Inflammatory and Immune Proteins in Umbilical Cord Blood: Association with Hearing Screening Test Failure in Preterm Neonates

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Objective. We aimed to determine whether elevated levels of various inflammatory and immune proteins in umbilical cord blood are associated with an increased risk of newborn hearing screening (NHS) test failure in preterm neonates. Methods. This retrospective cohort study included 127 premature singleton infants who were born at ≤33.6 weeks. Umbilical cord plasma at birth was assayed for interleukin (IL)-6, complement C3a and C5a, matrix metalloproteinase (MMP)-9, macrophage colony-stimulating factor (M-CSF), and endostatin levels using ELISA kits. Neonatal blood C-reactive protein (CRP) levels were measured within 2 hours of birth. The primary outcome measure was a uni- or bilateral refer result on an NHS test. Univariate and multivariate analyses were applied. Results. Fifteen (11.8%) infants failed the NHS test. In the univariate analyses, high IL-6 and low C3a levels in umbilical cord plasma, funisitis, and an elevated CRP level (>5 mg/L) in the immediate postnatal period were significantly associated with NHS test failure. However, the levels of umbilical cord plasma MMP-9, C5a, M-CSF, and endostatin were not significantly different between infants who passed and those who failed the NHS test. Multiple logistic regression analyses indicated that elevated umbilical cord plasma C3a levels were independently associated with a reduced risk of NHS test failure, whereas elevated levels of umbilical cord plasma IL-6 and high CRP levels in the immediate postnatal period were significantly associated with NHS test failure. Conclusions. Our data demonstrated that in preterm neonates, a systemic fetal inflammatory response reflected by umbilical cord plasma IL-6 and immediate postnatal CRP levels may contribute to the risk for NHS test failure, whereas the changes in complement activation fragments initiated in utero may have protective effect of hearing screen failure.

1. Introduction

Sensorineural hearing loss (SNHL) is one of the most common long-term disabilities worldwide in preterm infants, with an incidence of 0.7–17.5% for very preterm newborns (<32 weeks) [1–3]. Given the high prevalence and clinical relevance of SNHL in preterm neonates, the early detection and proper treatment of SNHL are important for normal speech development. Therefore, the identification of biomarkers that can ensure the early identification of preterm neonates at the
highest risk of SNHL, thus enabling early therapeutic intervention or auditory rehabilitation such as hearing aid or cochlear implantation, is urgently needed.

Although many perinatal and postnatal factors associated with SNHL have been reported in the literature [1–4], little is known regarding its prenatal risk factors. Importantly, recent studies by Leung et al. and our group have demonstrated that the presence of intra-amniotic infection (with reported incidences of 13.6% for preterm labor and 38% for preterm premature rupture of membranes) [5, 6], funisitis, and fetal inflammatory response syndrome (FIRS, defined as an elevated fetal plasma interleukin-6 level (>11 pg/mL) and/or the presence of funisitis/chorionic vasculitis) [7, 8] were significantly associated with an increased risk of hearing screening failure in very preterm neonates [9], suggesting that infection/inflammation in utero, including fetal infection/inflammation, may have a potentially deleterious effect on fetal auditory development. In this regard, an analysis of biomarkers in umbilical cord blood (UCB) may be useful for estimating the risk of SNHL because UCB can directly reflect fetal status, including the effects of the in utero milieu on the fetus, such as infection/inflammation, stress, and hypoxia. In fact, several studies have reported a significant association between elevated cytokine levels in the UCB and neurologic disability in very preterm infants [11, 12]. However, only one study to date has examined the relationship between UCB cytokines and hearing screen failure [9]. Moreover, this study was limited because sampling was not necessarily performed immediately after birth from UCB but rather in a broader time window of the 12-hour period after birth from umbilical cord or venous blood, resulting in inadvertent contamination of the results by the effects of postnatal factors [9]. The purpose of this study was to determine whether elevated levels of various inflammatory and immune proteins examined exclusively in the UCB are associated with an increased risk of newborn hearing screening (NHS) test failure in preterm neonates.

