Enterovirus 71-Induced Autoimmune Hemolytic Anemia in a Boy

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ABSTRACT: Autoimmune hemolytic anemia (AIHA) can be induced by recent or concomitant infections. Many infectious agents are postulated to be associated with this condition. Treatment of infection induced AIHA still varies. This report describes a previously healthy Thai boy who developed AIHA associated with enterovirus-71 infection. He was successfully treated with oral prednisone.

KEYWORDS: AIHA, pediatrics, enterovirus

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Introduction

Autoimmune hemolytic anemia (AIHA) is caused by autoantibodies against red blood cell surface antigens inducing hemolysis. The prevalence of AIHA is around 1 per 100 000/year[1] The severity can range from asymptomatic to life threatening condition. The cause of AIHA in most cases is idiopathic. However, in a small proportion of patients, AIHA can be the consequence of autoimmune disorders (eg, systemic lupus erythematosus,[2] infantile giant cell hepatitis[3]), malignancies[4] (eg, lymphoma,[5] solid tumors[6]) or infections. Infection contributes around 10% of cases.[7] Common infectious organisms that can trigger AIHA include, but not limit to, *Mycoplasma pneumoniae*[8] Epstein-Barr virus (EBV),[9] Cytomegalovirus (CMV),[10] Rubella virus,[11] Influenza virus,[12] Varicella zoster virus,[13] and Severe acute respiratory distress syndrome coronavirus 2 (SARS-CoV-2).[14,15] However, cases of Enterovirus-induced AIHA were rare[7,16] and cases of Enterovirus 71 (EV71)-induced AIHA have not been reported. Clinical manifestations of enterovirus infection are protean, ranging from non-specific febrile illness to severe myocarditis or severe CNS infection.[17,18] Common manifestations of EV71 infection are vesicular eruptions involving oral mucosa, palms and soles (hand foot and mouth disease). Rare but severe complications include autonomic dysregulation and brain stem encephalitis.[19] However, hematologic manifestations in patients with EV71 infection have been scarcely described. This report describes a case of an 11-year-old boy who developed AIHA after EV71 infection. Written consent from the patient's mother was provided before publication of this case.

Case Description

In October, 2020, a previously healthy 11-year-old boy presented with high grade fever, bilateral cervical swelling for 2 days and dark-colored urine for 1 day. There was no history of loose stool, cough, rash, arthritis, dysuria or abnormal bleeding and no history of vaccinations within 1 month prior to admission. He reported vertiginous sensation when walking. On examination, his body weight was 38.4 kg (at 75th percentile) and his height was 144 cm (at 75th percentile). He was fully conscious with moderate pallor and icteric sclerae. Non-tender bilateral cervical lymphadenopathy (sized 1.5-2 cm in diameter) and a tender right cervical lymph node (0.5 cm in diameter) were noted. Hepatosplenomegaly was absent. Cardiovascular, respiratory, and central nervous system examinations were unremarkable. The patient had no prior history of anemia or jaundice. Family history was unremarkable for any underlying hematological diseases. He denied taking any medications prior to the onset of his symptoms.

His complete blood count showed hemoglobin (Hb) 7 g/dL, hematocrit (Hct) 20.9%, white blood count 32 710/mm^3 (N78, L12, M4%), platelet count 493 000/mm^3, reticulocytes 3.4%, D-dimer 16 027 ng/mL, haptoglobin 0.05 mg/mL (normal range, 0.32-1.97), lactate dehydrogenase (LDH) 1682 U/L, and total bilirubin 6.4 mg/dL with direct bilirubin level of 0.5 mg/dL. G6PD screening was normal. Serum electrolytes were within normal range (sodium 132, potassium 3.91, chloride 103, carbon dioxide 18.6 mmole/L). Blood urea nitrogen and serum creatinine were 23 and 0.66 mg/dL, respectively. Liver function test revealed alkaline phosphatase (ALP) 284, aspartate aminotransferase (AST) 104, alanine aminotransferase (ALT) 14 and gamma glutamyl transferase (γGT) 23 U/L. Serum protein and albumin were 88.2 and 35.5 g/L, respectively. Peripheral blood smear showed microsperocyte 1-2+ with few polychromasia and no schistocytes (Figure 1). Red blood cell autoagglutination was not significantly noticed. Urinalysis showed dark-colored urine, positive 4+ of protein,

