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미숙아의 전신 염증 시 신경 발달의 관련인자: 전향적 코호트 연구

Factors associated with Neurodevelopment in Preterm Infants with Clinical Presentation of Systematic Inflammation : A Prospective Cohort Study

2020년 8월

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이 논문을 의학석사학위논문으로 제출함

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2020년 7월

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ABSTRACT

Predictors of Neurodevelopment during Systemic Inflammation in Preterm Infants: A Prospective Cohort Study

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Background: Several clinical studies have suggested that adverse neurodevelopment could be induced by systemic inflammation in preterm infants.

Objective: To investigate whether preterm infants with systemic inflammation would have impaired neurodevelopment, and if biomarkers and neurophysiologic studies during inflammation could be predicted neurodevelopment.

Methods: This prospective cohort study enrolled infants born before 30 weeks of gestation, or birth weight <1,250 g. Participants were assigned to groups according to the presence of systemic inflammation: Control (no inflammation, n=49), I (systemic inflammation, n=45). Blood, cerebrospinal fluid (CSF) samples and amplitude-integrated electroencephalography (aEEG) were performed at the time of inflammation. Brain MRI and aEEG were evaluated at near-term age were evaluated, and the Bayley Scales of Infant and

Toddler Development III (Bayley-III) was performed at a corrected age (CA) of 18 months. The association of laboratory markers including CBC, CRP, cytokines (IL-1 beta, IL-8, IL-6, TNF-alpha), and brain injury markers (S100B, Enolase, MBP) with head circumference, EEG, brain MRI, and Bayley-III was analysed.

Results: I group had more white matter injuries (2 vs. 26.7 %, Control vs. I, respectively), lower brain functional maturation (9.5 vs. 8), and smaller head size (30.5 vs. 29.3) at near-term age, and poorer neurodevelopment at 18 months of corrected age, than the control group ($p<0.05$). Seizure spike on aEEG (D0) had significant relationship with motor (F 0.581, p = 0.007) and social-emotional (F 0.544, p = 0.013) domains of Bayley-III. I/T ratio were negatively correlated with the motor (F -0.530, p = 0.016), and social (F -0.467, p = 0.038) domains. CRP (D0) was negatively correlated with the language (F -0.330, p = 0.033) and motor (F -0.330, p = 0.033) domains. TNF-alpha (D0) of plasma showed a significant correlation with most domains: cognitive (F -0.662, p = 0.037), motor (F -0.749, p = 0.013), and adaptive (F -0.783, p = 0.007).

Conclusions: Systemic inflammatory conditions affect neurodevelopment in preterm infants. Brain injury due to systemic inflammation can be monitored in real time through aEEG. The seizure spike on aEEG, I/T ratio, CRP, and TNF-alpha during inflammatory episodes can help predict neurodevelopmental outcomes.

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Keywords: Premature, White matter injury, Sepsis, Necrotizing enterocolitis, Inflammation,
Amplitude-integrated electroencephalography, Cytokine

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LIST OF ABBREVIATIONS

NEC	Necrotizing enterocolitis
aEEG	Amplitude-integrated electroencephalography
CRP	C-reactive protein
I/T ratio	Proportion of immature neutrophils
WMI	White matter injury
NICU	Neonatal intensive care unit
IVH	Intraventricular hemorrhage
CBC	Complete blood count
CSF	Cerebrospinal fluid
PM	Postmenstrual age
CA	Corrected age
Bayley-III	Bayley Scales of Infant and Toddler Development - the 3rd edition
GA	Gestational age
HC	Head circumference
RDS	Respiratory distress syndrome
SGA	Small for gestational age
BPD	Bronchopulmonary dysplasia
PDA	Patent ductus arteriosus
ROP	Retinopathy of prematurity

INTRODUCTION

As the survival rate of preterm infants has increased, the management of long-term complications, especially neurodevelopmental impairment, becomes important [1]. The most common brain injury in premature infants is periventricular leukomalacia [2], and its pathogenesis involves the effect of hypoxia, ischemia, and inflammation of the progenitor oligodendrocyte cells present during weeks 23-32 of gestation [3].

Preterm infants are at high risk for infection, and infection-induced inflammation were found to contribute to adverse neurodevelopment in several clinical and animal studies [4-7]. Based on this concept, there have been attempts to modulate systemic inflammation as well as using antibiotic therapy for infections [7]. However, premature infants who have systemic inflammation do not exhibit developmental impairment uniformly. If the infants with inflammation-associated white matter injury (WMI) at the time of inflammatory illness could be identified, they will make the target population for neuroprotection.