2. Materials and Methods

2.1. Study Design. This single-center retrospective cohort study included infants admitted to the neonatal intensive care unit at Seoul National University Bundang Hospital (Seongnam, Korea) between June 2004 and January 2015. The inclusion criteria were (1) singleton birth at 23+0 to 33+6 weeks gestation, (2) survival at least 90 days after birth, (3) underwent hearing screening test, and (4) an aliquot of UCB available for analysis. We excluded twins or higher-order infants, those for whom a histologic examination of the placenta was not performed, outborn infants, and those with major structural or chromosomal abnormalities. Gestational age was calculated based on the last menstrual period and ultrasound information obtained in the first or second trimester. The study was approved by the local ethics committee of Seoul National University Bundang Hospital (IRB no. B-1006/103-102). Written informed consent was obtained from the parents of all infants (participants) whose samples and data were used for the study.

2.2. Hearing Screening. Electronic medical records on unilateral or bilateral hearing screen failure of the included preterm singleton infants were reviewed by one otolaryngologist (Y. J. S.) who was blinded to the results of umbilical cord plasma analysis and the details of mothers and their infants. The conventional methods for hearing screening and the follow-up in our hospital were previously described in detail elsewhere [10, 13]. In brief, the automated auditory brainstem response (AABR) test was the most commonly performed (n = 107) NHS test while the otoacoustic emission (OAE) test was performed in 20 cases in which the AABR was not available. If the infant failed either the AABR or OAE test, the same test was repeated. Infants who failed two consecutive screenings of the AABR or OAE in one or both ears were classified as hearing screen failure. The results were recorded as either “refer” (further confirmation tests, such as an auditory brainstem response threshold test, needed) or “pass” (normal). Therefore, the primary outcome measure was a uni- or bilateral refer result on an NHS test.

2.3. Clinical Data and Definitions of Risk Factors for Hearing Screen Failure. The following maternal factors were extracted from the database: maternal age, parity, gestational age at admission, causes of preterm birth, delivery mode, antenatal use of medications (tocolytics, steroids, and antibiotics), and clinical diagnosis of chorioamnionitis. Perinatal/neonatal characteristics retrieved from the database were as follows: gestational age at birth, sex, birth weight, 1 and 5 min Apgar scores, pathologic diagnoses of the placenta, umbilical artery pH, neonatal blood C-reactive protein (CRP) levels and white blood cell (WBC) counts obtained within 2 hours of birth, use of surfactant, use of mechanical ventilation, proven neonatal sepsis, respiratory distress syndrome (RDS), bronchopulmonary dysplasia (BPD), intraventricular hemorrhage (IVH), periventricular leukomalacia (PVL), and necrotizing enterocolitis (NEC).

Clinical chorioamnionitis was diagnosed in accordance with the criteria of Gibbs et al. [14]. Proven sepsis, RDS, BPD, IVH, PVL, and NEC were diagnosed according to the definitions previously described in detail [10, 15]. Acute histologic chorioamnionitis was defined as the presence of an acute inflammatory change in any tissue sample (amnion, chorion-decidua, umbilical cord, or chorionic plate) using the criteria published previously [16]. Funisitis was diagnosed by the presence of neutrophil infiltration into the umbilical vessel walls or Wharton’s jelly. Fetal inflammatory response syndrome (FIRS) is defined as the presence of funisitis or elevated levels of umbilical cord plasma IL-6 (>11 pg/mL) [7, 17]. Neonatal blood CRP levels up to 2 hours postdelivery were analyzed as categorical variables because several CRP measurements were performed qualitatively and grouped by value (>5 vs ≤5 mg/L). Thus, a CRP level >5 mg/L was considered elevated; that is, it exceeded the 95th percentile for CRP at birth based on the data for healthy term and near-term infants [18].

2.4. Analysis of Inflammatory-Related Proteins in the Umbilical Cord Plasma. UCB samples were obtained from the umbilical vein at birth and collected into ethylenediaminetetraacetic acid
Mediators of Inflammation

Table 1: Maternal and obstetric characteristics of the study population according to newborn hearing screening test results.