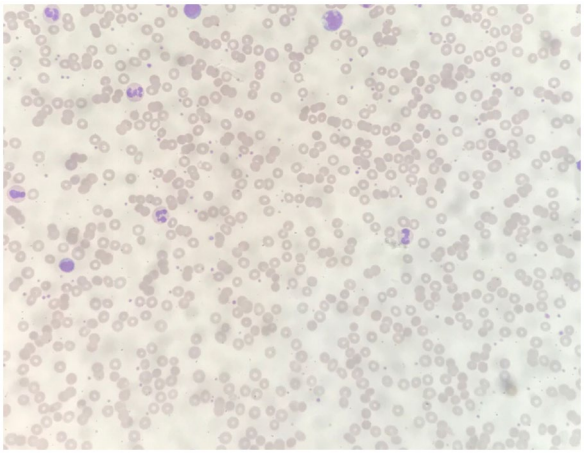

Figure 1. Peripheral blood smear of the patient. The peripheral blood smear of the patient performed upon admission shows a few agglutinations of red blood cells, microsperocyte 1 to 2+ with few polychromasia and without schistocytes.

negative glucose, negative bilirubin, marked positive of blood with 0-1/HPF of red blood cells. This indicated hemoglobinuria supporting the evidence of intravascular hemolysis. Direct Coombs test was positive 2+ for C3c, positive 4+ for C3d, but negative for IgG, IgM, and IgA. AIHA was diagnosed.

In order to find the etiology of AIHA, associated infectious diseases were sought. *Mycoplasma pneumoniae* antibody test (combined IgM and IgG detection by gelatin particle agglutination method; SERODIA®) was negative. EBV serology test (by lateral flow technique; vircell company) showed negative viral capsid antigen IgM, positive viral capsid antigen IgG, and positive Ebstein-Barr nuclear antigen (EBNA) IgG, indicating remote EBV infection. Blood and urine cultures were sterile. A multiplex PCR testing (QIAGEN) performed on nasopharyngeal swab was positive for rhinovirus/enterovirus. Because enterovirus infection was prevalent around the time of the patient's illness, enterovirus infection was suspected. The microneutralization test against EV71 in serum obtained on the admission day showed significantly elevated titers of neutralizing antibody at 1:640, while the neutralizing antibody titer against coxsackievirus A16 (CA16) was <1:10. The technique of microneutralization test is described in Appendix 1. Since there was no local transmission of SARS-CoV2 in Thailand at that time, screening for SARS-CoV2 was not performed.

Given his severe symptoms as hemoglobin level being 7 g/dL with vertiginous symptoms which may be attributed by anemia and specific type of AIHA could not be definitely determined at that time, he was treated with 60 mg per day of oral prednisolone (1.5 mg/kg/day), 2 units of red cell transfusion, intravenous fluid hydration and other supportive treatment. Fever subsided within a day and hemoglobinuria disappeared 2 days after initiation of treatment. Hemoglobin increased from 7 to 14.1 g/dL, and reticulocyte count decrease from maximum of 6% to 1.9% after 35 days of first presentation. Prednisolone was continued and gradually tapered to complete a total of 10 weeks. At 10-week follow-up visit, the patient's hemoglobin level was 14.1 g/dL with negative Coombs test and normalization of reticulocyte count and LDH level (Table 1). The clinical course of the patient is demonstrated in Figure 2.

Discussion

This previously healthy boy presented with acute febrile illness, cervical lymphadenopathy and pallor. The nasopharyngeal sample was positive for Rhino/Enterovirus and microneutralization test was strongly positive for Enterovirus 71. Autoimmune hemolytic anemia was concurrently diagnosed based on clinical presentation of anemia and laboratory findings of reticulocytosis, increased LDH level, indirect hyperbilirubinemia, low haptoglobin and positive direct Coombs test. To our knowledge, this is the first reported case of AIHA associated with EV71 infection. This report demonstrates that hematological complication can follow or be associated with EV71 infection. In addition to monitoring of cardiopulmonary and neurological complications following EV71 infection, monitoring of hematological complications may also be necessary.

AIHA can be categorized into many types. The types and etiology of AIHA have been recently reviewed.[20,21] In order to determine the type of AIHA, additional tests are needed.[20,22] Since we could not perform cold agglutinin titer to rule out cold agglutinin disease or cold agglutinin syndrome (CAS) and Donath-Landsteiner's test to rule out paroxysmal cold hemoglobinuria (PCH),[20] we could not determine the exact phenotype of AIHA in this patient. This patient had intravascular hemolysis supported by the evidence of positive urine hemoglobin and low blood haptoglobin level. The direct Coombs test was positive for complement but negative for IgG and IgM. PCH has been proposed to be associated with infections in children.[20,23,24] These pieces of evidence made PCH the most likely diagnosis in this patient. Donath-Landsteiner's test is indeed needed to confirm this diagnosis. The fact that the patient had intravascular hemolysis and very few red blood cell agglutination in peripheral blood smear disfavors the diagnosis of CAS. Nasal swab multiplex PCR testing for respiratory viruses and serological tests for Ebstein Barr virus and *Mycoplasma pneumoniae* were performed because of their common association with AIHA. In this case, the respiratory virus PCR test showed evidence of rhino/enterovirus infection even though the patient had not had clinical presentation of hand foot and mouth disease. Enterovirus infection may manifest as non-specific febrile illness or may be asymptomatic.[25] We believe that fever and swollen cervical lymph nodes might be the clinical manifestations of EV71 infection in this case. Evidence of recent EV71 infection was established by a significant rise in serum neutralization antibody titer against EV71. The neutralizing antibody against enterovirus can be used to identify enterovirus to species level[26] and it was used to confirm

Table 1. Laboratory data of the patient.

