 14, and 21. Maximum IVH grade in the first 28d, and shunt surgery or ventricular dilation on subsequent ultrasound (28d -36 w PMA) were determined.

Results—Of 902 infants in the NICHD NRN Cytokine study who survived to 36w/discharge, 3.1% had shunts. Of the 12% of infants with severe (Gr III–IV) IVH, 26% had a shunt associated with elevated TNF- α . None of the infants without IVH (69%) or with Gr I (12%) or II (7%) IVH received shunts, but 8.4% developed ventricular dilation, associated with lower IFN- γ and higher IL-18.

Conclusion—Statistically significant but clinically non-discriminatory alterations in blood cytokines were noted in infants with severe IVH who received shunts and in those without severe IVH who developed ventricular dilation. Blood cytokines are likely associated with brain injury but may not be clinically useful as biomarkers for white matter damage.

Keywords

Infant; premature; Cytokines; Hydrocephalus; Intraventricular hemorrhage; Intracranial hemorrhage

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Conflicts of interest: None

INTRODUCTION

Post-hemorrhagic ventricular dilation (PHVD) occurs in a significant proportion of extremely low birth weight (ELBW) infants developing intraventricular hemorrhage (IVH).¹ Although generally proportional to the magnitude of the intraventricular hemorrhage, the pathogenesis of PHVD remains uncertain.

Various cytokines have been implicated in the pathogenesis of PHVD. In a rat model of intracerebral hemorrhage, elevated expression of tumor necrosis factor- α (TNF- α) is noted in neutrophils, macrophages, and microglia surrounding the hemorrhage, and TNF- α inhibition improved neurobehavioral outcome compared to the untreated group.² In a mouse model of stroke, deficiency or neutralization of TNF- α attenuated brain damage and mortality.³ Transforming growth factor - β (TGF- β) has also been considered a possible mediator of PHVD.⁴ In adults with subarachnoid hemorrhage, TGF- β in CSF correlates with ventricular dilation and with the need for shunt.^{5,6} Interferon- γ (IFN- γ), IL-1 β , and IL-18 are the other main cytokines which have been associated with white matter damage.^{7,8}

Following brain injuries, there is passage of TGF- β from the periphery to the intrathecal compartment across the blood brain barrier.⁹ Studies in preterm infants have not shown significant correlation between paired CSF and plasma concentrations for IL-6, IL-8, IL-10, IFN- γ , and TNF- α ,¹⁰ suggesting that CSF concentration of these cytokines may be more dependent upon primary CNS/CSF production.

There is currently limited knowledge on which of the many known cytokines mediate response to brain injury in premature infants. Infants who develop PHVD may demonstrate alterations in the postnatal temporal profile of these critical cytokines, either due to causal involvement of the cytokine in brain injury and subsequent development of PHVD, or due to the cytokine serving as a marker of brain injury and white matter damage.

Our objective in this study was (a) to determine if elevated blood IFN- γ , IL1- β , IL18, TNF- α , or TGF- β are associated with need for shunt after severe IVH, or with ventricular dilation in infants with milder grades /no IVH, and (b) to determine temporal profiles of the cytokines over the first three postnatal weeks, as most IVH occurs in the first few days and ventricular dilation occurs in the subsequent weeks, and therefore evaluation of the changes over time in the cytokines may yield additional information. It was hypothesized that these pro-inflammatory cytokines would be elevated in infants who needed a shunt or developed ventricular dilation compared to infants surviving without PHVD.

PATIENTS AND METHODS

This study was an ancillary analysis of data from a prospective cohort study (the “Cytokine Study”) performed in the 17 centers of the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD) Neonatal Research Network, which was approved by the Institutional Review Boards at all participating centers (Cincinnati Children’s Hospital Medical Center, Duke University, Emory University, Indiana University, Rainbow Babies & Children’s Hospital, Stanford University, University of Alabama at Birmingham, University of California – San Diego, University of Miami, University of New Mexico, University of Tennessee, University of Texas Southwestern Medical Center at Dallas, University of Texas Health Science Center at Houston, Wake Forest University, Wayne State University, Women & Infants Hospital of Rhode Island, Yale University) and with written informed consent obtained from the parent(s).¹¹

Infants 401–1000 g at birth (including SGA infants¹²) of both genders and all racial/ethnic groups were screened for eligibility. Infants were excluded if they were >72 hours of age.