There have been no studies on the predictive indicators for adverse neurodevelopment during systemic inflammation. The objective of this study are two folds: first, to investigate whether preterm infants who experienced systemic inflammation are more susceptible to impaired short and long term neurodevelopment. Second, to examine the usefulness of amplitude-integrated electroencephalography (aEEG), an easily accessible technique to monitor the electrocortical activity in the neonatal intensive care unit (NICU), and laboratory markers including inflammatory cytokines and brain injury markers during inflammation in predicting neurodevelopmental problems.

MATERIALS AND METHODS

Study design

This was a prospective cohort study. We recruited infants born before 30 weeks of gestation, or whose birth weights were less than 1250 g, and were admitted to the NICU of the Seoul National University Children's Hospital from December 2013 to June 2017. We excluded infants with congenital anomalies and grade IV intraventricular hemorrhage (IVH).

The subjects were assigned to the control group at enrolment and re-assigned according to the clinical courses. During the NICU stay, if systemic infection was clinically suspected if there were signs such as fever, apnea, difficulty in breathing, tachycardia or bradycardia, hypotension, irritability, lethargy, and feeding intolerance, and blood tests including blood culture, complete blood count (CBC), and measurement of C-reactive protein (CRP) levels were performed subsequently for evaluation. If at least one of the following abnormal laboratory findings is revealed: (1) elevation of CRP > 1.0 mg/dL, (2) abnormal CBC of elevated proportion of immature neutrophils (I/T ratio ≥ 0.2), or platelets ≤ 100 × 109/L, and parenteral antibiotics were administered for more than 5 days, they were designated to the systemic inflammation group (I). If infants did not develop any septic episodes or show negative laboratory results after the sepsis workup, they remained in the control group. In the I group, CBC with differential counts and CRP level were evaluated on day zero (the day of symptom onset), two, and six of the inflammatory episode along with an aEEG. In addition, plasma and cerebrospinal fluid (CSF) samples were obtained for cytokine and brain injury marker analysis. The CSF tapping was performe

d for patients suspected of having sepsis or central nervous system i nfection. For all infants, an aEEG was done at 35 weeks of postmens trual age (PMA), a brain MRI was performed before discharge from t he NICU, and the 18 months of corrected age (CA) follow-up informa tion was collected.

This study was approved by the Institutional Review Board of Seo ul National University Hospital, and informed consent was obtained fr om the parents (IRB No. 1301-058-458).

aEEG

A portable electroencephalogram (EEG) monitor, Olympic Cerebral F unction Monitor (Olympic Medical, Seattle, USA) was used. Continuou s impedance was measured, and the electrodes were deemed to be we ll applied when the impedance was less than 10 kΩ. Continuous recor ding was done over 3 hours [8-10]. The aEEG scoring system was u sed to evaluate a maturation score. Four components of the aEEG rec ord including continuity, cycling, amplitude of the lower border, and b andwidth span were evaluated and the summed score ranged from 0 to 13 [8]. In addition, we classified the aEEG background activity as normal amplitude, the upper margin of band of aEEG activity >10 μV and the lower margin >5 μV; moderately abnormal amplitude, the upp er margin of band of aEEG activity >10 μV and the lower margin ≤ 5 μV; and suppressed amplitude, the upper margin of the band of aE EG activity <10 μV and lower margin <5 μV [11]. We monitored the seizure spike at a rapid rise in both the lower and upper margins of the trace, and evaluated simultaneous raw EEG [12].

Cytokine and brain injury marker analysis (plasma and CSF)

The analyses of IL-1 beta, IL-6, IL-8, Myelin Basic Protein (MBP), Tumor Necrosis Factor-alpha (TNF-alpha), S100B, Glial fibrillary acidic protein (GFAP), and Enolase 2 were performed. The enzyme-linked immunosorbent assay kit (Cloud-Clone Corp, Houston, Texas, USA) and Quantikine® (R&D systems, Minneapolis, Minnesota, USA) were used for the quantitative determination of cytokines and brain injury markers.

Brain MRI

Experienced pediatric radiologists assessed the degree of WMI by scoring five categories on MRI, including the nature and extent of white matter signal abnormality, periventricular white matter volume loss, cystic abnormalities, ventricular dilatation, and thinning of the corpus callosum [13]. These five scores were summed and WMI on MRI was categorised as none (total score 5-6), mild (total score 7-9), and moderate to severe (total score 10-15).

Follow-up outcomes at 18 months of CA

The body measurement data and the diagnosis of cerebral palsy were collected. The diagnosis of cerebral palsy was made at the discretion of rehabilitation specialists [14]. The patients were assessed using the Bayley Scales of Infant and Toddler Development - the 3rd edition (Bayley-III). The Bayley-III generates scores for five composite domains including cognitive, language, motor, social-emotional, and adaptive behavior [15].