	HB (G/DL)	RETICULOCYTE (%)	TOTAL WBC (/MM^3)	DIFFERENTIAL WBC (%)	PLATELET COUNT (/MM^3)	LDH (IU/L)	DIRECT COOMBS	SERUM ELECTROLYTES (MMOLE/L)	BUN/CR (MG/DL)	LFT (U/L)
Day 1	7	3.4	32710	N 78 L 12 M 4 Band 2 Myelo 2 Blast 2	493000	1682	Positive	Na 132 K 3.91 Cl 103 CO_2 18.6	23/0.66	ALP 234 AST 104 ALT 14 γGT 23
Day 2	6.2	4.7	20450	N 79 L 14 M 6 B 1	446000			Na 139 K 4.87 C 111 CO_2 19.9	17/0.52	
Day 3	7.6	4.3	20939	N 79 L 14 M 7	458000	1681				
Day 4	7.5	6	17140	N 72 L 24 M 3 Myelo 1	474000	1100		Na 139 K 3.59 Cl 108 CO_2 22.9	12/0.46	ALP 166 AST 25 ALT 16 γGT 24
Day 14	12.2	5.7	11860	N 84 L 11 M 5	499000	282	Weakly positive			
Day 35	14.1	1.9	11600	N 75 L 20 M 4 E 1	402000	285				
Day 77	14.1	1.1	6500	N 30 L 51 M 11 E 4 B 1 Atyp L 3	343000	288	Negative			

Abbreviations: γGT, gamma glutamyl transferase; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; Atyp L, atypical lymphocyte; B, basophil; BUN, blood urea nitrogen; Cl, chloride; CO_2, carbondioxide; Cr, creatinine; E, eosinophil; g/dL, gram per decilter; Hb, haemoglobin; IU/L, international unit per liter; K, potassium; L, lymphocyte; LDH, lactate dehydrogenase; LFT, liver function test; mg/dL, milligram per decilitre; mm^3, cubic millimetre; M, monocyte; Myelo, myelocyte; N, neutrophil; Na, sodium; U/L, unit per liter; WBC, white blood count. Day1 represents the day of admission.

the diagnosis of recent EV71 infection in this case. In our case, we then postulated that AIHA was triggered by the EV71 infection according to this temporal relationship.

In general, EV71 is an important pathogen of hand foot and mouth disease and herpangina. The common complication of EV71 is aseptic meningitis, poliomyelitis-like paralysis, brainstem encephalitis, autonomic dysregulation and pulmonary edema.[27] However, its role as a cause of hematologic complications is rare. There was a report of hemolytic-uremic syndrome associated with enterovirus infection,[28] while a few cases of enterovirus infection induced hemolytic anemia have been reported in the literature. A review of literature reporting a total case of 265 children with AIHA showed that 10% of cases were post-infectious and only one case was from enterovirus.[7] However, detailed descriptions of the case were not available. Marxgut et al reported another case of a girl presenting with enterovirus-related AIHA[16] but the detailed serotype of the etiologic virus and type of AIHA were not mentioned. In addition, this reported case was suspected to have underlying primary immune deficiency which was cured by bone marrow transplantation. To the best of our knowledge, our case is the first case of AIHA following definite EV71 infection.

Although the mechanism of EV71 infection induced AIHA remains unclear, the production of autoantibodies recognizing erythrocyte antigen has been considered. It is postulated that virus can modify host immune responses such as increasing macrophage ability to phagocytose antibody-coated erythrocytes leading to hemolysis, enhancing the pathogenicity of preexisting autoantibodies,[29] and exacerbating erythrophagocytosis through macrophage activation by gamma-interferon.[30]

The management of infection associated AIHA was based on the etiologies and severity of anemia. Most patients can be managed supportively with appropriate antimicrobials and transfusion for symptomatic anemia. Some patients received corticosteroid for severe persistent hemolysis.[31] In a review of 26 pediatric cases of AIHA,[1] 81% of patients responded well to oral corticosteroid therapy. Currently, monoclonal antibodies