Whole blood spots on filter paper were collected within four hours of birth (day 0), and on days 3±1, 7±1, 14±3, and 21±3 (date of sampling ± range of days within which sample could be obtained), and immediately frozen. Clinical data were collected by trained research coordinators, and all analyses were performed at a central data coordinating center (RTI International). The stored blood spots were analyzed in a batch for 25 cytokines using a multiplex Luminex assay (Luminex Corp., Austin, TX).^{13,14} This multiplex assay using approximately 6 µL of whole blood from blood spots has been validated, has low variation (<10% intra-assay; 7–23% inter-assay), and is sensitive with limits of detection lower than median concentrations observed in normal newborns.¹³ Cytokine concentrations that were undetectable were entered as “0”.

For this ancillary study, infants from the cytokine study were included if they survived to 36 weeks' post-menstrual age or earlier discharge, as infants who died earlier may have died before identification of PHVD. Clinical variables (Table 1) and the most severe findings on cranial ultrasound within the first 28 days (initial ultrasound) and on a subsequent ultrasound between 28 days of age and 36 weeks' post-menstrual age (late ultrasound) were analyzed.

IVH on the initial ultrasound was classified as: Grade 0 = no IVH; Grade 1 = blood/echodensity in the germinal matrix/subependymal area; Grade 2 = blood/echodensity in the ventricle (ventricular size not enlarged); Grade 3 = blood/echodensity in the ventricle AND ventricular size enlarged; Grade 4 = blood/echodensity in the parenchyma. The maximum grade of IVH on either side was recorded. Subsequent ventricular dilation was defined as ventricular dilation on the late ultrasound. A shunt was defined as the surgical placement of a shunt (either a subgaleal shunt, a ventricular reservoir, a ventriculoperitoneal shunt, or other form of surgical CSF drainage).

Statistical analysis

To determine if blood concentrations of the cytokines were higher in the infants with severe IVH (Grade 3 or 4) who received a shunt compared to those with severe IVH who were not shunted, the peak values of these cytokines over the period of measurement were compared using the Wilcoxon two-sample test, t-approximation. The peak rather than median values were analyzed as it was considered that pathophysiology may be more closely associated with the more extreme values. Logistic regression analyses were done for the dependent variable of shunt surgery, adjusting for birth weight, gender, and antenatal steroids.

To determine if concentrations of the selected cytokines were higher in infants with mild or no IVH (Grades 0, 1, or 2) who developed ventricular dilation compared to those who did not develop ventricular dilation, the peak values of each of these cytokines were also compared. Logistic regression analyses were done for the dependent variable of ventricular dilation, adjusting for birth weight, gender, and antenatal steroids.

As Grade IV IVH and periventricular leukomalacia (PVL) may be considered distinct entities, similar analyses were done for infants with Grade IV IVH and/or PVL who received a shunt compared to those who were not shunted.

The c-statistic (corresponding to the area under the curve) was calculated for regression models. To determine the contribution of the cytokines to the c-statistic, models were developed including peak values of all cytokines, as well as excluding cytokines. These models were adjusted for birth weight, gestational age, gender, race, antenatal steroids, and prophylactic indomethacin.

Changes in cytokine concentrations over time in infants with severe IVH who received shunts, infants with severe IVH who did not receive shunts, infants with no/mild IVH who

developed ventricular dilation, and infants with no/mild IVH who did not develop ventricular dilation were plotted using population median values in order to evaluate temporal profiles of the cytokines over the first three postnatal weeks.