<table>
<thead>
<tr>
<th>Abnormal finding on newborn hearing screening test</th>
<th>Absent (n = 112)</th>
<th>Present (n = 15)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age (years)</td>
<td>31.6 ± 3.6</td>
<td>31.8 ± 3.8</td>
<td>0.970</td>
</tr>
<tr>
<td>Nulliparity</td>
<td>49 (43.8%)</td>
<td>10 (66.7%)</td>
<td>0.107</td>
</tr>
<tr>
<td>Membrane status</td>
<td></td>
<td></td>
<td>0.270</td>
</tr>
<tr>
<td>Intact membranes</td>
<td>49 (43.8%)</td>
<td>4 (26.7%)</td>
<td></td>
</tr>
<tr>
<td>Preterm PROM</td>
<td>63 (56.3%)</td>
<td>11 (73.3%)</td>
<td>0.407</td>
</tr>
<tr>
<td>Cesarean delivery</td>
<td>44 (39.3%)</td>
<td>4 (26.7%)</td>
<td></td>
</tr>
<tr>
<td>Antenatal corticosteroids</td>
<td>107 (95.5%)</td>
<td>13 (86.7%)</td>
<td>0.193</td>
</tr>
<tr>
<td>Antenatal antibiotics</td>
<td>87 (77.7%)</td>
<td>13 (86.7%)</td>
<td>0.737</td>
</tr>
<tr>
<td>Antenatal tocolytics</td>
<td>91 (81.3%)</td>
<td>12 (80.0%)</td>
<td>1.000</td>
</tr>
<tr>
<td>Gestational age at admission (weeks)</td>
<td>29.3 ± 3.3</td>
<td>28.4 ± 3.6</td>
<td>0.356</td>
</tr>
<tr>
<td>Histologic chorioamnionitis</td>
<td>74 (66.1%)</td>
<td>10 (66.7%)</td>
<td>1.000</td>
</tr>
<tr>
<td>Funisitis</td>
<td>22 (19.6%)</td>
<td>7 (46.7%)</td>
<td>0.043</td>
</tr>
<tr>
<td>Clinical chorioamnionitis</td>
<td>5 (4.5%)</td>
<td>3 (20.0%)</td>
<td>0.078</td>
</tr>
</tbody>
</table>

Values are given as mean ± standard deviation or n (%). PROM: premature rupture of membranes.

3. Results

During the study period, a total of 127 women with either preterm labor (n = 54) or preterm premature rupture of membranes (n = 73) and their neonates who met the inclusion criteria were ultimately included in the analysis. The mean gestational age at birth of the cohort was 30.7 weeks (SD, 1.9 weeks; range, 24.5–33.5 weeks), and the mean birth weight was 1627 g (SD, 431 g; range, 700–2620 g). One hundred twelve neonates (88.2%) passed the NHS test bilaterally, whereas 15 (11.8%) failed the NHS test. Among those 15 neonates, 10 (7.8%) had unilateral failure, while the other five (3.9%) had bilateral failure. Breaking down the failure cases in terms of the screening tests, nine ears of seven neonates (4.20% (9/214) and 6.54% (7/107)) had a “refer” result on the automated ABR, while 11 ears of eight neonates (27.5% (11/40) and 40% (8/20)) had a “refer” result on the automated OAE.

3.1. Univariate Relationship of Clinical and Laboratory Factors with Hearing Screen Failure. The maternal and obstetric characteristics of the study population according to NHS test results are shown in Table 1. Mothers delivering neonates with a refer result on the NHS test had a significantly higher rate of funisitis (P = 0.043) and tended to have a higher tendency of clinical chorioamnionitis (P = 0.078) than mothers who delivered neonates who passed the NHS
Table 2: Umbilical cord plasma levels of inflammatory and immune proteins according to newborn hearing screening test results.