Statistical analysis

Statistical analysis was performed using the SPSS Statistics 20.0 software (SPSS Inc., Chicago, IL, USA). The chi-squared test was used to compare qualitative data between the groups, while the Mann-Whitney U tests were used to compare quantitative data between the groups. P-values were derived from ANCOVA adjusted for gestational age (GA), apgar score, respiratory distress syndrome (RDS), bronchopulmonary dysplasia (BPD), patent ductus arteriosus (PDA) operation, retinopathy of prematurity (ROP) (≥Grade III), and intraventricular haemorrhage (IVH) (≥ Grade III).

Pearson's partial correlation analysis (adjusted for PMA and GA) and multiple linear regression analysis were used to evaluate the relationship between the biomarkers and neurological outcome in the inflammation group. And correlation analysis was performed to assess the relationship between the results of neurological evaluation and Bayley score at 18months of corrected age (CA). P-values < 0.05 were considered statistically significant.

RESULTS

Study population

Of the 100 preterm infants enrolled in the study, five infants who died before 35 weeks of PMA and one infant with IVH grade IV were excluded. There were 49 infants in the control and 45 infants in the I group (11 for sepsis and one for NEC) (Figure 1). No infants were diagnosed with meningitis. The median PMA at the inflammatory episode were 29+6 (24+0-34+6) weeks.

Perinatal characteristics and neonatal morbidities

The GA was younger (29+2 vs. 26+6; Control vs. I respectively if not mentioned otherwise, $p < 0.001$) and the birth weight was less (1090 vs. 770, $p < 0.001$) in the I group compared to the control. The apgar score was significantly lower in I group and neonatal morbidities including RDS, BPD, PDA operation, and ROP ($\geq$stage III) were significantly more frequent in the I groups (Table 1).

Short-term and long-term neurological outcome

Head circumference (HC)s at 36 weeks PMA were significantly smaller in the I group. (30.5 vs. 29.3; Control vs. I respectively if not mentioned otherwise, $p < 0.049$). HCs at CA 18months (Z-score) were significantly smaller in the I group, too (0.32 vs. -0.88, $p < 0.005$).

Infants in the I group had significantly more WMI on the MRI at term equivalent age (TEA) than those in the control group (2 % vs. 26.7 %, $p = 0.036$). At 35 weeks of PMA, significantly lower maturation scores of aEEG were revealed in the I group (9.5 vs. 8, $p = 0.017$)

(Table 2). When comparing Bayley-III at 18 months of CA to the control group, correcting only for gestational age, I group had significantly lower scores in all domains except the social-emotional. However, when correcting for neonatal morbidities, only total score and motor domain showed significant differences (100 vs. 91, p = 0.029) (Table 2).

The patients with culture-proven sepsis or stage II or higher NEC had no significantly different neurologic outcomes when compared with the rest of I group.

aEEG for predicting neurodevelopment during systemic inflammation

At the onset of systemic inflammation (D0), there was a significant correlation between I/T ratio and aEEG. When the I/T ratio was high, aEEG showed lower maturation score (Pearson correlation coefficient with p-value, F -0.496, p = 0.026) and more frequent seizure spikes (F -0.584, p = 0.007). IL-1 BETA (CSF) also showed significant association in maturation (F -0.662, p = 0.019), seizure (F -0.858, p = 0.001), and voltage based classification (F 0.608, p = 0.036). Among brain injury markers, MBP showed significant association with seizure (F -0.566, p = 0.044) (Table 3).

Seizure spike of aEEG (D0) had significant correlation with motor (F 0.581, p = 0.007) and social-emotional (F 0.544, p = 0.013) domains of Bayley-III at 18months of CA. The cognitive (F 0.376, p = 0.034), language (F 0.408, p = 0.021), and social-emotional (F 0.356, p = 0.045) domain scores were lower in infants whose aEEG at 35weeks PMA exhibited seizure spikes. In addition, maturation score of aEEG at 35weeks of PMA had positive correlation with adaptive domain (F 0.45

4, p = 0.009) (Table 4).

Biomarkers for predicting neurodevelopment during systemic infl ammation

The CBC and CRP of blood sample were done for all infants of the G group. A CSF exam was performed in 14 of 45 infants (31%) in th e inflammation groups. The plasma biomarker analyses were performe d in 23 of 45 infants (51%) with day zero samples according to samp le availability.