RESULTS

Patient characteristics

1067 ELBW infants were enrolled in the study from 1999 to 2001, of whom 902 infants survived to 36w/discharge (Table 2). 895 infants had IVH data that could be analyzed. Patient characteristics and peak cytokine concentrations in infants classified by the grade of IVH are shown in Table 3. 68.6% of infants did not have any IVH, 12% had grade I, 7.4% grade II, 6% grade III, and 6% grade IV IVH. Approximately 20% of the infants with grade III IVH, and 32% of the infants with grade IV IVH received a shunt. None of the infants with grade 0–2 IVH had placement of a shunt (Table 3).

Shunt in infants with severe IVH

107 infants with severe IVH survived to 36 weeks/discharge, and 28 of them received a shunt. Peak TNF- α was higher in infants who received a shunt by both unadjusted and adjusted analyses (Table 4).

Ventricular dilation among infants with mild or no IVH

Of 622 infants (not all the 787 infants with milder or no IVH had subsequent sonograms), 52 (8.4%) developed ventricular dilation after 28 days of age (Table 5). None received a shunt. Infants who developed subsequent ventricular dilation had lower peak IFN- γ and increased peak IL-18, which did not remain significant after adjustment for birth weight, gender, and antenatal steroids (Table 5).

Shunt in infants with Grade IV IVH and/or PVL

81 infants with Grade IV IVH and/or PVL survived to 36 weeks/discharge, and 20 of them received a shunt. None of the cytokine concentrations were associated with a shunt, either on un-adjusted or adjusted analysis (Table 6).

Comparison of models including cytokines and clinical variables to those including only clinical variables

Comparison of the models using the peak cytokine values and clinical variables to models employing only clinical variables showed that addition of cytokine data did not improve the c-statistic, although trends for improvement ($0.05 < p < 0.25$) were noted for severe IVH as well as shunt following severe IVH (from 0.58 to 0.67 by addition of cytokine data) (Table 7).

Temporal profiles (Figure)

An evaluation of the temporal profiles of the median blood concentrations of the cytokines in the ELBW infants classified by ventricular dilation and need for shunt (Figure) showed complex changes over time, with IFN- γ reduced both initially (day 3) and at days 14 and 21 in infants with severe IVH who were shunted, as well as in infants with mild IVH who developed ventricular dilation. Infants with severe IVH who were shunted had trends of increased IL-1 β on day 14 days, increased IL-18 on day 7, and increased TNF- α on days 7, 14, and 21. TGF- β did not demonstrate any visually appreciable trends by grade of IVH and ventricular dilation/need for shunt.

DISCUSSION

The major findings of this study involving a large number of extremely low birth weight infants from multiple tertiary care centers were the alterations in blood cytokines associated with shunts following severe IVH or ventricular dilation following milder IVH. Other observations include (a) the incidence of different grades of IVH, (b) the incidence of shunts following severe IVH (20% in grade III and 32% in grade IV), and (c) the substantial proportion of infants with minor grades of IVH who developed subsequent ventricular dilation (8.4%).

In this study, 31% of surviving infants had IVH, and the IVH was severe in 12%. 20% of infants with grade III IVH, and 32% of those with grade IV IVH received shunts. These results are consistent with those obtained by other investigators in earlier cohorts,^{15,16} suggesting that the incidence of severe IVH and the perceived need for a shunt have not changed over the past two decades.

In this study, TNF- α was the only cytokine significantly higher in infants who received a shunt following severe IVH. Savman et al.¹⁷ showed that TNF- α was more often detected (43%) in the CSF of infants with PHVD compared to those without PHVD (11%, $p < 0.05$). Increased TNF- α has been seen in PVL lesions,^{18,19} and polymorphisms associated with higher TNF- α increase cerebral palsy risk²⁰ indicating possible involvement of TNF- α in white matter damage. Adults with hydrocephalus have increased intrathecal TNF- α which is eliminated by shunt surgery.²¹ It is therefore possible that increased TNF- α may be the result of ventricular dilation, and not its cause. This speculation may be supported if future studies show that shunt surgery in preterm infants reduces TNF- α .