<table>
<thead>
<tr>
<th>Abnormal finding in newborn hearing screening test</th>
<th>Absent (n = 112)</th>
<th>Present (n = 15)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Umbilical cord plasma IL-6 (pg/mL)</td>
<td>11.0 ± 15.1</td>
<td>19.0 ± 18.7</td>
<td>0.040</td>
</tr>
<tr>
<td>Umbilical cord plasma C3a (µg/mL)</td>
<td>11.8 ± 5.9</td>
<td>8.0 ± 5.0</td>
<td>0.017</td>
</tr>
<tr>
<td>Umbilical cord plasma C5a (ng/mL)</td>
<td>30.3 ± 22.8</td>
<td>23.6 ± 10.8</td>
<td>0.390</td>
</tr>
<tr>
<td>Umbilical cord plasma MMP-9 (ng/mL)</td>
<td>108.0 ± 714.0</td>
<td>83.9 ± 71.2</td>
<td>0.124</td>
</tr>
<tr>
<td>Umbilical cord plasma M-CSF (pg/mL)</td>
<td>715.4 ± 390.8</td>
<td>749.7 ± 509.5</td>
<td>0.946</td>
</tr>
<tr>
<td>Umbilical cord plasma endostatin (ng/mL)</td>
<td>82.9 ± 16.3</td>
<td>85.6 ± 17.4</td>
<td>0.497</td>
</tr>
<tr>
<td>Umbilical cord plasma IL-6 &gt;11 pg/mL</td>
<td>33 (29.5%)</td>
<td>8 (53.3%)</td>
<td>0.063</td>
</tr>
<tr>
<td>Fetal inflammatory response syndromea</td>
<td>43 (38.4%)</td>
<td>9 (60.0%)</td>
<td>0.110</td>
</tr>
</tbody>
</table>

Values are given as mean ± standard deviation or n (%). IL: interleukin; MMP: matrix metalloproteinase; M-CSF: macrophage colony-stimulating factor. *Fetal inflammatory response syndrome is defined as the presence of funisitis or elevated levels of umbilical cord plasma IL-6 (>11 pg/mL).

tests. However, there were no significant intergroup differences in maternal demographics, antenatal medications, or the rate of histologic chorioamnionitis.

The proportions of umbilical cord plasma samples with detectable protein levels were 98.4% for M-CSF and 100% for C3a, C5a, IL-6, MMP-9, and endostatin. Of these six proteins measured in the umbilical cord plasma, MMP-9 levels were significantly positively correlated with those of all proteins but M-CSF ($r = 0.233–0.342$, $P < 0.01$), whereas endostatin levels were significantly positively correlated with all proteins but C3a and C5a ($r = 0.198–0.236$, $P < 0.05$). M-CSF levels in the umbilical cord plasma were significantly correlated with endostatin levels only ($r = 0.227$, $P = 0.01$). Regarding the correlation between C3a, C5a, and IL-6 levels in the umbilical cord plasma, positive significant correlations were found only between C3a and C5a ($r = 0.401$, $P < 0.001$) and between C5a and IL-6 ($r = 0.264$, $P = 0.003$).

Table 2 shows the umbilical cord plasma levels of the inflammatory and immune proteins by NHS test results. Neonates who failed the NHS test had a significantly higher median umbilical cord plasma IL-6 level and lower median umbilical cord plasma C3a level than neonates who passed the NHS test. However, there were no significant intergroup differences in umbilical cord plasma C5a, MMP-9, M-CSF, and endostatin levels, FIRS rates, or elevated umbilical cord plasma IL-6 (>11 pg/mL) levels.

Table 3 shows the neonatal characteristics by NHS test results. The rate of an elevated CRP level (>3 mg/L) in the immediate postnatal period was significantly higher in the neonates who failed the NHS test than those who passed the NHS test. However, no significant differences were found between neonates who passed and those who failed the NHS test in terms of neonatal characteristics and morbidities, including gestational age at birth, umbilical artery pH, major treatments (i.e., continuous positive airway pressure, mechanical ventilation, and surfactant use), and major neonatal morbidities (i.e., proven sepsis, RDS, BPD, IVH, PVL, or NEC).

3.2. Multivariate Analysis. Multiple logistic regression analyses were performed to further examine the relationship between the various proteins in the umbilical cord plasma and NHS test failure after adjusting for the effects of baseline variables. The following variables were assessed in the multivariate logistic regression analysis as significant predictors in the univariate analyses ($P < 0.05$): umbilical cord plasma IL-6 and C3a levels, funisitis, and an elevated CRP level (>3 mg/L) in the immediate postnatal period. Prior to performing logistic regression analysis for testing the model, tests for multicollinearity among the independent variables were performed using bivariate analyses (e.g., $\chi^2$ test, Spearman’s rank correlation test, and the Mann-Whitney U test). Significant correlations were found among funisitis, umbilical cord plasma IL-6 levels, and an elevated CRP level in the immediate postnatal period in bivariate analyses ($P = 0.001$ to <0.001), whereas umbilical cord plasma C3a level was correlated with none. Therefore, funisitis, umbilical cord plasma IL-6 levels, and elevated CRP levels in the immediate postnatal period were analyzed in separate models (Table 4). After adjustments for umbilical cord plasma C3a levels, elevated umbilical cord plasma IL-6 level, funisitis, and elevated blood CRP levels in the immediate postnatal period were significantly associated with NHS test failure. When these four variables were simultaneously entered into logistic regression analysis, elevated umbilical cord plasma C3a levels were independently associated with a reduced incidence of NHS test failure (Table 4).

