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Cytokine Profiles Before and After Exchange Transfusions in Severe Late-Onset Neonatal Group B Streptococcus Meningitis: A Case Report

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Streptococcus agalactiae or group B streptococcus (GBS) is a pathogen that causes severe neonatal infections, resulting in sepsis, pneumonia, and meningitis. Neonatal GBS meningitis has a poor neurological prognosis and a high mortality rate. GBS disease is classified as early- and late-onset if the onset age is 0-6 and 7-89 days after birth, respectively. There is currently no effective preventive strategy against late-onset GBS (LOGBS) disease. Here, we report a case of female infant with LOGBS meningitis who recovered from the septic shock by two exchange transfusions (ExTs) but still experienced severe neurological sequela. She was born at a gestational age of 39 weeks via caesarian section due to oligohydramnios and had fever 11 days after birth. GBS was detected in her cerebrospinal fluid (CSF) and blood but not in the vaginal or breast-milk cultures of the mother. The patient was treated with intravenous antibiotic administration; however, she suddenly developed pulseless ventricular tachycardia and asystole the next day. Her heart rate was normalized via cardiopulmonary resuscitation. We also performed two ExTs, and she recovered from the septic shock. Cytokine-profile analysis revealed that the serum and CSF levels of various pro-inflammatory and anti-inflammatory cytokines were elevated before the ExTs, after which the serum levels of several of these cytokines decreased. Two ExTs were effective in saving the life of the patient but did not improve the neurological prognosis. Given that neonatal GBS meningitis has high fatality and sequela rates; thus, it is necessary to establish a preventive strategy.

Keywords: anti-inflammatory cytokines; cytokine profile; exchange transfusion; late-onset group B streptococcus meningitis; septic shock
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Introduction

Streptococcus agalactiae or group B streptococcus (GBS) causes invasive infections among neonates and infants, resulting in sepsis, pneumonia, and meningitis. This pathogen is resident in the vagina and/or rectum of healthy women (Schuchat 1999). Approximately 11% of Japanese women harbor GBS (Gomi et al. 2019). Neonatal GBS disease is classified as early-onset GBS (EOGBS) and late-onset GBS (LOGBS) disease if the disease onset occurs during the first 6 days and days 7-89 after birth, respectively. The mean global incidence of EOGBS and LOGBS diseases is 0.41 and 0.26 per 1,000 live births, respectively (Madrid et al. 2017). However, the numbers of institutionalized cases of EOGBS and LOGBS diseases in Japan are lower than the world averages and estimated at 0.09 and 0.12 per 1,000 livebirths, respectively. In Japan, both EOGBS and LOGBS diseases have mortality rates of 4-5%. Additionally, 26% of EOGBS- and 45% of LOGBS-disease cases are meningitis. Furthermore, 29% of cases of GBS meningitis show a permanent neurological sequela, which still has a poor prognosis (Matsubara et al. 2017). Intrapartum antibiotic prophylaxis (IAP) is effective against EOGBS disease associated with maternal rectal/vaginal GBS colonization detected via microbiological screening or clinical risk factors. However, this strategy is ineffective against LOGBS disease (Puopolo et al. 2019). There is currently no effective preventive strategy against LOGBS dis-

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ease.

Exchange transfusion (ExT) against severe neonatal sepsis is a relatively safe and effective treatment for eradicating bacteria, bacterial toxins, and circulating pro-inflammatory cytokines (Pugni et al. 2016). However, to our knowledge, there are no reports on before and after ExT cytokine-profiles in LOGBS meningitis. We had a case of severe LOGBS meningitis that was treated with two ExTs. We evaluated the cytokine-profiles in the cerebrospinal fluid (CSF) and serum before and after the ExTs.

Case Presentation

An 11-day-old female infant developed fever and was transferred to our neonatal intensive care unit (NICU). She was born to a 29-year-old mother (gravida 0, para 0) via cesarean section at a gestation age of 39 weeks, 4 days due to oligohydramnios. There were no abnormalities in the course of pregnancy other than a mild intrauterine growth retardation. Her birth weight, length, and head circumference were 2,336 g, 48.0 cm, and 32.0 cm, respectively. Her Apgar scores were 8 and 9 at 1 and 5 minutes, respectively. Her cord-blood gas analysis resulted pH 7.35 and BE-0.0 mmol/L. She received phototherapy at day 6 for neonatal jaundice. On the morning of day 11, lethargy and poor milk-sucking power were noted, and her body temperature was 38.3°C. She was transferred to our NICU 8 hours after the fever developed.

