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Perinatal Coxsackievirus B3 Infection with Transient Thrombocytopenia

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Coxsackievirus (Cox) B is the second common picornaviruses, after echovirus, detected from children younger than 2 months of age. Neonates who present with Cox B3 infection in the first week are known to have severe illness such as myocarditis or menigoencephalitis. Severity is commonly associated with perinatal vertical transmission. Here, we report a neonatal case of Cox B3 infection with severe thrombocytopenia through horizontal transmission. The patient was a preterm infant born without asphyxia by selective cesarean section. From his 6th day of life, the patient had recurrent episodes of apnea. At that time, the laboratory investigations revealed a profound thrombocytopenia without any evidence of inflammation. Thus, neonatal alloimmune thrombocytopenia (NAIT) was suspected, and the patient received transfusion of immunoglobulin and platelets. Thereafter, the patient had no further episodes of apnea, and platelet counts of the patient increased gradually. Later, the possibility of NAIT was ruled out by the result of the platelet antigen genotyping of the patient and his parents. Culture obtained from his nasopharynx was positive for Cox B3. We thus speculate that the patient was exposed to the virus from his mother because she had a febrile episode at her 5th day after delivery, and her Cox B3 infection was confirmed by serology. Assuming that the thrombocytopenia was a complication of Cox B3 infection, the immunoglobulin transfusion might have provided a neutralizing antibody against Cox B3. It is important to consider the possibility of enterovirus infection as a differential diagnosis whenever unexplained thrombocytopenia was observed in neonates.

Keywords: apnea; coxsackievirus B3; perinatal infection; preterm infant; thrombocytopenia
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Introduction

Severe thrombocytopenia is a life threating hematological condition among neonates admitted to intensive care units. One of the most critical conditions of thrombocytopenia is intracranial hemorrhage, and prompt initiation of treatment is necessary. The cause of neonatal thrombocytopenia is diverse; from alloimmune and autoimmune diseases, infection, placental insufficiency, asphyxia, to metabolic diseases. Thus, diagnosis of thrombocytopenia requires a comprehensive approach. In general, infection should be ruled out at the initial step of diagnosis. The common causative pathogens are cytomegalovirus, rubella virus, human immunodeficiency virus, toxoplasma as in congenital infection, and gram-negative bacilli as in perinatal infection (Roberts et al. 2008). Owing to the recent progress of methodology for detecting pathogens, *Picornaviridae*, non-enveloped viruses causing variety of infection in children, had been recognized as additional viral pathogens. Among *Picornaviridae*, Coxsackievirus (Cox), which is divided into Cox A and Cox B, is known to cause perinatal infection (James 2014). Cox A tends to infect the skin and mucous membranes, causing herpangina and hand-foot-and-mouth disease, while Cox B tends to infect the heart, and liver, causing myocarditis, pericarditis, and hepatitis. Neonates who present with Cox B3 infection in the first week are known to have severe illness such as myocarditis or menigoencephalitis (Javett et al. 1956; Rantakallio et al. 1970). These viruses are usually transmitted by fecal-oral route, but perinatal infection does occur via vertical or horizontal route. The vertical transmission has been associated with fetal or neonatal loss. On the other hand, postpartum neonatal infection is relatively common and asymptomatic, or occur as self-limited febrile ill-

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ness with non-specific signs of infection (Jenista et al. 1984). Here, we report a neonatal case of Cox B3 infection with severe thrombocytopenia through horizontal transmission.

Case Presentation

The patient was a Japanese boy born at 35 weeks and 5 days of gestation with a birth body weight of 2,560 g. His mother was 30 years old with a history of Gravida 1, Para 1. At the 35 weeks and 5 days of pregnancy, the mother was hospitalized for threatened premature delivery, which was difficult to suppress. The patient was delivered by a selective cesarean section with Apgar scores of 9 at 1 minute and 9 at 5 minutes. A complete blood count of umbilical cord blood revealed leukocytes as 12,800/μL (neutrophils 60.7%, lymphocytes 26.9%, monocytes 4.9% and eosinophils 6.7%), red blood cells as $3.42\times10^6/\mu$L, hemoglobin as 12.4 g/dL, hematocrit as 36.1%, and platelets as 179,000/μL. The IgM level in the umbilical cord blood was normal (13 mg/dL), and C-reactive protein (CRP) was negative (0.00 mg/dL) indicating no evidence of intra-uterus infection. Since the patient was born at preterm, he was admitted to the neonatal care unit for observation. The patient stabilized within 6 hours, and tube feeding was initiated by 24 hours of age.