3.3. ROC Curve Analysis. Figure 1 displays the ROC curves for the umbilical cord plasma C3a and IL-6 levels in predicting NHS test failure. The area under the curve (AUC) for umbilical cord plasma IL-6 and C3a levels for predicting NHS test failure was 0.663 (95% confidence interval (CI), 0.526–0.801, $P = 0.040$) and 0.690 (95% CI, 0.552–0.829, $P = 0.017$), respectively. The best cutoff values (sensitivity and specificity) for predicting failure in the NHS test were 3.37 pg/mL for umbilical cord plasma IL-6 (80.0% sensitivity, 51.8% specificity) and 10.63 µg/mL for umbilical cord plasma C3a (73.3% sensitivity, 55.4% specificity) (Figure 1).

4. Discussion

4.1. Principal Findings of This Study. Our data demonstrate that in preterm neonates, a systemic fetal inflammatory response reflected by umbilical cord plasma IL-6 and immediate postnatal CRP levels may contribute to the risk for NHS
test failure, whereas the changes in complement activation fragments initiated in utero may have protective effect of hearing screen failure. These findings support the hypothesis that a systemic fetal inflammatory response and changes in complement activation fragments initiated in utero might be involved in the pathophysiological mechanism of hearing loss in preterm infants and suggest that the optimal timing for therapeutic strategies (e.g., antimicrobial therapy for the prevention and treatment of fetal infection) intended to prevent hearing loss in preterm infants may be prior to delivery.

4.2. Meaning of This Study. In the literature, elevated IL-6 UCB levels at birth were reportedly associated with an increased risk of neonatal morbidity and mortality, including neonatal sepsis, systemic inflammatory response syndrome, PVL, and NEC [19–21]. However, little is known about the associations among elevated levels of UCB IL-6, abnormal NHS results, and subsequently confirmed SNHL in preterm infants. In the context of abnormal NHS results, Leung et al. showed an elevated IL-6 level in neonatal blood obtained within 12 hours of birth but not necessarily at birth was a risk factor for hearing screen failure [9]. In accordance with the previous report, our results also demonstrated that elevated IL-6 levels in UCB, reflecting an in utero initiation of the fetal response to perinatal events, were significantly associated with NHS test failure. Given that IL-6 is a well-known important mediator of host response to infection [22], which in turn is significantly involved in the pathogenesis of sensorineural hearing impairment [10, 23], these observations are not unexpected and support the hypothesis that a systemic fetal inflammatory response may be detrimental to auditory function in preterm infants.

<table>
<thead>
<tr>
<th>Table 3: Neonatal characteristics and morbidities according to newborn hearing screening test results.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Abnormal finding on newborn hearing screening test</strong></td>
</tr>
<tr>
<td>Gestational age at birth (weeks)</td>
</tr>
<tr>
<td>Birth weight (kg)</td>
</tr>
<tr>
<td>Male gender</td>
</tr>
<tr>
<td>Apgar score &lt; 7</td>
</tr>
<tr>
<td>1 min</td>
</tr>
<tr>
<td>5 min</td>
</tr>
<tr>
<td>Umbilical artery pH</td>
</tr>
<tr>
<td>CRP level &gt; 5 mg/L in immediate postnatal period</td>
</tr>
<tr>
<td>WBC count in immediate postnatal period (10^3 cells/mm^3)</td>
</tr>
<tr>
<td>Continuous positive airway pressure</td>
</tr>
<tr>
<td>Mechanical ventilation</td>
</tr>
<tr>
<td>Use of surfactant</td>
</tr>
<tr>
<td>Proven sepsis</td>
</tr>
<tr>
<td>Respiratory distress syndrome</td>
</tr>
<tr>
<td>Bronchopulmonary dysplasia</td>
</tr>
<tr>
<td>Intraventricular hemorrhage, grade 2 or more</td>
</tr>
<tr>
<td>Periventricular leukomalacia</td>
</tr>
<tr>
<td>Necrotizing enterocolitis</td>
</tr>
</tbody>
</table>

Values are given as mean ± standard deviation or n (%). CRP: C-reactive protein; WBC: white blood cell.

