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Cytokine profiles of newborns exposed to chorioamnionitis who developed chronic lung disease

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Abstract

INTRODUCTION : Neonatal chronic lung disease (CLD) is caused by intrauterine infections, pressure damage, and oxygen toxicity caused by postnatal respiratory management. Inflammatory cytokines are strongly induced in CLD, particularly when intrauterine infections, such as chorioamnionitis (CAM) occurs. In this study, serum cytokine levels of newborns with CLD were comprehensively analyzed at birth, and the association between respiratory prognosis and severity were investigated.

METHODS : A total of 18 patients, who were given a diagnosis of CLD36 (CLD36 was defined as requiring oxygen at a corrected gestational age of 36 weeks, i.e., the need of auxiliary ventilation, such as positive pressure ventilation) after being admitted to the Neonatal Intensive Care Unit (NICU) between March 2018 and October 2019 were included in the study, and their cytokine levels were analyzed. The subjects were classified into 2 groups ; 9 CAM patients and 9 non-CAM patients, and their backgrounds were analyzed. Serum cytokines from samples on day 0 was measured and compared between the 2 groups. Levels of cytokines interluekin (IL)-1β, IL-6, IL-8, IL-10, and tumor necrosis factor (TNF)-α were measured using the Bio-Plex suspension array (Bio-Rad Laboratories). The number of white blood cells (WBC), C-reactive protein (CRP) and serum IgM, duration of respiratory management were also compared between the 2 groups.

RESULTS : In the CAM group, there were 4 patients who received home oxygen therapy and 1 death. IL-8 levels were significantly higher in the CAM group than in the non-CAM group ($P < 0.05$). The IL-8/IL-10 cytokine ratio tended to be higher in the non-CAM group, but the difference was not statistically significant.

CONCLUSION : IL-8 levels were significantly higher in the CAM group than in the non-CAM group. Patients with increased serum IgM levels or IL-8/IL-10 tended to require a longer duration of respiratory management. Therefore, IL-8/IL-10 may be a useful biomarker to predict the respiratory prognoses of extremely preterm infants.

Introduction

Neonatal chronic lung disease (CLD) is caused by intrauterine infections, pressure damage, and oxygen toxicity resulting from postnatal respiratory management[1-4]. Particularly in cases associated with chorioamnionitis (CAM), the production of chemotic substances, such as leukotrienes, chemokines, and complements, as well as the production of various cytokines by macrophages is increased, leading to hypercytokinemia. Furthermore, when chronic lung damage occurs owing to pressure damage from artificial ventilation, the production of transforming growth factor (TGF)-β leads to dysplasia and fiber proliferation in alveoli. Furthermore, it has been reported that alveolar formation is eventually inhibited, leading to the inhibition of vascular development[5]. Intrauterine infections, such as vaginitis and cervical inflammation cause inflammatory responses in the

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Key words : cytokine profiling, chronic lung disease (CLD), chorioamnionitis (CAM), home oxygen therapy (HOT), Interleukin (IL)-8/IL-10

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mother and fetus, resulting in the release of inflammatory cytokines and chemokines. Interleukin (IL)-1 and tumor necrosis factor (TNF)-α induce the production of prostaglandins and cause uterine contractions, and IL-6, IL-8, and TNF-α cause rupture of the cervical membrane and opening, resulting in premature birth. These cytokines are thought to cause periventricular leukomalacia and lung damage in the fetus[6].

Cytokines, which are glycoproteins secreted from stimulated and activated cells, control the survival, proliferation, and differentiation of cells by acting on cells expressing their receptors and cells in the vicinity, causing a variety of reactions, such as immunity and inflammation[7]. The production of disease-specific cytokines have been reported in a variety of diseases, and in recent years, the association of CLD and cytokines has been reported. This appears to be associated with the fact that it has become possible to measure and profile various cytokines, even in trace samples.

Patients with CLD have been reported to have high levels of the inflammatory cytokines IL-1, IL-6, IL-8, and TNF-α in their endotracheal aspirates and serum[8]. Takahashi et al reported a positive association between CLD and levels of IL-6, IL-8, and Monocyte Chemotactic Protein-1 (MCP-1)[9]. Thus, CLD is thought to be strongly associated with cytokines. Clarifying the relationship between CLD and cytokines reveals the pathology of CLD and leads to the development of new treatments.