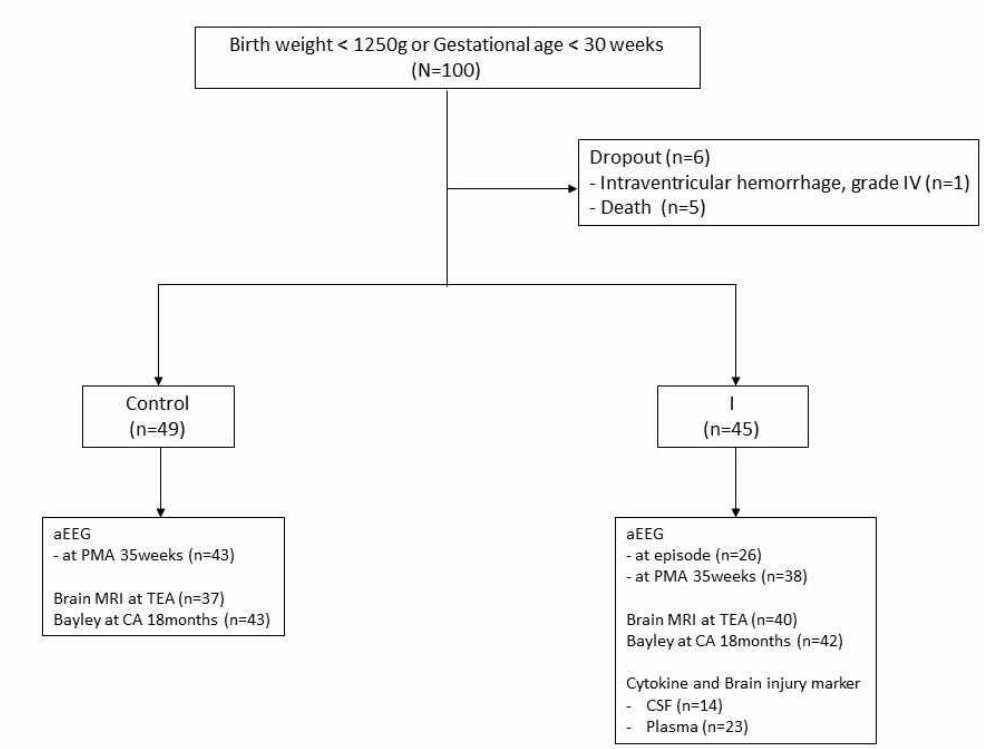
In the correlation analysis, the higher the I/T ratio (D0), the more white matter injury (WMI) in brain MRI at TEA (F 0.483, p = 0.03 1). And I/T ratio was negatively correlated with the motor (F -0.530, p = 0.016), and social (F -0.467, p = 0.038) domains in Bayley-III. CR P (D0) was negatively correlated with the language (F -0.330, p = 0. 033) and motor (F -0.330, p = 0.033) domain. In multivariate linear re gression analysis, CRP at day zero ([p = 0.011], β -0.433; 95% CI, - 2.934 to -0.427) was significantly associated with the motor score in t he inflammation group (p = 0.047, R2 = 0.573) (adjusted for GA, birth weight, sex, IVH [≥ Grade III], PMA at the time of the episode, pre mature rupture of membranes, histologic chorioamnionitis, antenatal st eroids, SGA, RDS, BPD, PDA, and ROP).

Several biomarkers in CSF showed a significant negative correlatio n with HC of 36 weeks of PMA: IL-8 (F -0.742, p = 0.014), TNF-al pha (F -0.771, p = 0.009), S100B (F -0.792, p = 0.006), and Enolase 2 (F -0.729, p = 0.017). Among plasma samples, only Enolase-2 present ed a negative correlation with HC. Among the biomarkers, plasma T NF-alpha particularly showed a significant correlation with most dom ains of Bayley-III: cognitive (F -0.662, p = 0.037), motor (F -0.749, p

= 0.013), and adaptive (F -0.783, p = 0.007). In addition, IL-1 BETA and MBP showed significant negative correlation with social emotiona l domain (Table 3).

The infants with WMI on MRI at TEA had significantly poor Bayl ey scores in four domains except language domain. After correcting f or GA and PMA of inflammation episode, the HC at 36 weeks of PM A and 18 months of CA were correlated (F 0.740, $p < 0.001$). In addi tion, there was a significant positive correlation between the HC at 3 6 weeks of PMA and Bayley scores (motor, F 0.345, p = 0.032; adapti ve, F 0.368, p = 0.012) (Table 4).

Figure 1. Flow diagram of the cohort study.



aEEG, amplitude-integrated electroencephalography; PMA, post menstrual age; TEA, term equivalent age; CA, corrected age.