IL-18 is a pro-inflammatory cytokine that may mediate CNS injury.²² Animal studies indicate that IL-18 contributes to neonatal white matter injury, with IL-18 expressed by astrocytes and microglia, and the IL-18 receptor found in astrocytes localized in and around the subventricular white matter.²³ Higher cord blood levels of IL-18 have been associated with white matter damage and subsequent CP.²⁴ IL-1 β is another pro-inflammatory cytokine that has been associated with neonatal brain white matter lesions.²⁵ Higher CSF IL-18 and IL-1 β have been noted in infants with PHVD.⁸ Our results indicate a trend (statistically non-significant) towards increased IL-18 and IL-1 β in infants who received a shunt.

Infants who developed subsequent ventricular dilation following mild/no IVH had lower peak IFN- γ . Evaluation of the temporal profiles suggested that lower IFN- γ was associated with PHVD requiring shunt, as well as ventricular dilation following milder IVH. This result is particularly interesting as it has been considered that higher serum IFN- γ is associated with WMD.^{7,26,27} Therefore, the reduction of IFN- γ in PHVD in our study is difficult to explain. One possible explanation is that higher levels of IFN- γ may be a beneficial response to injury, rather than a contributor to injury as IFN- γ (but not TNF- α) promotes neuronal differentiation and neurite outgrowth in neural stem cells,²⁸ and reduces reactive astrocytosis.²⁹

Our study did not show associations of TGF- β with PHVD. Previous studies have implicated TGF- β in animal models of PHVD,^{30,31} but inhibition of TGF- β activity did not reduce PHVD.³² Similar to IFN- γ , it is possible increased TGF- β may reflect a beneficial response to injury, as TGF- β is a survival factor for neonatal cortical neurons.³³

A limitation of this study is that whole blood cytokine concentrations may not reflect cytokine concentrations in the brain parenchyma or CSF. Blood cytokines generated at other sites of inflammation may have limited effect on the brain due to variable passage across the blood-brain barrier. Also, cytokines generated in the brain following IVH may not be a

major fraction of circulating cytokines. Other limitations include the fact that while the studied cytokines were the ones implicated in the literature, other mediators may possibly be more important. IVH may be either unilateral or bilateral, and the size of a grade III and IV IVH may also vary substantially, but such information was not available. It is also not possible to determine the exact relationship between the peak cytokine level and the occurrence of IVH, dilatation, or shunting, as we do not have data on when exactly IVH, dilatation, or shunting occurred in patients in this dataset. Another limitation is that our *a priori* hypothesis included the analysis of peak cytokine concentrations over days 1–21, but an evaluation of the temporal profiles suggests that a more focused evaluation of cytokines at specific time points (e.g. IFN- γ on days 14–21, or IL-1 β on day 14), or an analysis of the nadir rather than the peak, may have proven more strongly associated with outcome.

Infants with Grade IV IVH and/or PVL who received a shunt had cytokine concentrations not significantly different from those who did not get a shunt. One explanation is that the incidence of PVL is rather low (compared to IVH) with much variability in ultrasound interpretation. Another is that PVL is often followed by resolution of the cysts and ventricular dilation, and we may be identifying and analyzing these infants as those with ventricular dilation (Grade III IVH).

A strength of this study is the prospective evaluation of a relatively large number of ELBW infants from multiple centers. In addition, cytokines were measured at multiple time points, including the period of maximum risk for IVH (days 1–3) and PHVD (days 3–21). Cytokines selected based upon the existing literature were evaluated, rather than an analysis restricted to a single cytokine, and both unadjusted analyses as well as analyses adjusting for major clinical variables were performed.

In conclusion, PHVD and the need for ventricular shunting is a serious morbidity³⁴. Knowledge of the peak cytokine concentrations did not improve identification of infants are most at risk (as compared to models with clinical data alone), but these data provide an opportunity for hypothesis-generation, especially regarding the roles of increased TNF- α and reduction of IFN- γ in PHVD, or different temporal patterns in cytokine concentrations in PHVD. Identification of the precise role of the cytokines in PHVD may lead to new therapeutic strategies aimed at inhibiting their activity or receptors.