In this study, we analyzed serum cytokines at birth in CLD infants and examined the relationship between respiratory prognosis and severity.

Objective

Comprehensive analysis of serum cytokines at birth in CLD infants were analyzed, and differences in cytokine levels in the presence and absence of intrauterine infections were compared. We revealed that CLD is a condition centered on inflammation and we also inferred the respiratory prognosis from cytokine values at birth.

The association between respiratory prognosis and severity of CLD was also analyzed.

Materials and methods

A total of 40 preterm infants, gestational age less than 33 weeks, who were admitted and treated in our Neonatal Intensive Care Unit (NICU) between March 2018 and October 2019 were included in this study. Serum cytokine levels of 18 out of 40 patients who met the diagnosis of CLD36 were measured at birth. CLD36 was defined as requiring oxygen at a corrected gestational age of 36 weeks, i.e., the need of auxiliary ventilation, such as positive pressure ventilation. Cytokine levels were measured using the Bio-Plex suspension array (Bio-Rad Laboratories), after obtaining the parents' informed consent at the time of hospitalization. The background characteristics of the patients, such as the number of weeks of gestation, birth weight, sex, and Apgar score were determined, and patients were classified into 2 groups ; CAM or non-CAM. Serum cytokine levels (IL-1β, IL-6, IL-8, IL-10, and TNFα), the number of white blood cells (WBCs), and C-reactive protein (CRP) and serum IgM levels from day 0 (day of birth) samples were measured and compared between the 2 groups. In this study, we could not measure bronchoalveolar lavage (BAL) samples and the number of neutrophils. The duration of intubation, presence or absence of respiratory distress syndrome (RDS), time to the observation of foam-like shadows on X-ray, and the duration of respiratory management were also compared between the 2 groups. RDS was diagnosed by the attending physician from the patient's clinical course, gastric fluid, chest X-ray results, and the need for surfactant administration. We sprayed the surfactant (1 V=120 mg/kg) in three directions during hospitalization as RDS treatment. The appearance of foam-like shadows is a characteristic X-ray finding in chronic lung disease, indicating emphysema changes in the lungs. CAM was defined as higher than Blanc classification II in placental pathology. A foam-like appearance on chest X-ray was determined by the author. Home oxygen therapy (HOT) was performed in patients who required oxygen even at the corrected gestational age of 40 weeks. Statistical analyses were performed using Statcel4 software, and the Mann-Whitney U test was used to analyze the difference between 2 groups. A *P*-value of less than 0.05 was considered to indicate a statistically significant difference between 2 groups. This study was carried out with the approval of the Ethics Association of Tokyo Medical University Hospital (study approval no. : SH3648).

Results

The total number of patients given a diagnosis of CLD36 was 18 (13 male and 5 female infants). Nine patients were in the CAM group. The number of median gestational weeks and birth weight were 25.6 weeks and 770 g in the CAM group, and 28.4 weeks and 948 g in the non-CAM group, respectively. The gestational weeks of patients in the CAM group were lower than the non-CAM group ($P = 0.04$), and the birth weights of patients in the CAM group tended to be lighter. The median Apgar scores at 1 min and 5 min were 4 points and 7 points in the CAM group, and 8 points, 9 points in the non-CAM group, respectively. RDS was observed in 8 patients in the CAM group, and 8 patients in the non-CAM group. Four patients received HOT, and there was 1 death. In addition, foam-like shadows appeared earlier in the CAM group,

and the intubation period was longer, but there was no statistical significance. There were 4 patients who received HOT in the CAM group. One patient with necrotizing enteritis, short bowel syndrome, and liver failure in the CAM group died. WBC number, and CRP and IgM levels showed no statistical significance between the 2 groups (Table 1).

Serum cytokine levels are shown in Table 2. IL-8 levels were significantly higher in the CAM group than in the non-CAM group ($P = 0.015$). There were no statistically significant differences in IL-1β, IL-6, TNF-α, and IL-10 levels between the 2 groups. The ratios of inflammatory cytokines and inhibitory cytokines are shown in Table 3.