From his 6th day of life, the patient had recurrent episodes of apnea (Fig. 1). At that time, the patient was slightly pale and hypoactive. The body temperature, heart rate, and blood pressure remained stable. The patient was not icteric, and liver and spleen were not palpable. Muscular tonus was appropriate, and neurological findings were normal. There were no signs of respiratory distress or bowel diseases. Laboratory investigations of the patient revealed a profound thrombocytopenia (13,000/μL) with a normal leukocyte count (15,200/μL) and no clear evidence of inflammatory reaction (CRP 0.04 mg/dL). There was no apparent petechiae or purpura, and intracranial hemorrhage was ruled out by ultrasonography. Screening of congenital infections by maternal antibodies against toxoplasma, cytomegalovirus, herpes simplex virus, varicella-zoster virus, and syphilis showed no recent infection or re-activation of the pathogens. The levels of CRP remained low throughout his clinical course. From these findings, neonatal alloimmune thrombocytopenia (NAIT) was suspected. Based on the guideline for management of NAIT (Ouwehand et al. 2000), the patient received intravenous transfusion of immunoglobulin (freeze-dried sulfonated human normal immunoglobulin 1 g/kg; Kaketsuken Laboratories, Kumamoto, Japan) and concentrated platelet suspension on his 10th day of life. Without additional treatments, no

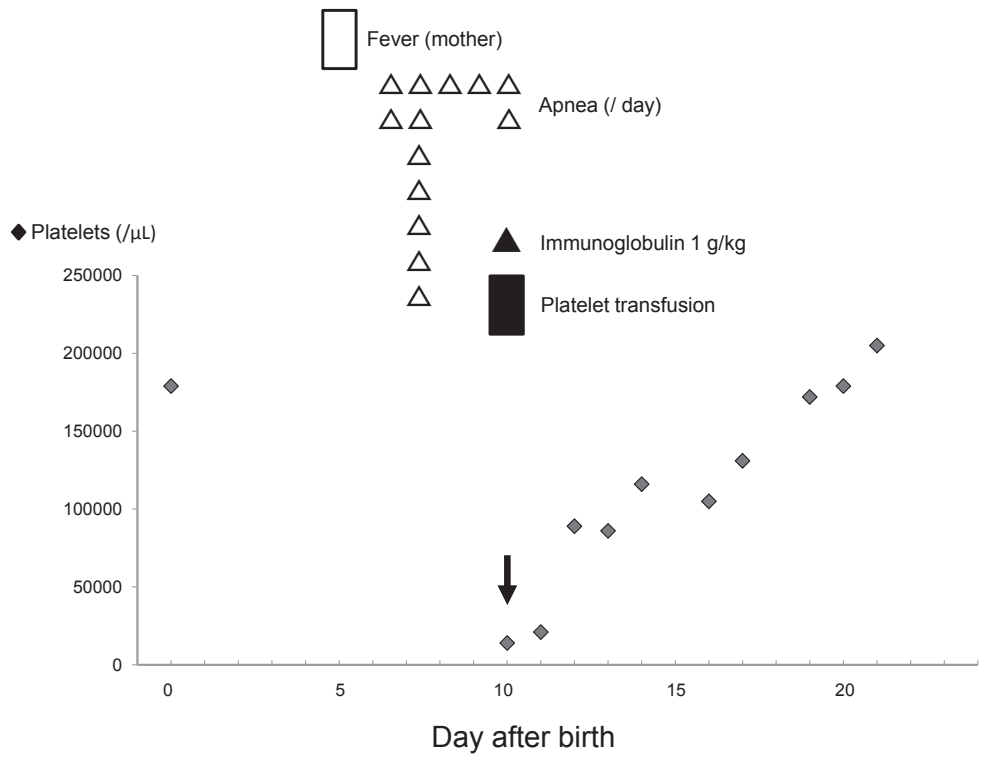

Fig. 1. Clinical course of the case and his mother.
The mother of the patient had a febrile episode on the 5th day after her delivery (open square: □) and had contact with the patient on the same day. The patient had recurrent episodes of apnea from his 6th day of life (open triangle: △). The patient received intravenous transfusion of immunoglobulin (closed triangle: ▲) and concentrated platelet suspension (closed square: ■) on his 10th day of life because laboratory investigations of the patient revealed a profound thrombocytopenia (13,000/μL; black arrow: ↓). Thereafter, platelet counts of the patient increased gradually.

further episode of apnea was observed by heart rate monitoring from his 11th day of life, and the platelet count gradually increased to 116,000/μL by his 25th day of life, and the patient was discharged from our hospital.

However, the possibility of NAIT was ruled out by the results of genotyping of the human platelet antigens of the patient and his parents. From the non-specific symptoms of our patient and a profound thrombocytopenia with no clear evidence of inflammatory reaction, enterovirus infection was suspected. Nasopharyngeal swab was submitted on his 12th day of life, and it was positive for Cox B3.