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Data collected at participating NRN sites were transmitted to RTI International, the data coordinating center (DCC) for the NRN, which stored, managed, and analyzed the data for this study. On behalf of the network, Dr. Abhik Das (DCC PI) and Mr. Scott A. McDonald (DCC Statistician) had full access to all the data in the study and take responsibility for the integrity of the data and accuracy of the data analysis.

Abbreviations

ELBW	extremely low birth weight
PHVD	Post-hemorrhagic ventricular dilation
PVL	Periventricular leukomalacia

The following investigators participated in this study

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What is already known on this topic

- Post-hemorrhagic ventricular dilation occurs in a significant proportion of extremely low birth weight infants developing intraventricular hemorrhage
- Although generally proportional to the magnitude of the intraventricular hemorrhage, the pathogenesis of the ventricular dilation remains uncertain
- Various cytokines have been implicated in the pathogenesis of brain injury but their role in post-hemorrhagic ventricular dilation is not known

What this study adds

- Alterations in blood cytokines were noted in infants with severe intraventricular hemorrhage with shunts, and in those with no/minor hemorrhage who developed ventricular dilation
- Blood cytokines are likely associated with brain injury but may not be clinically useful as biomarkers for white matter damage

Temporal profile of cytokines in relation to ventricular dilation or need for shunt

Figure 1.

Temporal profile of cytokines in ELBW infants with (a) Grade I/II IVH and no ventricular dilation, (b) Grade I/II IVH and ventricular dilation, (c) severe IVH and no shunt, (b) severe IVH and shunt. Values shown are median values, unadjusted for other cytokine or clinical variables.

Table 1

Clinical variables used and their definitions.

Variable	Definitions of clinical variables
Birth weight	Birth weight in grams
Gestational Age	Gestational age in completed weeks
SGA	Small for gestational age (below the 10 th percentile). This is determined by the combination of gender, gestational age, and birthweight.
Male gender	Male gender. No infants with ambiguous genitalia were noted.
Black race	Black, not Hispanic
Antenatal steroids	Administration of antenatal steroids to accelerate pulmonary maturity (1 dose)
Pregnancy Induced Hypertension	Hypertension/pre-eclampsia/eclampsia
Cesarean delivery	Cesarean delivery of infant
1 min Apgar (median; 25–75 th)	Apgar score at 1 minute
5 min Apgar (median; 25–75 th)	Apgar score at 5 minutes
Severe Intraventricular Hemorrhage (IVH)	IVH Grades III (Blood/echodensity in ventricle AND ventricular size enlarged) and Grade IV (Blood/echodensity in parenchyma)
Mechanical Ventilation (IMV)	Use of either conventional or high frequency ventilator

Table 2

Patient characteristics

	All infants enrolled in study N=1067	Infants surviving to 36w/discharge N=902
Birth Wt (g)	761 ± 141 (Median 760)	776 ± 136 (Median 779)
GA (w)	25.8 ± 2.0 (Median 26)	26.1 ± 1.9 (Median 26)
Male Gender	48.6 %	47.3 %
Black Race	47.7 %	47.9 %
Premature ROM (>24 hr)	22.1 %	23.0 %
Cesarean delivery	58.8 %	61.3 %
Antenatal steroids (Any)	75.8 %	78.3 %
Apgar 1	4 ± 2 (Median 4)	5 ± 2 (Median 5)
Apgar 5	7 ± 2 (Median 7)	7 ± 2 (Median 7)
Prophylactic Indocin	22.0 %	23.1 %
No IVH	63.8 %	68.6 %
IVH – Gr 1	11.8 %	12.0 %
IVH – Gr 2	7.6 %	7.4 %
IVH – Gr 3	6.8 %	6.0 %
IVH – Gr 4	9.9 %	6.0 %
Shunt surgery	2.7 %	3.1 %
PVL	4.6 %	4.2 %
TNF-α (peak) (pg/ml)	136 ± 260 Median 87	139 ± 278 Median 89
TGF-β (peak) (pg/ml)	2770 ± 1668 Median 2413	2934 ± 1681 Median 2544
IFN-γ (peak) (pg/ml)	331 ± 619 Median 232	341 ± 661 Median 244
IL-1β (peak) (pg/ml)	372 ± 431 Median 201	357 ± 390 Median 195
IL-18 (peak) (pg/ml)	2630 ± 1515 Median 2281	2642 ± 1497 Median 2278

Table 3

Classification of infants* by Grade of IVH and cytokine peak serum concentrations (median, 25–75th centiles; in pg/ml) and demographics.