A statistically significant difference in IL-8/IL-10 was not observed between the 2 groups, but it tended to be higher in the CAM group ($P = 0.085$) (Figure 1). Serum IgM levels and IL-8/IL-10 ratios at the time of hospitalization of 4 patients who received HOT are shown in Table 4. All 4 HOT cases were RDS. Patients with a high IgM level or IL-8/IL-10 tended to require HOT. Tracheotomy was performed in patient 4 because of intolerance to extubation owing to subglottic stenosis, and HOT was performed.

Table 1 Characteristics of CAM and non-CAM patients

	CAM ($n = 9$)	Non-CAM ($n = 9$)	P-value
Gestational age (weeks)	25.6 (25.3, 27.8)	28.4 (26.9, 29.4)	**0.04**
Birth weight (g)	770 (649.5, 920)	948 (754.5, 1,359.5)	0.14
Male	6	7	0.69
Apgar (1 min)	4 (1.5-6)	7 (3-7.5)	0.07
Apgar (5 min)	8 (7-8.5)	9 (6.5-9)	0.83
RDS	8	8	1
Intubation duration (days)	23 (1.5, 83.5)	6 (1, 37.5)	0.35
Foam-like appearance	3	0	0.23
HOT	4	0	0.11
Mortality	1	0	0.69
WBC (/μL)	9,600 (6,500, 12,350)	5,700 (4,250, 8,200)	0.06
CRP (mg/dL)	0.05 (0, 0.5)	0.03 (0.01, 0.2)	0.66
IgM (mg/dL)	7 (3.5, 21.5)	5 (2.5, 7)	0.13

Gestational age, birth weight, Apgar score, intubation duration, WBC number, and CRP and IgM levels are described as the median (interquartile range). RDS : respiratory distress syndrome, HOT : home oxygen therapy, CAM : chorioamnionitis

Table 2 Serum cytokine levels of CAM and non-CAM patients on day 0

	CAM ($n = 9$)	Non-CAM ($n = 9$)	P-value
Proinflammatory			
TNFα (pg/mL)	31 (24.2, 35.9)	23 (13.4, 30.1)	0.23
IL-1β (pg/mL)	0.2 (0.09, 0.7)	0.2 (0.1, 0.27)	0.54
IL-6 (U/mL)	63.6 (17.6, 693.9)	15.2 (6.1, 141.4)	0.27
Chemokines			
IL-8 (ng/mL)	153 (69.4, 176)	39.1 (26.8, 73.7)	**0.015**
Inhibitory			
IL-10 (pg/mL)	8.3 (3.0, 23.2)	8.8 (3.8, 18.2)	0.83

Cytokine levels are described as the median (interquartile range).

Table 3 Ratios of inflammatory cytokines to inhibitory cytokines

	CAM ($n = 9$)	Non-CAM ($n = 9$)	P-value
TNFα/IL-10	2.7 (1.6, 13.1)	2.6 (1.1, 7.3)	0.66
IL-1β/IL-10	0.05 (0.03, 0.07)	0.02 (0.01, 0.06)	0.20
IL-6/IL-10	3.4 (0.9, 72.5)	5.2 (0.3, 23.0)	0.40
IL-8/IL-10	9.8 (5.3, 13.0)	3.7 (1.8, 16.6)	0.085

Cytokines are shown as the median (interquartile range).