Table 1. Perinatal Characteristics, Neonatal Morbidities and Laboratory Result

	Control (n=49)	I (n=45)	P-value
Gestational age (week)	29^{+2} (24^{+1}, 34^{+2})	26^{+6} (24^{+0}, 33^{+1})	***<0.001****
Birth weight (g)	1090 (540, 1340)	770 (420, 1400)	***<0.001****
Male	20 (40.8)	28 (62.2)	*0.100*
Multiple birth	21 (42.9)	20 (44.4)	*0.877*
Apgar score			
At 1min	5 (1, 8)	3 (0, 8)	***0.028****
At 5min	7 (1, 9)	7 (0, 9)	***0.004****
Cesarean section	33 (67.3)	28 (62.2)	*0.440*
PROM	10 (20.4)	7 (15.6)	*0.541*
HCA	19 (38.8)	21 (46.7)	*0.440*
Preeclampsia	2 (4.1)	3 (6.7)	*0.577*
Antenatal steroid	42 (85.7)	41 (91.1)	*0.294*
SGA	14 (28.6)	15 (33.3)	*0.618*
RDS	26 (53.1)	35 (77.8)	***0.012****
Moderate to severe BPD	11 (22.4)	30 (66.7)	***<0.001****
PDA operation	2 (4.1)	14 (31.1)	***<0.001****
ROP ≥ stage Ⅲ	2 (4.1)	15 (33.3)	***<0.001****
IVH ≥ grade III	0 (0.0)		*0.136*
Culture Proven Sepsis	0 (0.0)	11 (24.4)	***<0.001****
NEC ≥ stage II	0 (0.0)	2 (4.44)	***<0.001****
Inflammation episode		1 (1, 6)	-
WBC ($\times 10^3/\mu\ell$)	-	11.44 (1.00, 39.00)	-
I/T ratio	-	0.00 (0.00, 0.89)	-
Platelet ($\times 10^3/\mu\ell$)	-	157 (30, 811)	-
CRP (mg/dL)	-	1.96 (0.01, 18.21)	-

Values are presented as median (min, max), or number (%).
PROM, premature rupture of membranes; HCA, histologic chorioamnionitis; SGA, small for gestational age; RDS, respiratory distress syndrome; BPD, bronchopulmonary dysplasia; PDA, patent ductus arteriosus; ROP, retinopathy of prematurity; IVH, intraventricular hemorrhage; I/T ratio, immature to total neutrophil ratio; CRP, C-reactive protein
* $p < 0.05$

Table 2. Short-term and Long-term Neurologic Findings in the Groups

	Control (n=49)	I (n=45)	P-value†	P-value§
HC at PMA 36 weeks (cm)	30.5 (26.5, 33.0)	29.3 (24.5, 32.5)	**<0.001**	**0.049***
aEEG at PMA 35 weeks				
Maturation score	9.50 (4, 13)	8 (4, 12)	***0.002***	***0.017****
WMI on MRI at TEA			***0.008***	***0.036****
None	48 (98)	33 (73.3)		
Mild	1 (2)	8 (17.8)		
Moderate to severe	0 (0)	4 (8.9)		
HC at CA 18 months (z-score)	0.32 (-2.42, 2.36)	-0.88 (-3.91, 2.13)	**<0.001**	***0.005****
Bayley-III and CP at CA 18 months				
Cognitive	95 (85, 125)	90 (55, 120)	***0.006***	*0.076*
Language	97 (74, 121)	89 (50, 121)	***0.015***	*0.108*
Motor	100 (82, 118)	91 (46, 110)	***0.002***	***0.029****
Social Emotional	95 (80, 110)	90 (55, 115)	*0.066*	*0.311*
Adaptive Behavior	92 (61, 115)	84 (48, 111)	***0.005***	*0.055*
Cerebral palsy	2 (4.0)	6 (13.3)	*0.080*	*0.240*

Values are presented as average (min, max), or number (%).
†P-values are adjusted for gestational age.
§P-values are adjusted for gestational age, apgar score, RDS, BPD, PDA operation, ROP (≥Grade III), and intraventricular hemorrhage (≥ Grade III).
HC, head circumference; aEEG, amplitude-integrated EEG; PMA, Postmenstrual age; WMI, White matter injury; TEA, Term equivalent age; CP, cerebral palsy; CA, corrected age
* $p < 0.05$

Table 3. Correlation between biomarkers (D0) and Neurological outcome in the inflammation Group