	No IVH N=614	Grade I N=107	Grade II N=66	Grade III N=54	Grade IV N=54
Birth Wt (g)	780.4 ± 134.6 Median 780	788.1 ± 139.7 Median 805	758.3 ± 137.2 Median 743.5	757.2 ± 140.8 Median 737.5	736.4 ± 136.2 Median 724
GA (w)	26.3 ± 1.9 Median 26	25.9 ± 1.7 Median 26	25.3 ± 1.8 Median 25	25.4 ± 1.5 Median 25	25.2 ± 2.2 Median 25
Male Gender	43.3%	62.6%	54.6%	57.4%	48.2%
Black Race	46.9%	54.2%	45.5%	42.6%	55.6%
Antenatal steroids (Any)	79.5%	77.6%	69.7%	83.3%	75.5%
Apgar I	5 ± 2 Median 5	4 ± 2 Median 4	4 ± 2 Median 4	4 ± 2 Median 4	3 ± 3 Median 2.5
Apgar 5	7 ± 2 Median 7	6 ± 2 Median 7	6 ± 2 Median 7	6 ± 2 Median 6.5	6 ± 2 Median 6
Prophylactic Indocin	25.1%	26.2%	15.2%	20.4%	9.3%
Shunt surgery	0%	0%	0%	20.4%	32.1%
PVL	1.8%	5.6%	4.6%	14.8%	18.5%
TNF-α (peak)	89 (58 – 156)	75 (46 – 151)	88 (56 – 145)	110 (75 – 203)	87 (64 – 159)
TGF-β (peak)	2696 (1896 – 3881)	2364 (1674 – 3436)	2375 (1588 – 3158)	2547 (1786 – 3338)	2132 (1329 – 3120)
IFN-γ (peak)	253 (144 – 436)	283 (128 – 432)	241 (101 – 391)	181 (114 – 355)	240 (161 – 330)
IL-1β (peak)	190 (93 – 541)	193 (85 – 530)	207 (96 – 445)	239 (117 – 620)	197 (101 – 524)
IL-18 (peak)	2185 (1563 – 3107)	2192 (1672 – 3340)	2449 (1868 – 3171)	2901 (1858 – 4193)	2528 (1764 – 3310)

* Only infants who survived to 36 weeks / discharge are included.

Table 4

Need for shunt in infants* with Grade III–IV IVH in relation to cytokine concentrations (Median (25th–75th centile); in pg/ml)

Cytokines	Un-adjusted comparisons			Logistic regressions for shunt (adjusted for birthweight, gender, and antenatal steroids)		
	No shunt N=79	Shunt done N=28	p**	OR (95% CI)	p	Significant covariates
TNF- α (peak)	90 (64 – 157)	120 (84 – 223)	0.02	1.55 (OR per 100 pg/ml) (1.00 – 2.40)	0.049	None
TGF- β (peak)	2340 (1575 – 3120)	2482 (1439 – 3796)	0.63	1.08 (OR per 1000 pg/ml) (0.82 – 1.44)	0.57	None
IFN- γ (peak)	223 (139 – 349)	184 (114 – 324)	0.34	0.89 (OR per 100 pg/ml) (0.73 – 1.09)	0.26	None
IL-1 β (peak)	191 (102 – 506)	354 (124 – 626)	0.14	1.10 (OR per 100 pg/ml) (0.96 – 1.27)	0.17	None
IL-18 (peak)	2502 (1764 – 3426)	3102 (2001 – 4012)	0.14	1.28 (OR per 1000 pg/ml) (0.95 – 1.74)	0.11	None

* Only infants who survived to 36 weeks / discharge are included.