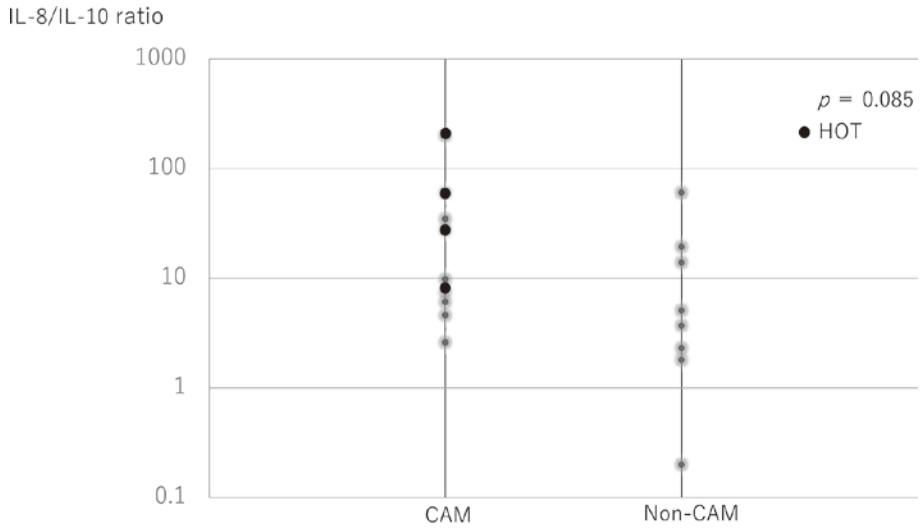

Fig. 1 IL-8/IL-10 levels between CAM and non-CAM patients
Patients in the CAM group tended to have higher IL-8/IL-10 ratios, and there were more patients requiring HOT, but the difference was not statistically significant ($P = 0.085$).

Table 4 Characteristics of the 4 patients receiving HOT

Patient	Gestational age (weeks)	Birth weight (g)	IgM (mg/dL)	IL-8/IL-10
1	24w0d	546	34	200
2	25w0d	712	56	27.5
3	28w1d	1,122	9	60
4	26w3d	832	3	7.6

HOT : home oxygen therapy

Discussion

In this study, we measured cytokine levels of CLD36 patients at birth and analyzed their respiratory prognoses. Although there are many studies on the association of CLD and cytokines, only a few reports have analyzed the association between the cytokine network and the clinical course of patients. As a cytokine can affect the production and activity of other cytokines and form a complex cytokine network, inflammation can lead to a disruption in the cytokine network balance, resulting in increased inflammation and the progression of tissue disorders[10)11)]. Sakai et al analyzed the cytokine-producing ability of very preterm infants and preterm infants after 32 weeks of gestation, and reported that the production of inflammatory and inhibitory cytokines was sufficient and balanced. On the other hand, in extremely preterm infants with CAM, although their ability to produce inflammatory cytokines, such as IL-8, is maintained, their ability to produce inhibitory cytokines is very low, and hence excessive fetal inflammation persists in the uterus, and they are more likely to develop fetal inflammatory response syndrome[12)]. In our present study, the reason why there was a statistically significant difference between the 2 groups only in IL-8 level is thought to be due to the difference in cytokine-producing ability in infants of different gestational weeks. Patients from 24 weeks to 32 weeks of gestation are included in this study, and cytokine-producing ability is different among the infants owing to differences in their maturity. Therefore, we believe a statistically significant difference was detected only for IL-8 levels because the ability to produce this cytokine is not affected by gestational age. We analyzed IL-8/IL-10 ratios, focusing on the disruption of the balance of inflammatory and inhibitory cytokines. Although there was no statistically significant difference in IL-8/IL-10 ratios between the 2 groups, IL-8/IL-10 tended to be relatively high in the CAM group, and foam-like shadows appeared earlier in CAM patients leading to the need of HOT. In addition, a study analyzing cytokines of serum and bronchoalveolar lavage from subjects in their early stages of life demonstrated that an increase in IL-8 level and IL-8/IL-10 causes severe RDS and progression to CLD[13)]. In this study, IL-8/IL-10 of the serum and BAL was higher in the CLD than non-CLD, and a statistically significant difference was found in serum. In other words, IL-8 may move to the lungs due to blood circulation and lead to alveolar injury. It is necessary to examine the correlation between IL-8 and neutrophils. Huang et al have

similarly reported an association between the increase in IL-8 levels and the onset of CLD[14]. However, it has been reported that a decrease in the levels of the inhibitory cytokine IL-10 is involved in CLD development[15-18]. These studies have shown that IL-10 inhibits IL-8 production by monocytes and granulocytes.