On admission to our NICU, lethargy and hypotonia of the muscles were noted. Her body weight was 2,340 g, and the vital signs on arrival included a body temperature of 37.5°C, heart rate of 162 beats per minute, blood pressure of 85/59 mmHg, respiratory rate of 68 breaths per minute, and an oxygen saturation level of 100% in room air. Her skin color was pale, with reduced skin turgor. The anterior fontanelle was distended, measuring 6 × 6 cm. She had a peripheral cold and erythema on her back and right ear. Laboratory examination results showed a hemoglobin count of 17.4 g/dL, platelet count of 29.2 × $10^4/\mu$L, white blood cell (WBC) count of 1,600/μL (neutrophils, lymphocytes, eosinophils, basophils, and monocytes were 52.5%, 36.7%, 1.9%, 1.9%, and 7.0%, respectively), and C-reactive protein (CRP) concentration of 6.19 mg/dL. CSF analysis revealed a markedly increased WBC count (250/μL), with 96% segmented neutrophils, a protein concentration of 432 mg/dL, and a glucose concentration of < 2 mg/dL. Gram staining of the CSF showed gram-positive cocci in chains. No abnormalities were found on urinalysis, ultrasonography, or chest/abdominal radiography. GBS was detected in the cultures of the CSF and blood. The capsular type of GBS was serotype III according to the immunological reactivity of the capsular polysaccharides in GBS. The maternal vaginal and stool cultures at 36 gestational weeks, and postnatal–breast-milk culture were negative for GBS.

The patient was diagnosed with late-onset neonatal meningitis and administered intravenous ampicillin (200 mg/kg/day), cefotaxime (150 mg/kg/day), and gamma globulin (Fig. 1). Because tachypnea and bradycardia were noted, ventilation was started 4 hours after admission to our NICU. Eleven hours after the admission, she suddenly developed pulseless ventricular tachycardia and asystole. We performed cardiopulmonary resuscitation and administered intravenous adrenaline, and subsequently her heart rate normalized. We performed an ExT 18 hours after admission against septic shock. Tachycardia and hypoxia

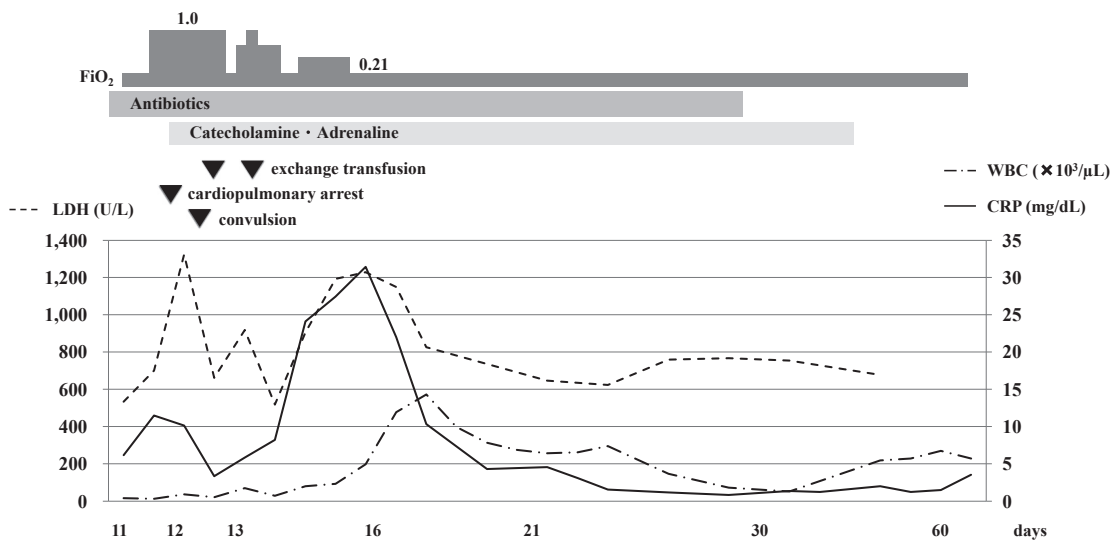

Fig. 1. Clinical course during the first 60 days after admission.
Arrows indicate cardiopulmonary arrest, convulsions, and exchange transfusions. After exchange transfusion, the oxygenation improved and the LDH level decreased. After two exchange transfusions, administration of oxygen, adrenaline, and catecholamines could be reduced and discontinued. In addition, antibiotic treatment reduced the serum CRP concentration and restored the white-blood-cell count.
FiO_2, fraction of inspiratory oxygen; LDH, lactate dehydrogenase; CRP, C-reactive protein; WBC, white blood cell.

gradually improved during the first ExT. Her levels of lactate dehydrogenase and CRP decreased after the ExT (from 1,324 to 662 U/L, and 10.16 to 3.39 mg/dL, respectively). However, tachycardia and hypoxia recurred after the first ExT. Thirty-one hours after the admission, convulsive seizures characterized by stiffened limbs developed, but these symptoms were controlled with phenobarbital and benzodiazepines. We performed a second ExT 42 hours after the admission. Subsequently, her fever, hypoxia, tachycardia, and low blood pressure improved, followed by recovery from the septic shock, and thus we gradually reduced and discontinued the administration of catecholamines and adrenaline. However, she did not resume spontaneous breathing. Brain magnetic resonance imaging on day 65 revealed extensive cerebral necrosis and brain atrophy. She was tracheotomized 4 months after birth and was discharged home 10 months after birth. She is now 5 years old and continues to be ventilated at home without spontaneous breathing or motor activity.