<table>
<thead>
<tr>
<th>Table 4: Risk factors associated with newborn hearing screening test failure according to logistic regression analyses.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Risk factors</strong></td>
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<tr>
<td></td>
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<tr>
<td></td>
</tr>
<tr>
<td>Umbilical cord plasma IL-6 (pg/mL)</td>
</tr>
<tr>
<td>Funisitis</td>
</tr>
<tr>
<td>Elevated blood CRP levels (&gt;5 mg/L) in immediate postnatal period</td>
</tr>
<tr>
<td>Umbilical cord plasma C3a (μg/mL)</td>
</tr>
</tbody>
</table>

OR: odds ratio; CI: confidence interval; IL: interleukin; CRP: C-reactive protein.
has profound e
biology of sensorineural hearing loss. Moreover, from a thera
support cells in the cochlea underlying a subset of pathophys-
microglia, and oligodendrocytes) [28, 29] corresponding to 
\cite{27} and a neuroprotective e
C3a has been shown to have an anti-in
injury, especially in the immature brain \cite{25, 26}. Indeed,
protects the brain tissue against neonatal hypoxia 
relationship between neonatal hypoxic
previously reported clinical evidence and animal data on the

\textbf{Figure 1:} Receiver operating characteristic curves for umbilical 
cord plasma interleukin-6 (IL-6) “line” and C3a “broken line” for 
predicting newborn hearing screening test failure (cord plasma 
IL-6: area under the curve, 0.663; standard error, 0.070; cord 
plasma C3a: area under the curve, 0.690; standard error, 0.071; 
no differences (P = 0.778) between cord plasma IL-6 and C3a).

reduced risk of NHS test failure. This finding is similar to the 
previously reported clinical evidence and animal data on the 
relationship between neonatal hypoxic–ischemic brain injury 
and complement peptide C3a, which have shown that C3a 
protects the brain tissue against neonatal hypoxia–ischemia 
injury, especially in the immature brain \cite{25, 26}. Indeed, 
C3a has been shown to have an anti-inflammatory effect 
\cite{27} and a neuroprotective effect \textit{in vitro} via acting on many 
types of glia in the central nervous system (e.g., astrocytes, 
microglia, and oligodendrocytes) \cite{28, 29} corresponding to 
support cells in the cochlea underlying a subset of pathophys-
ology of sensorineural hearing loss. Moreover, from a therapeu-
tic perspective, recent studies by Morán et al. and 
Järlestedt et al. demonstrated that the application of C3a 
has profound effects on the amelioration of neonatal hyp-
oxic–ischemic brain injury in a mouse model \cite{25, 30}. There-
fore, it is likely that C3a could also serve as a potential new 
therapeutic substance targeting treatment for SNHL. Further 
studies are needed to determine whether abnormal NHS 
results and hearing impairment could be reduced by early 
C3a treatment in an animal model and subsequent clinical 
trials in preterm neonates at high risk of developing SNHL.

A body of research has suggested that prenatal infection 
and resultant fetal inflammatory response contribute to the 
pathogenesis of severe neonatal neurologic illness, such with 
white matter injury (WMI) \cite{31, 32}, which in turn was asso-
ciated with an increased risk of neurosensory impairment 
(hearing or vision) \cite{33, 34}, and elevated blood CRP levels 
at birth were associated with WMI in preterm infants \cite{35}. 