In our present study, there was no statistically significant difference in the number of WBCs, as well as CRP and IgM levels between the 2 groups, but foamy shadows appeared early in 2 patients with high IgM levels, who subsequently required HOT. In summary, if there is an increase in IL-8/IL-10 or IgM levels indicating intrauterine infection and decreased biological defense ability, early CLD onset is expected, which is likely to lead to the need of HOT.

At present, the American Academy of Pediatrics and the Canadian Pediatrics Society state that routine steroid administration for the prevention and treatment of CLD is not recommended, except in severe cases, in which it should be performed in short-term doses[19]. In addition, large-scale comparative studies on inhaled steroids suggest that steroid inhalation may reduce the incidence of CLD, but it is not effective compared with systemic steroid administration[20][21]. The treatment for CLD has not been established, hence we hope that this cytokine profiling method will lead to the development of effective treatments in the future.

This study has several limitations. The number of cases was small and the variation was large in IL-8/IL-10, therefore it is necessary to increase the number of cases in the future to confirm our results. In addition, the measurement of cytokine levels was only performed at 1 timepoint, and treatment course was not considered and should be clarified in the future.

Conclusions

IL-8 was significantly higher in the CAM group. Systemic hypercytokinemia, especially increased IL-8 activity, by CAM plays a key role in the development of CLD. Respiratory management of patients with increased IL-8/IL-10 and IgM levels was difficult and such patients tended to require HOT. IL-8/IL-10 may hence be a biomarker to predict the respiratory prognoses of extremely preterm infants, which will be useful for comparing preterm infants of different gestational ages. Further studies are required to clarify useful respiratory prognostic markers for CLD.

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絨毛膜羊膜炎合併の新生児慢性肺疾患児における血清サイトカインの検討

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【要旨】【はじめに】 新生児慢性肺疾患は、絨毛膜羊膜炎（CAM）に代表される子宮内感染症や、長期の呼吸管理に伴う圧損傷や酸素毒性により肺胞組織の炎症や繊維化が惹起され生じる。慢性肺疾患（CLD）は炎症性サイトカインが強く影響していることが示唆されており、CAM などの子宮内感染症が関連した場合ではよりその影響を強く受け、肺胞障害が重度となることが報告されている。今回、新生児慢性肺疾患児における出生時の血清サイトカインを網羅的に解析し、呼吸予後や重症度との関連性について検討した。

【対象と方法】 2018 年 3 月から 2019 年 10 月までに当院 NICU に入院し、CLD36 の診断に至り、サイトカインを測定することができた 18 例を対象とした。胎盤病理で CAM を合併した 9 例と非合併 9 例の 2 群に分類し、それぞれ在胎週数や出生体重などの背景を調査した。また出生時（日齢 0）における血清サイトカインを測定し 2 群間で比較検討を行った。サイトカインの測定には Bio-Plex suspension array（Bio-Rad Laboratories）を用い、IL-1β、IL-6、IL-8、IL-10、TNFα を測定した。さらに入院時検査所見（WBC, CRP, IgM）や呼吸管理期間、レントゲンにおける泡沫状陰影の出現時期についても検討を行った。

【結果】 CAM 合併群では早期に泡沫状陰影が出現し、挿管日数が長い傾向にあった。CAM 合併群で在宅酸素 4 例、死亡 1 例を認めた。入院時の WBC、CRP、IgM は両群で統計学的有意差は認めなかった。血清サイトカインは IL-8 のみ CAM 群において有意に高値であった（$p<0.05$）。炎症性サイトカインと抑制系サイトカインの比である IL-8/IL-10 は統計学的有意差は認めなかったが、CAM 合併群で非合併群と比べ高い傾向にあった。

【結論】 CAM 合併群では非合併群と比較して IL-8 が有意に高値であった。血清 IgM の上昇または IL-8/IL-10 の上昇がある症例は、より長期の呼吸管理を要し重症化する傾向にあった。IL-8/IL-10 が超早産児の呼吸予後を予測するマーカーとなる可能性がある。

〈キーワード〉 サイトカインプロファイル、新生児慢性肺疾患（CLD）、絨毛膜羊膜炎（CAM）、在宅酸素療法（HOT）、IL-8/IL-10