Cytokine profiles

We performed cytokine-profile analysis of both the CSF and serum on admission and of only the serum before and after the ExTs. CSF and serum cytokine levels were measured using a MINIPLEX MAP Human Cytokine/Chemokine Magnetic Bead Panel-Immunology Multiplex Assay (Merck KGaA, Darmastadt, Germany). The cytokines evaluated include tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-1 receptor antagonist (IL-1Ra), IL-4, IL-6, IL-8, IL-10, C-X-C motif chemokine ligand (CXCL)-10, granulocyte macrophage colony-stimulating factor (GM-CSF), hepatocyte growth factor (HGF), soluble IL-1 receptor (sIL-1R)1, sIL-1R2, transforming growth factor (TGF) β1, TGFβ2, and TGFβ3 (Table 1; for sIL-1R1 and TGFβ3, the data are not shown). TNF-α, IL-1β, IL-1Ra, IL-6, IL-8, IL-10, CXCL-10, GM-CSF, HGF, sIL-1R2, TGFβ1, and TGFβ2 levels were quite high in both the CSF and serum upon admission. Among them, the levels of TNF-α, IL-1β, IL-1Ra, IL-6, IL-10, HGF, sIL-1R2, TGFβ1, and TGFβ2 decreased after the two ExTs (from 535 to 344, 48.8 to 39.2, > 10,000 to 4,558, 86,900 to 3,299, 34,752 to 13,044, 1,507 to 416, 17,100 to 14,217, 364 to 169, and 23.0 to 10.1 pg/mL, respectively).

Discussion

GBS remains a leading cause of morbidity and mortality in neonates and infants worldwide. EOGBS disease develops when a newborn is exposed in the birth canal to the GBS present in the vagina and/or rectum of the mother. In contrast, LOGBS disease infection routes are poorly understood. It is suspected that LOGBS disease is transmitted postnatally through various sources, including breastmilk, community sources, or the hospital environment (Le Doare and Heath 2013). The present case was diagnosed as LOGBS meningitis and was at death risk due to several factors, including septic shock, coma, assisted ventilation, high CSF protein levels, and leukopenia in blood (Georget-Bouquinet et al. 2008). In addition, the source of infection was unknown because the maternal vaginal and stool cultures at 36 gestational weeks and postnatal–breast-milk culture were negative for GBS. Although appropriate antibacterial treatment was administered within 10 hours of fever development, severe neurological sequela remained.

The basic treatment for GBS meningitis is intravenous administration of ampicillin. As IAP is less effective in LOGBS disease, and mortality and sequela rates are high in GBS meningitis even with appropriate antibiotic treatment, infection prevention is paramount in LOGBS disease.

Table 1. Cytokine concentrations in the CSF (on admission) and serum (on admission, and before and after ExT).

	Cut-off	CSF	Serum		
		On admission	On admission	Before ExTs	After ExTs
TNF-α (pg/mL)	< 3.2	9,329.98	894.82	535.18	344.45
IL-1β (pg/mL)	< 4.3	> 10,000	818.52	48.81	39.16
IL-1Ra (pg/mL)	105-1,062	> 10,000	> 10,000	> 10,000	4,558.22
IL-4 (pg/mL)	< 6.0	5.14	< 3.2	< 3.2	< 3.2
IL-6 (pg/mL)	< 4.0	4,894.26	2,987.10	8,6900.0	3,299.15
IL-8 (pg/mL)	< 2.0	5,213.71	6,275.59	> 10,000	N/A
IL-10 (pg/mL)	< 5.0	15,945.51	5,511.20	34,752.32	13,044.74
CXCL10 (pg/mL)	690-2,500	14,683.47	N/A	> 10,000	> 10,000
GM-CSF (pg/mL)	< 8.3	533.22	161.97	115.44	114.47
HGF (pg/mL)	< 390	2,170.84	129.53	1,507.75	416.48
sIL-1R2 (U/mL)	No data	4,327.86	2,280.09	17,100.38	14,217.72
TGFβ1 (ng/mL)	1.56-3.24	16.10	2,577.82	363.87	168.67
TGFβ2 (pg/mL)	No data	21.06	64.15	22.99	10.05

CSF, cerebrospinal fluid; ExT, exchange transfusion; TNF, tumor necrosis factor; IL, interleukin; Ra, receptor antagonist; CXCL, C-X-C motif chemokine ligand; GM-CSF, granulocyte macrophage colony-stimulating factor; HGF, hepatocyte growth factor; sIL-1R, soluble interleukin-1 receptor; TGF, transforming growth factor; N/A, not available.