** P-values are from Wilcoxon two-sample test, t-approximation.

Subsequent[†] ventricular dilation among infants^{*} with no IVH or grade I–II IVH within 28 days, in relation to cytokine concentrations (Median (25th–75th centile); in pg/ml)

Table 5

	Un-adjusted comparisons			Logistic regressions for ventricular dilation (adjusted for birthweight, gender, and antenatal steroids)		
	No subsequent ventricular dilation N=570	Subsequent ventricular dilation N=52	p ^{**}	OR (95% CI)	p	Significant covariates
TNF- α (peak)	89 (58 – 154)	85 (63 – 160)	0.85	1.00 (OR per 100 pg/ml) (0.92–1.09)	1.00	None (BW < 0.06)
TGF- β (peak)	2537 (1809 – 3686)	2413 (1643 – 3452)	0.49	0.99 (OR per 1000 pg/ml) (0.83 – 1.19)	0.93	None (BW < 0.07)
IFN- γ (peak)	252 (143 – 423)	170 (98 – 338)	0.03	0.93 (OR per 100 pg/ml) (0.81 – 1.05)	0.24	None (BW < 0.06)
IL-1 β (peak)	196 (95 – 547)	229 (88 – 668)	0.75	1.04 (OR per 100 pg/ml) (0.99 – 1.10)	0.14	None (BW < 0.06)
IL-18 (peak)	2264 (1617 – 3177)	2399 (2043 – 3456)	0.03	1.09 (OR per 100 pg/ml) (0.92 – 1.30)	0.33	None (BW < 0.09)

^{*} Only infants who survived to 36 weeks / discharge are included.

^{**} P-values are from Wilcoxon two-sample test, t-approximation.

[†] Infants whose subsequent sonogram was also within 28 days (N=23) are excluded.

Table 6

Need for shunt in infants* with Grade IV IVH and/or PVL in relation to cytokine concentrations (Median (25th–75th centile); in pg/ml)

Cytokines	Un-adjusted comparisons			Logistic regressions for shunt (adjusted for birthweight, gender, and antenatal steroids)		
	No shunt N=61	Shunt done N=20	** p	OR (95% CI)	p	Significant covariates
TNF- α (peak)	89 (59 – 189)	118 (81 – 193)	0.29	1.01 (OR per 100 pg/ml) (0.55 – 1.84)	0.98	None
TGF- β (peak)	2302 (1498–3368)	2023 (1439–4094)	1.0	0.98 (OR per 1000 pg/ml) (0.73 – 1.31)	0.88	None
IFN- γ (peak)	203 (126 – 304)	187 (148 – 324)	0.88	0.96 (OR per 100 pg/ml) (0.77 – 1.19)	0.68	None
IL-1 β (peak)	223 (135 – 503)	479 (95 – 626)	0.51	1.04 (OR per 100 pg/ml) (0.88 – 1.22)	0.66	None
IL-18 (peak)	2319 (1604 – 3231)	2929 (2229 – 3714)	0.08	1.23 (OR per 1000 pg/ml) (0.90 – 1.67)	0.19	None

* Only infants who survived to 36 weeks / discharge are included.

** P-values are from Wilcoxon two-sample test, t-approximation.

Table 7

Comparison of C statistics, with and without cytokines in models. All models include birthweight, GA, gender, race, antenatal steroids, and prophylactic indocin. Cytokines in the models are peak levels of TNF- α , TGF- β , IFN- γ , IL-1 β , and IL-18.

Outcome	C statistic with cytokines in model	C statistic without cytokines in model	p-value
Severe IVH (Grades III–IV)	0.67	0.66	0.15
Need for shunt among infants with IVH Grades III–IV	0.67	0.58	0.21
Subsequent ventricular dilation among infants with no IVH or IVH Grades I–II	0.68	0.67	0.39