Cytomegalovirus-Associated Severe Direct Antiglobulin Test Negative Hemolytic Anemia: A Case Report

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Cytomegalovirus is a common virus that is mostly asymptomatic when infected, but rarely causes life-threatening hemolysis especially in immunocompromised children. We report a case of antiglobulin test negative severe hemolytic anemia caused by cytomegalovirus infection developed in an immune competent 9-year-old girl. The patient’s hemoglobin level was 4.8 g/dL on the day of admission. The diagnosis was achieved by exclusion of other causes of hemolytic anemia and serological evidence of recent CMV infection. The patient was successfully treated with anti-viral agents and steroids resulting in recovery from anemia. Clinicians should consider cytomegalovirus infection in the differential diagnosis of hemolytic anemia in pediatric patients.

Key Words: Hemolytic anemia, Cytomegalovirus

Introduction

Autoimmune hemolytic anemia (AIHA) is rarely observed in infants and young children. Most of the cases are associated with viral or bacterial infection, but the immunologic events leading to hemolysis are poorly understood [1-3]. Cytomegalovirus (CMV) is a common viral agent responsible for a wide range of clinical manifestations that vary according to the immunologic status of the patient with a compromised immune system in adults and children. Most immune competent hosts infected present no symptoms, whereas mild to moderate symptoms, such as prolonged fever, lymphadenopathy and arthralgia may also occur, a syndrome looking a lot alike to infectious mononucleosis [4]. In immune compromised patient, CMV can cause severe disease either via reactivation of latent CMV infection or via acquisition of primary CMV infection, Clinical syndromes that may be observed in this setting include encephalitis, pneumonitis, hepatitis, uveitis, retinitis, colitis, and...
graft rejection [4]. Severe hemolysis is a rare, but potentially life threatening complication of CMV infection, seen mostly in immune compromised adults and children [3,5]. However, hemolytic anemia due to acute CMV infection was previously reported in an immune competent adult [6,7] and immune competent infant [3,8,9]. We describe here a case of antiglobulin test negative severe hemolytic anemia developed in an immune competent 9-year-old girl with acute CMV infection who was treated successfully with steroids and ganciclovir.

Case Report

The patient was a 9 year old girl, with no significant past medical history. She arrived in the outpatient clinic complaining of pale appearance and fever. One week earlier, she had a cough, rhinorrhea, sore throat, and dizziness. She had a fever five days earlier and was treated with oseltamivir under suspicion of influenza. The fever persisted and she looked pale, she was transferred to our hospital. Her blood pressure was 109/72 mmHg, her heart rate was 121 times/min, her respiratory rate was 20 times/min, and her body temperature was 36.6°C. During a physical exam, her conjunctiva was anemic and there were no oropharyngeal lesions, heart murmurs, hepatosplenomegaly, rashes or joint abnormalities. The blood examination showed hemolytic anemia: red blood cells (RBCs), 1,570,000/μL; hemoglobin (Hb), 4.8 g/dL; hematocrit, 15%; mean corpuscular volume (MCV), 98 fL, mean corpuscular hemoglobin (MCH), 30 pg; mean corpuscular hemoglobin concentration (MCHC), 31 g/dL; red blood cell distribution (RDW), 19.8%; reticulocyte count, 21.41%; corrected reticulocyte 7.13%; white blood cell (WBC), 8,280/μL; platelet, 541K; lactate dehydrogenase (LDH), 651 U/L; total bilirubin, 2.41 mg/dL; direct bilirubin, 0.74 mg/dL; and undetectable serum haptoglobin. A peripheral blood smear (PBS) showed normocytic normochromic anemia, anisocytosis, poikilocytosis. The direct antiglobulin test using polyspecific antibodies against human IgG and complement C3 was negative, and repeat testing confirmed the negative result, although was mildly positive for cold agglutinins (1:4). No specificity, including anti-I or anti-i, could be detected. Infection with Mycoplasma pneumoniae, which is a well known cause of cold agglutinins, was also excluded. Other test showed alanine aminotransferase, 40 U/L; aspartate aminotransferase, 32 U/L; alkaline phosphatase, 129 U/L; C-reactive protein (CRP), 0.7 mg/L (reference value: 0.5 mg/dL), erythrocyte sedimentation rate, 8 mm/h.

Serologic and virologic examination showed CMV IgM, Positive; CMV antigenemia, positive (3/200,000); CMV urine culture, positive; CMV viremia, positive (731 copies/mL); parvovirus blood polymerase chain reaction, negative; Epstein-barr virus viral capsid antigens IgM, negative.

Noninfectious cause of hemolytic anemia, including hemoglobinopathies, drug toxicity, autoimmune diseases, and malignancies, were excluded. An abdominal ultrasonography was negative for solid tumors, revealing only splenomegaly 12.5 cm (upper limit 11 cm). Osmotic fragility was not increased and hemoglobin electrophoresis pattern was within the reference range. Glucose-6-phosphate dehydrogenase activity was 20.6 Ug/Hb (reference value: >10 Ug/Hb). Pyruvic acid level was 0.09 mmol/L (reference value: 0.034-0.014 mmol/L). Ceruloplasmin level was also in a normal range.

The Hb level was 4.2 g/dL at day two and packed red blood cell (PRC) transfusion (3 cc/kg) was prescribed. At day 3, Hb level was 5.7 g/dL. The patient had no fever, dyspnea, or dizziness and an echocardiogram showed that the heart function was normal. The Hb level was decreased 4.9 g/dL at day four and another PRC transfusion (3 cc/kg) was prescribed. At day five on admission anti-viral treatment with ganciclovir, 900 mg/day was administered to the patient because hemolytic anemia caused by cytomegalovirus infection could not be ruled out. Steroid therapy with methylprednisolone, 1 mg/kg/day intravenous was also done. The Hb levels were ranging between 5 and 6 g/dL. The patient no longer received blood transfusions. But at day 10, Hb levels began to rise to 6.9 g/dL (Fig. 1).