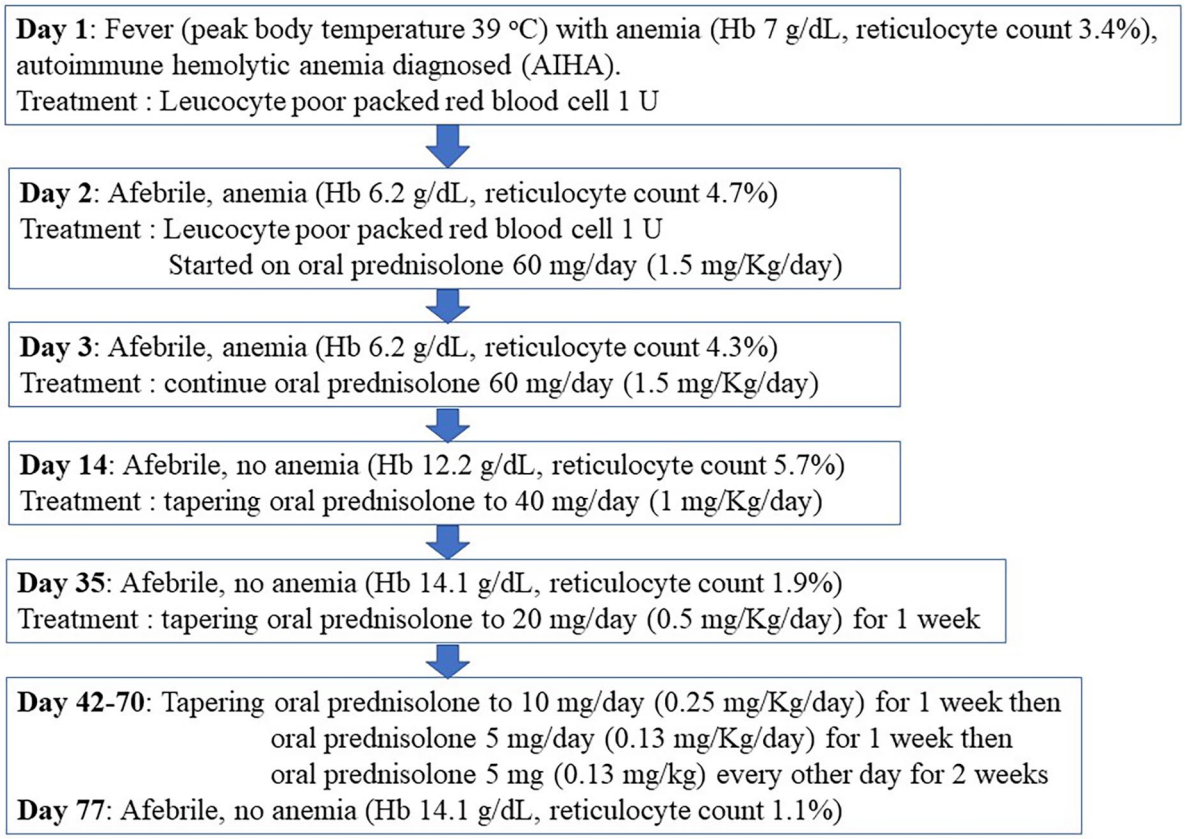

Figure 2. Clinical course of the patient. The diagram demonstrates the symptoms of the patients, hemoglobin level and reticulocyte count correlating with the treatment.
Abbreviations: AIHA, autoimmune hemolytic anemia; dL, deciliter; Hb, hemoglobin; Kg, kilogram; mg, milligram; U, unit.

targeting specific effector cells or molecules responsible for AIHA are being developed[32] and successful treatment with these monoclonal antibodies have been increasingly reported.[33-36] For example, complement can be responsible for opsonization of red blood cells leading to phagocytosis of these cells, complement inhibition with eculizumab was reported to be effective in treatment of PCH in one case report.[35] Although PCH was the most likely diagnosis in this patient and corticosteroid efficacy in PCH is uncertain,[37] this patient received prednisone upon AIHA diagnosis because the type of AIHA could not be determined initially and the patient had very low hemoglobin level and anemic symptom. Steroid could be considered in cases with severe PCH.[21] The patient rapidly recovered upon treatment though PCH may also spontaneously recover with only supportive treatment.[21,24] The patient received 10 weeks of prednisolone without adverse events.

In conclusion, this reported case demonstrate the association of EV71 infection and AIHA in a previously healthy young child. EV71 infection was confirmed by microneutralization test. Apart from known neurological and cardiopulmonary complications, EV71 infection can be associated with AIHA. EV71 should be considered as another possible cause of virus-induced AIHA in any patients presenting with hemolytic anemia, especially during the season of EV71 outbreak.

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Author Contributions

PP and NA drafted the manuscript.
PP, NS and NA took care of the patient.
BT, TP and PK provided the laboratory data.

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Supplemental Material

Supplemental material for this article is available online.

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