Currently, GBS vaccines are being developed as a new strategy to prevent GBS disease in neonates and infants. A suitable vaccine against GBS given to pregnant women could provide effective prophylaxis against invasive GBS diseases that cannot be prevented with IAP or where IAP is not feasible or is incomplete (Carreras-Abad et al. 2020).

ExT is a relatively safe and effective treatment to eradicate bacteria, bacterial toxins, and circulating pro-inflammatory cytokines, to improve perfusion and tissue oxygenation, to correct the plasma coagulation system, and to enhance the immunological defense mechanisms in neonates (Pugni et al. 2016). ExT has been effective in infants with *H. influenzae* meningitis and pneumococcal meningitis (Takizawa and Nakajima 1985; Kameyama et al. 2016). In the present case, the blood pressure of the patient increased back and her oxygenation improved after two ExTs without any side effects, and she recovered from the septic shock.

The cytokine-profiles revealed that the levels of pro-inflammatory cytokines, such as TNF-α, IL-1β, IL-6, IL-8, CXCL-10, and GM-CSF, and those of anti-inflammatory cytokines, such as IL-10, IL-1Ra, and TGF-β, were elevated in both the CSF and serum. In neonatal sepsis and bacterial meningitis, immune responses associated with secretion of various cytokines are involved in the pathology, affecting disease severity and mortality (Barichello et al. 2013; Machado et al. 2014). Several studies have reported that serum TNF-α, IL-1β, IL-6, and IL-10 levels are elevated in GBS infection (Hashimoto et al. 1999; Al Hazzani et al. 2018), whereas serum IL-1α and CSF IL-6 levels are elevated in GBS meningitis (Fida et al. 2006; Takahashi et al. 2014). In severe neonatal sepsis, the serum IL-10 level is significantly higher (39.7 vs. 13.2 pg/mL) than the levels in non-severe sepsis, whereas neonates who died or developed severe sequela presented significantly high levels of GM-CSF (80.7 vs. 53.6 pg/mL, respectively) compared with those in individuals who were healthy when discharged (Leal et al. 2019). Regarding bacterial meningitis, in patients with a fatal outcome, CSF levels of TNF-α, IL-1β, IL-1Ra, and IL-6 are significantly higher than the levels in nonfatal cases (Grandgirard et al. 2013). As mentioned previously herein, the cytokines reported to be elevated during severe sepsis and bacterial meningitis associated with poor prognosis were elevated in the present case, indicating that the case was severe bacterial meningitis.

IL-10, IL-1Ra, and TGF-β1 are anti-inflammatory cytokines. Plasma levels of TNF-α are positively correlated with levels of IL-1Ra and IL-10 during a septic shock (Kasai et al. 1997). The levels of these anti-inflammatory cytokines are correspondingly elevated in response to elevated levels of pro-inflammatory cytokines and suppress an overzealous inflammatory response (Adib-Conquy and Cavaillon 2009). In the present case, IL-1Ra and IL-10 levels remained elevated, while TNF-α and IL-1β levels decreased from admission to the next day. It is considered that IL-1Ra and IL-10 levels were elevated in response to high levels of pro-inflammatory cytokines. When these anti-inflammatory cytokines are continuously overproduced, the body falls into the compensatory anti-inflammatory response syndrome (CARS) state (Bone 1996). In CARS, immunocompetent cells become dysfunctional, resulting in a condition called immunoparalysis, which leads to opportunistic infections (Patricio et al. 2019). In the present case, not only the levels of pro-inflammatory cytokines but also the elevated levels of anti-inflammatory cytokines were reduced by two ExTs, and approaching normal cytokine levels via the two ExTs may have improved general condition.

In conclusion, we experienced a case of severe LOGBS meningitis, in which the patient underwent two ExTs. These procedures reduced the levels of both overproduced pro-inflammatory and anti-inflammatory cytokines and thus saved the life of the patient from a septic shock but did not improve the neurological prognosis. Therefore, ExT may be used as an adjunct treatment in LOGBS meningitis, but additional cases are needed to validate the therapeutic effect of this procedure. There is currently no effective preventive strategy against LOGBS disease, which has a high frequency of meningitis with poor prognosis. Thus, it is necessary to establish a preventive strategy.

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Conflict of Interest

The authors declare no conflict of interest.

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