At discharge (day 15) blood examination showed RBCs, 2,620,000/μL; Hb, 9.0 g/dL; reticulocyte count, 22.21% corrected reticulocyte, 14.31%; total bilirubin, 1.40 mg/dL; CMV viremia was negative (<100 copies/mL). Treatment with 900 mg/qd oral ganciclovir, and oral prednisone, 1 mg/kg/day (30 mg), was continued.
At day 22, the patient was asymptomatic with a hemoglobin level of 10.7 g/dL (corrected reticulocyte, 12.32%). Steroid was tapered to 25 mg for 3 days, followed by 10 mg for 3 days, followed by 5 mg for 3 days, and then stopped.

At day 39, hemoglobin level was 11.0 g/dL and corrected reticulocyte count was 5.33%, CMV viremia was negative (<100 copies/mL). Ganciclovir was stopped (Fig. 1).

**Discussion**

This is an uncommon case of severe hemolytic anemia during primary CMV in an immune competent child. An interesting finding in our case was the negativity of the anti-globulin test. A positive antiglobulin test could have helped in identifying an autoimmune mechanism, thus making the patient eligible for early steroid therapy. However, the presence of an underlying autoimmune mechanism could not be ruled out; based only on the negativity of antiglobulin test. The literature provides evidence of the onset of hemolysis in patients with negative antiglobulin test during CMV infection [6,10]. In our specific case, the presence of cold agglutinins may be a possible explanation for the onset of hemolysis [11]. There were five direct antiglobulin test (DAT) negative transfusion requiring hemolytic anemia adult patients after CMV infection [6,10,12-14]. Interestingly, two patients were identical to our case with mild elevation

![Graph A](image1.png)  
**Fig. 1.** Changes of laboratory data according clinical course. (A) The changes of hemoglobin and hematocrit during admission and follow up. Packed red blood cell (PRC) transfusion was prescribed at day 2 and day 4. (B) The changes of total bilirubin and lactate dehydrogenase (LDH) during hospitalization.
Table 1. Hemolytic anemia during acute cytomegalovirus infection in immune competent child: data from the literature

| Age of patient | Antiglobulin test | BT | Steroids | IVIG | CMV immune globulin | Ganciclovir | Rituximab | Outcome | Reference |
|----------------|------------------|----|----------|------|---------------------|-------------|-----------|---------|-----------|
| Case 1: 11 mo  | Case 1: Pos      | Yes| Yes      | Yes  | Yes                 | No          | No        | Cured   | [3]       |
| Case 2: 4 mo   | Case 2: Pos      | Yes| Yes      | Yes  | Yes                 | No          | No        | Cured   | [8]       |
| 4 mo           | Pos              | Yes| Yes      | Yes  | No                  | Yes         | Yes       | Died    | [9]       |
| 6 mo           | Pos              | No | No       | Yes  | No                  | No          | No        | Cured   |           |

BT, blood transfusion.

of cold agglutinin [6,10]. One case was all negative serology test [12]. The other case have initial negative DAT, but positive conversion of DAT in recovery period [13]. The final one have negative IgG DAT, but positive IgA DAT [14]. After CMV infection, we think in some cases, the cause of hemolysis may be not antibodies against human IgG and complement C3d, but hemolysis by cold agglutinins via complement activation. Hemolysis by cold agglutinins via complement activation could be mechanism explaining hemolysis in negative DAT patient. The production of cold agglutinin after CMV infection is rarely known about. The cold agglutinin disease after infection like as Mycoplasma pneumonia or Epstein-Barr virus are usually transient. Cold Agglutinin Disease is characterized by an auto-antibody which is not IgG but IgM has the capability to agglutinate red blood cells subsequently to induce complement activation, as a consequence of which there is lysis of red blood cells [15]. The DAT is positive for C3 only. We could not detect antibodies against C3d because the production of autoantibody is transient and some patients (2-10%) could not have antibodies against for C3d. The typical treatments for AIHA are less effective in cold agglutinin disease (i.e. corticosteroids, and splenectomy). It is stated that corticosteroid therapy is sometimes necessary for the treatment of marked anemia due to decrease production of autoantibody [16].

We performed a review of the literature by PubMed for relevant articles regarding hemolytic anemia during acute CMV infection in apparently immunocompetent infant and children, published between 1990 and 2017. Only 4 cases have been reported [3,8,9,10]. Among the four cases reported in the literature, the antiglobulin test was positive in all cases (Table 1). Regarding therapeutic management, two patients were treated with steroids, intravenous immunoglobulin (IVIG) therapy (and were dependent on transfusion) but were unresponsive and ultimately treated with intravenous CMV immune globulin, with subsequent improvement [3]. One six month old infant was treated with steroids and IVIG therapy (He also had no blood transfusions) and recovered completely [8]. The other, a four month old infant was treated with steroids, ganciclovir and rituximab (and was dependent on transfusion) succumbed to death [9].

As shown in previous reports, no conclusive statements regarding specific treatment of hemolytic anemia during acute CMV infection in immune competent patients can be made. Steroid, anti-globulin and ganciclovir treatment have been used for treatment of patients with severe CMV induced hemolysis.

Randomized controlled trials are needed for conclusive answers regarding the specific treatment of hemolytic anemia due to CMV infection.

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