	I/T ratio	Plate let	CRP	IL-1 BETA		IL-8		TNF alpha		S100B		Enolase2		MBP	
				C	P	C	P	C	P	C	P	C	P	C	P
aEEG: Day 0															
Maturation score	**0.496* (0.026)**	0.048	0.037	**-0.662* (0.019)**	0.055	-0.264	0.063	0.240	-0.169	-0.130	0.115	-0.247	0.224	-0.101	0.018
Seizure[†]	**-0.584* (0.007)**	-0.086	0.080	**-0.858* (0.001)**	0.215	0.092	0.015	0.220	-0.405	0.253	0.056	0.208	0.199	0.042	**-0.566* (0.044)**
Voltage based[§]	0.407	-0.094	-0.092	**0.608* (0.036)**	0.281	0.138	0.744	-0.173	0.147	-0.019	0.183	0.049	-0.148	0.049	-0.012
aEEG: PMA 35 weeks															
Maturation score	-0.621 (0.055)	0.314	-0.208	-0.278	-0.109	0.046	-0.280	-0.338	-0.252	-0.013	0.321	-0.012	0.075	-0.056	-0.148
Seizure[†]	0.172	-0.376	-0.597	0.312	1.000	0.219	-0.417	0.256	0.179	-0.053	0.179	0.116	-0.030	0.219	0.362
Voltage based[§]	0.085	-0.295	0.359	0.021	0.030	0.037	-0.253	0.391	0.474	0.200	0.310	0.146	0.249	0.174	0.425
HC: 36 weeks (cm)	0.048	0.283	-0.1	0.010	-0.405	**-0.742* (0.014)**	0.408	**-0.771* (0.009)**	-0.493	**-0.792* (0.006)**	0.174	**-0.729* (0.017)**	**-0.725* (0.018)**	-0.286	-0.405
Brain WMI at TEA[¶]	**0.483* (0.031)**	-0.027	-0.098	0.426	-0.185	-0.104	0.128	-0.073	-0.195	-0.278	-0.374	-0.303	-0.007	-0.122	0.077
HC: 18 months of CA (z-score)	-0.018	0.200	-0.032	-0.110	-0.388	-0.343	0.403	-0.562	**-0.792* (0.006)**	-0.429	-0.216	-0.326	**-0.763* (0.010)**	-0.172	-0.309
Bayley score: 18months of CA															
Cognitive	-0.307	0.062	-0.294	-0.604	-0.086	0.246	-0.255	0.141	**-0.662* (0.037)**	0.127	0.134	0.209	-0.090	-0.287	-0.352
Language	-0.032	0.102	**-0.204 (0.050)**	-0.270	-0.133	0.208	-0.270	-0.117	**-0.617* (0.058)**	0.049	-0.151	0.177	-0.261	-0.319	-0.328
Motor	**-0.530* (0.016)**	0.101	**-0.330* (0.033)**	-0.649 (0.058)	-0.005	0.067	-0.221	-0.052	**-0.749* (0.013)**	0.008	0.267	0.100	-0.144	-0.120	-0.445
Social emotional	**-0.467* (0.038)**	0.019	-0.222	**-0.764* (0.016)**	0.383	0.420	-0.098	0.395	-0.452	0.554	-0.191	0.533	-0.252	-0.152	**-0.632 (0.050)**
Adaptive	-0.325	0.119	-0.300	-0.405	-0.186	0.202	-0.083	-0.161	**-0.783* (0.007)**	0.140	-0.043	0.255	-0.295	-0.074	-0.504

The values are Pearson correlation coefficient. If the p-value is less than 0.05, with (p-value). All values are adjusted for gestational age and postmenstrual age.

[†]Seizure (1: yes, 2: no), [§]Voltage based classification (1: normal, 2: moderate abnormal, 3: severe abnormal), [¶]Brain WMI (1: normal, 2: mild, 3: moderate to severe)

C, Cerebrospinal fluid; P, plasma; MBP, Myelin Basic Protein; TNF alpha, Tumor Necrosis Factor-alpha; aEEG, Amplitude-integrated electroencephalography; PMA, postmenstrual age; HC, head circumference; WMI, white matter injury; TEA, Term equivalent age; CA, corrected age

* $p < 0.05$

Table 4. Correlation between aEEG/Brain MRI/Head circumference and Neurodevelopment (CA 18 months) in the Inflammation Group

	aEEG at Day 0			aEEG at PMA 35 weeks			Brain WMI at TEA[¶]	Head circumference at 36 weeks
	Maturation score	Seizure spike[†]	Voltage base[§]	Maturation score	Seizure spike[†]	Voltage base[§]		
Bayley score								
Cognitive	0.124	0.040 (0.052)	-0.269	0.283	**0.376* (0.034)**	-0.133	**-0.371* (0.020)**	0.193
Language	-0.127	0.171	-0.102	0.246	**0.408* (0.021)**	-0.293	-0.271	0.210
Motor	0.141	**0.581* (0.007)**	-0.269	0.161	0.227	0.030	**-0.431* (0.006)**	**0.345* (0.032)**
Social emotional	0.024	**0.544* (0.013)**	-0.175	0.206	**0.356* (0.045)**	-0.098	**-0.350* (0.029)**	0.177
Adaptive	0.152	0.371	-0.288	**0.454* (0.009)**	0.229	**-0.349 (0.050)**	**-0.334* (0.038)**	**0.368* (0.021)**
HC at 18 months	-0.092	0.031	-0.002	0.177	-	-0.174	-0.167	**0.740* (<0.001)**

The values are Pearson correlation coefficient. If the p-value is less than 0.05, with (p-value).
All values are adjusted for gestational age and postmenstrual age.