In order to identify the origin of the infection, epidemiological and retrospective laboratory investigations were conducted. Within 10 days before and after the onset of Cox B3 infection of the patient, none of the healthcare workers in the neonatal care unit was complicated with respiratory or febrile illness. Moreover, no other patients in the unit showed clinical symptoms and laboratory findings comparable with the patient. However, the mother recalled a febrile episode without any specific symptoms on her 5th day after the delivery and had contact with the patient on the same day. Subsequently, maternal Cox B3 antibody titers were examined with stored sera collected a day before and 9 days after the delivery. By neutralization test, the antibody titer increased from negative to 4 folds indicating a recent Cox B3 infection of the mother. In addition, Cox B3 was not detected by CODEHOP approach (Nix et al. 2006), a reverse transcription-seminested PCR targeting the VP1 region of the enterovirus, using the stored serum of the mother a day before her delivery.

Discussion

We presented a case of Cox B3 infection in perinatal period with transient thrombocytopenia. Thrombocytopenia in neonate is caused by various reasons, and it is often classified based on the timing of the onset (Roberts et al. 2008). Fetal thrombocytopenia is mainly caused by NAIT, congenital infection, and aneuploidy. On the other hand, the early onset thrombocytopenia, which usually begins within 72 hours after the delivery, is mainly caused by placental insufficiency, perinatal asphyxia, perinatal infection, and NAIT. In our patient, NAIT was initially suspected by the laboratory findings of thrombocytopenia without any clear signs of inflammation.

NAIT is caused by maternally transferred antibodies against paternal platelet antigens expressed on the patient's platelets (Ouwehand et al. 2000; Roberts et al. 2008). To diagnose NAIT, genotyping of human platelet antigens of the patient and his parents was performed. Because the patient had a severe thrombocytopenia, a risk factor of intracranial hemorrhage, transfusion of immunoglobulin and platelets were performed promptly prior to the definitive diagnosis. However, the possibility of NAIT was ruled out by the result of the genotyping, and a diagnosis of Cox B3 infection was made by the result of the nasopharyngeal culture. Platelet counts of the patient recovered gradually. Assuming that the thrombocytopenia was a result of Cox B3 infection, the immunoglobulin therapy might have provided a neutralizing antibody against Cox B3.

Because Cox B3 was isolated from the nasopharyngeal culture of the neonate during the symptomatic phase, it was the pathogen of the infection. Non-polio enterovirus infection in neonates is common, and 12.8% of the neonate are infected with the virus within 4 weeks after birth (Jenista et al. 1984). Cox is classified into group A and B, and Cox B is the second common picornaviruses, after echovirus, detected from children younger than 2 months of age (Morens 1978). In our patient, apnea, pale appearance and hypoactive status were observed. These symptoms together with thrombocytopenia suggest that the patient was suffering from an infection although the laboratory data did not indicate clear sings of inflammations. In case of severe enterovirus infections, disseminated intravascular coagulation provoked by the virus results in anemia and thrombocytopenia. However, symptoms of typical perinatal Cox infection are mild, and the diagnosis is difficult unless the virus is identified. Thus, whenever unexplained thrombocytopenia were observed in neonates, it is important to consider a possible enterovirus infection.

There are several transmission routes of perinatal Cox B infection. Like other enterovirus infections, patients infected with transplacentally transmitted Cox B might present with myocarditis or menigoencephalitis, or even die in perinatal period (Ornoy and Tenenbaum 2006). However, symptoms of patients infected with Cox B after birth can be mild and non-specific as observed in our patient. The onset of symptoms of *in utero* transmitted Cox B3 is within a few days after delivery (Bendig et al. 2003). In contrast, the onset of our patient was his 6th day of life. For Cox B3 outbreak in a maternity home, the latent time of infection was 3 days, and the time interval between the onset of the mother and our patient was equivalent to the previous report (Javett et al. 1956). From laboratory investigation of the serum, mother did not have evidence of Cox B3 infection a day before the delivery. Taken together, we speculate that the patient was exposed to the virus from the mother during her febrile episode after the delivery.

Fortunately, there was no secondary infection of Cox B3, and any additional preventative measures were not needed in our care unit. The institutional outbreaks of Cox have been well documented, and virus has been often transmitted to child from mother (James 2016). In documented outbreak in the nursery, secondary infection may result in severe infections such as myocarditis and menigoencephalitis (Javett et al. 1956; Rantakallio et al. 1970), and great effort should be made to control the Cox infection in case of institutional outbreak. The common route of Cox transmission in perinatal period is oral-oral (respiratory) rather than fecal-oral, and it is essential to take appropriate preventative measure.

Conclusion

We report a patient with Cox B3 infection with transient thrombocytopenia. The patient was exposed to the virus from his mother after delivery. It is important to consider the possibility of enterovirus infection as a differential diagnosis whenever unexplained thrombocytopenia was observed in neonates.

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Conflict of Interest

The authors declare no conflict of interest.

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