However, to date, no study has evaluated the direct relation-
ship between immediate postnatal blood CRP levels (at or 
immediately after birth) and hearing status in preterm 
infants. However, of note, in a study that analyzed the CRP 
values reflecting rather postnatal inflammatory status due 
to infection (using the maximum CRP values obtained 
during the entire course before the auditory screening), 
Yoshikawa et al. found a significant association between high 
neonatal blood CRP levels and hearing screen failure \cite{36}. 
Similar to the findings in this study, we further found that 
elevated CRP levels in the immediate postnatal period, which 
may be more reflective of perinatal rather than postnatal 
events, were also significantly associated with NHS test fail-
ure. In fact, these observations are natural because CRP is 
predominantly secreted by the liver in response to an elevated 
IL-6 level \cite{37}, which was already reported to be a 
major independent risk factor for fetal and neonatal disor-
ders associated with neonatal hearing impairment, such as 
FIRS, funisitis, early onset neonatal sepsis, and WMI in pre-
term infants \cite{9, 10, 21, 33, 38}. It is most likely that the pres-
ence of a ripple effect of bacterial and viral infections such as 
inflammatory reaction in the cochlea as reflected by an 
increase in CRP levels could cause NHS test failure.

Our failure to obtain a statistical association between 
NHS test failure and MMP-9, C5a, M-CSF, and endostatin 
levels in the umbilical cord plasma in the present study 
merits attention. A famous tissue remodeling gene, the 
MMP-9 gene, was previously reported to be significantly 
upregulated after exogenous trauma to the cochlea such as 
cochlear implantation and was even qualitatively associated 
with a change in hearing thresholds after cochlear implant-
ation in guinea pigs \cite{39}. It could be that the expression of 
the tissue remodeling gene MMP-9 is more significantly 
affected by exogenous trauma than endogenous inflamma-
tion. Endostatin has been reported to show beneficial effects 
on the inflammatory disease and even sepsis as previously 
shown in a septic mouse model and a rheumatoid arthritis 
model in which endostatin reduced multiple organ dysfunc-
tion syndrome due to sepsis and angiogenesis, respectively 
\cite{40, 41}. However, we could not observe any protective 
effect of endostatin from hearing loss status in preterm neo-

nates in the present study. The cochlea might have a differ-
ent milieu for endostatin to play such a role. Alternatively, 
the main mechanisms of hearing loss from preterm neo-

nates might walk in a pathway less a

ective of perinatal rather than postnatal 
events, were also significantly associated with NHS test fail-
ure. In fact, these observations are natural because CRP is 
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IL-6 level \cite{37}, which was already reported to be a 
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ence of a ripple effect of bacterial and viral infections such as 
inflammatory reaction in the cochlea as reflected by an 
increase in CRP levels could cause NHS test failure.

4.3. \textit{Strengths and Limitations of the Study.} The current study 
has several limitations. First, this retrospective study was 
conducted at a single center with a limited number of sub-
jects, limiting our ability to extrapolate our results to the
general population. Second, the role of various immune-related proteins in the UCB in the development of SNHL could not be precisely evaluated because of the low prevalence of SNHL. The SNHL incidence in this study (1.5%, 2/127) was in accordance with those of other studies [1, 44]. Third, a full characterization was not performed on pro- and anti-inflammatory cytokines and MMP in the umbilical cord plasma in this study, which is not likely to reflect the entire picture of immune activation related to hearing loss status. The strength of our study is that it is the first to our knowledge to investigate the relationship between the levels of various inflammatory and immune proteins in the UCB and hearing screen failure that places a neonate at risk for SNHL. Finally, six proteins in the umbilical cord plasma were selected in the present study because their expressions are increased during preterm birth–associated inflammatory/immunological responses in the maternal blood, amniotic fluid, or UCB [12, 19, 45–47].

5. Conclusions

In conclusion, in preterm newborns, elevated levels of umbilical cord plasma C3a were independently associated with a reduced risk of NHS test failure, whereas elevated levels of umbilical cord plasma IL-6 and elevated CRP levels in the immediate postnatal period were significantly associated with NHS test failure. However, these measures are not sensitive or specific markers for hearing screen failure (Figure 1). Elevated umbilical cord plasma MMP-9, C5a, M-CSF, and endostatin levels were not associated with hearing screen failure. Further large longitudinal studies are needed to assess which time point provides the best predictive value of subsequent hearing impairment using the fetal and neonatal blood samples collected serially at predefined time points.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that there is no conflict of interest.

Authors’ Contributions

Ye Ji Shim and Byung Yoon Choi contributed equally to this work.

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