[†]Seizure (1: yes, 2: no), [§]Voltage based classification (1: normal, 2: moderate abnormal, 3: severe abnormal), [¶]Brain WMI (1: normal, 2: mild, 3: moderate to severe)
aEEG, amplitude-integrated EEG; PMA, Postmenstrual age; WMI, White matter injury; HC, Head circumference

DISCUSSION

Our study is the first to comprehensively follow head growth, aEEG, brain MRI and neurodevelopment in premature infants who experienced inflammation. Preterm infants who experienced a systemic inflammation showed poor brain growth, abnormal functional brain maturation and imaging at near-term age, and poor neurodevelopment at 18 months of CA. In addition, in analysis to find predictors for neurodevelopment, the level of I/T ratio, CRP, and plasma TNF-alpha and seizure spikes on aEEG at the initial phase of inflammatory episodes well correlated with neurodevelopmental outcomes.

The HC at 36 weeks of PMA was lower in inflammation group compared to the control. It was an important early index that group differences in HC were still maintained at 18 months of CA after adjusting for GA. The HC at 36 weeks of PMA also correlated with Bayley scores at 18 months of CA. This indicates that the prior brain injury from systemic inflammation does not fully recover and is connected to the long-term outcome. Compared to the control after adjusting gestational age, the inflammation group had significantly lower cognitive, language, motor, and adaptive behavior scores. However, when the morbidities were corrected, only the motor domain remained significant. Previous studies suggested that preterm infants with postnatal sepsis or NEC are more likely to have adverse neurodevelopment [4,5,16,17]. Interestingly, infants with systemic inflammation without proven sepsis or NEC also had delayed maturation on aEEG, more WMI, and poor long-term neurodevelopment outcomes indistinguishable from infants with culture-proven sepsis or NEC in our study. This was also suggested

previously by Stoll et al. reporting that infants with only clinical infection without any proven pathogen had a significantly smaller HC at 36 weeks of PMA and lower mental and psychomotor development indices on BSID-II [4]. Several studies have shown that systemic inflammation without a proven pathogen alone causes brain injury and is associated with high inflammatory cytokine levels [18-20].

We attempted to identify biomarkers of high risk for neurodevelopmental impairment among infants with systemic inflammation. Firstly, we analysed whether the aEEG could serve as the predictor of neurodevelopmental outcome. The seizure spike on aEEG at day zero had significant negative relationship with motor and social domains. In the I group infants, the increase in I/T ratio and CSF IL-1 beta, which are the indices of infection and inflammation, were significantly related to increased seizure spike and low maturation of aEEG. This suggests that systemic and central nervous system inflammation caused the abnormal aEEG findings to occur. The septic adult patients without central nervous system infection developed diffuse cerebral dysfunction and characteristic EEG findings, known as sepsis-associated encephalopathy [23]. Preterm infants showed acute electroencephalographic changes such as burst suppression during sepsis as well [10]. This results indicate that aEEG could be used to monitor brain injury during systemic inflammation and help define the patients who need therapeutic interventions.

Secondly, blood markers during the inflammatory episodes were analysed. It is well known that an increased I/T ratio and CRP, and thrombocytopenia suggest infection [22,23]. Using this indicators, a correlation analysis was conducted to determine whether

neurodevelopment can be predicted according to the degree of inflammation. CRP was negatively correlated with the language and motor domains, and the I/T ratio was also negatively correlated with motor and social domains. In addition, we analyzed proteins including cytokines and brain injury markers of plasma and CSF. CSF proteins such as IL-8, TNF-alpha, S100B, and Enolase 2 showed negative correlations with HC at 36 weeks of PMA, however they were not correlated with HC at 18 months CA or Bayley scales. In a previous study, concentrations of inflammation-related proteins including IL-6, TNF-R2, IL-8, MCP-1, and ICAM-1 in plasma were associated with risk of microcephaly [24]. Unlike other protein markers, plasma TNF-alpha was correlated with many domains of Bayley scales and HC at CA 18 months. Interestingly CSF TNF-alpha was not predictive of neurodevelopment and only correlated with HC at 36 weeks PMA as with other CSF cytokines and brain injury markers. This might imply the critical role of plasma TNF-alpha in inflammation-associated brain injury in preterm infants. The ELGAN study found elevations of cytokines including TNF-alpha to be associated with poor cognitive function [7,25]. In the study, elevated levels of TNF-alpha in blood collected on postnatal days 14 are associated with impaired mental and motor development at age two years [7]. And preterm infants who had sustained elevations of inflammation-related proteins including CRP, TNF-alpha, and IL-8 in the first postnatal month are more likely to have cognitive impairment at 10 years [25]. Our study differed from the ELGAN study in that it studied the relationship between cytokine and neurodevelopment at a suspected point of infection. These results suggest that the degree of inflammation reflected in clinically

available laboratory findings may aid in defining the high risk for brain injury during systemic inflammation. These results suggest that neurodevelopmental outcomes can be predicted by usual laboratory markers of I/T ratio and CRP rather than inflammatory cytokines or brain injury markers.

More research is needed to confirm the results of this study and other neurophysiologic monitoring such as detailed EEG and others during inflammatory episodes could give further insights into brain injury during inflammation.

CONCLUSION

Our prospective cohort study reconfirmed that systemic inflammation affects neurodevelopment in preterm infants. Brain injury due to systemic inflammation can be monitored in real time through aEEG. The seizure spike on aEEG, I/T ratio, CRP, and plasma TNF-alpha during inflammatory episodes can help predict neurodevelopmental outcomes.

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국문초록

서론: 미숙아의 전신염증이 신경발달에 부정적인 영향을 미친다는 선행 임상연구가 있다. 본 연구에서는 두위 성장, 임상 검체 및 영상 검사, 신경 생리학적 검사, 발달 검사 등의 포괄적인 평가를 통해 미숙아의 전신 염증 반응이 신경발달에 영향을 미치는지 보고자 하였으며 전신염증반응 당시 혈액검사 및 신경생리학적 검사를 통해 미숙아의 신경발달을 예측할 수 있을지에 대해 연구하였다.

방법: 전향적 코호트 연구로, 서울대학교 어린이병원 신생아중환자실에 2013년과 2017년 사이 입실한 임신 나이 30주 미만 또는 출생체중 1250 g 미만으로 출생한 미숙아를 대상으로 하였다. 등록된 미숙아는 전신염증의 유무에 따라 다음 두 개의 군으로 나누었다: I (전신염증반응을 경험한 미숙아), Control (전신 염증반응이 없었던 미숙아).

염증반응 당시 C 반응단백수치 (CRP), 전 혈구 검사, 사이토카인 (IL-1 beta, IL-8, IL-6, TNF-alpha), 뇌손상지표 (S100B, Enolase, MBP) 검사를 포함한 혈액검사 및 척수액 검사와 amplitude-integrated electroencephalography (aEEG)를 시행하였으며, 교정 주령 만삭 주변에 aEEG와 뇌 MRI를 시행하였다. 그리고 교정연령 18개월에는 베일리 검사로 발달평가를 진행하였다.

결과: I군에서 뇌백질 손상이 많았으며 (2 vs. 26.7 %, Control vs. I) 뇌파상 낮은 뇌 기능 성숙도 (9.5 vs. 8), 작은 머리 크기 (30.5 cm vs. 29.3 cm)를 보였다. 그리고 교정연령 18개월에 시행한 베일리 검사에서 전신염증이 있었던 군에서 낮은 점수를 보였다.

뇌척수액의 IL-beta와 미성숙한 호중구의 비가 전신염증반응 당일의 aEEG 소견과 연관이 있었으며, 경련 스파이크 소견이 있었던 경우 교정 18개월의 베일리 검사의 운동(F 0.581, p = 0.007), 사회 (F 0.544, p = 0.

013) 영역 점수가 낮았다. 또한 염증반응이 있었던 환아에서 시행한 혈액의 CRP, 미성숙한 호중구의 비, 혈액의 TNF-alpha가 베일리점수와 음의 상관관계를 보였다.

결론: 전신 염증 상태가 미숙아의 전반적인 신경발달에 영향을 미친다. 전신염증에 의한 뇌손상이 비침습적으로 aEEG를 통해 보여 지며, 염증 당시의 경련스파이크, C 반응단백 수치, 미성숙한 호중구의 비, 그리고 혈액의 TNF-alpha 수치가 신경발달 예측에 도움이 된다.

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주요어: 미숙아, 뇌백질 손상, 패혈증, 괴사성 장염, 전신 염증, 사이토카인

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