Author’s response to reviews

Title: Parvovirus B19 infection presenting with severe erythroid aplastic crisis during pregnancy in a woman with autoimmune hemolytic anemia and alpha-thalassemia trait: a case report

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Version: 3
Date: 17 December 2014

Author's response to reviews: see over
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**Version:** 1  **Date:** 18 December 2014

**Author's response to reviews:** see over
Reviewer's report

Title: Parvovirus B19 infection presenting with severe erythroid aplastic crisis during pregnancy in a woman with autoimmune hemolytic anemia and alpha-thalassemia trait: a case report

Version: 2 Date: 19 November 2014

Reviewer: Michinori Mayama

Reviewer's report:

General:
This is an interesting article referring to a rare case of transient aplastic crisis caused by Parvovirus B19 infection in thalassemia trait complicated by autoimmune hemolytic anemia in a pregnant patient. The case is clearly described and the discussion is relevant and clear. The prevention of fetal hydrops caused by Parvovirus B19 is really important in a pregnant case, and this article can give a useful experience of IVIG treatment for the management of Parvovirus B19 infection in a pregnant woman.

Specific comments:

1. If you conducted polymerase chain reaction for Parvovirus B19 after IVIG treatments, you should show the result to support efficacy of IVIG treatment.

Answer:
We are sorry to report that we did not conduct polymerase chain reaction for Parvovirus B19 after IVIG treatments. But we believe that IVIG was very effective in eradication of the virus as the cause of the patient’s transient aplastic crisis since the percentage of her peripheral blood reticulocytes recovered dramatically one week after IVIG therapy.

2. Cold hemagglutinin titer was relatively low for hemolysis on the admission. Did this patient show the signs of hemolysis after her
reticulocyte counts recovered.  
Was it reasonable for such blood transfusion dependent anemia?

Answer:
The patient did have hemolysis after her reticulocytes recovered because we could still observe slightly elevated serum indirect bilirubin level and rapid fall of hemoglobin levels after erythrocytes transfusion as shown in Figure 10 of our manuscript. The cold type AIHA was only partially controlled by high dose dexamethasone. We have to confess that the patient’s cold hemagglutinin titer was not very high at admission as you point out. This might be due to a technique error since the cold antibodies are most reactive in low temperatures and our laboratory condition probably did not meet this strict requirement very well when we examined cold hemagglutinin titer. However, the patient’s cold antibody was found to be strongly positive in both direct and indirect antiglobulin tests. On the other hand, the reactivity of cold agglutinins, not the titer of them, most actually predicts the severity of hemolysis according to Leslie Silberstein and Melody Cunningham (page 383 in Chirstopher Hillyer et al: Blood Banking and Transfusioin Medicine, Basic Principles & Practice, Churchill Livinstone, 2003). We like to modify our manuscript and emphasize the extraordinarily strong reactivity of cold antibodies in antiglobulin tests. Thank you for your raising this important question which somehow has also puzzled us also.

The change of manuscript regarding this point in red color:

*** Case presentation section. Now it reads “Both direct and indirect antiglobulin tests were strongly positive.”

*** Case presentation section. Now it reads “Although her peripheral blood reticulocyte percentage increased dramatically after IVIG treatment (Figure 9), her hemolytic anemia did not improve much.”
Reviewer's report

Title: Parvovirus B19 infection presenting with severe erythroid aplastic crisis during pregnancy in a woman with autoimmune hemolytic anemia and alpha-thalassemia trait: a case report

Version: 2 Date: 5 December 2014

Reviewer: Amita Jain

Reviewer's report:

General:
The topic of the case report is quite important and Parvovirus B19 infection should be suspected in every case of transient aplasia. However, certain questions need to be answered before consideration for publication:

Specific comments:

1. How was the diagnosis of Parvovirus B19 infection made?

   Answer:
   The diagnosis of Parvovirus B19 infection was made from a combination of the following findings: typical giant erythroblasts in bone marrow smear, typical intranuclear viral inclusions within erythroblasts in bone marrow biopsy, positive nuclear immunohistochemical staining of Parvovirus B19 antigen in erythroblasts and positive PCR results for Parvovirus B19 in specimens from bone marrow, plasma, and nasal cavity.

2. Was EBV infection excluded in the patient? EBV has been associated with both transient aplasia and cold antibody autoimmune hemolytic anemia. Additionally, a high incidence of protective antibodies in adults makes Parvovirus B19 a less frequent cause of aplasia in this age group.

   Answer:
Serum antibodies for EB virus were not checked in the patient so EBV infection could not be totally excluded. But as we have shown in our manuscript and mentioned above, the evidence of Parvovirus B19 infection in our patient is so persuasive that we feel hard to consider EBV as the main cause of her disease. Besides, the patient did not present symptoms and signs of infectious mononucleosis or lymphoproliferative disorders with which most cases of EBV associated AIHA were reported. Nonetheless, your opinions are welcome and we will add this point in our discussion to make the manuscript perfect.

The change of manuscript regarding this point in red color:

*** Case presentation section. Now it reads “Antibodies against Epstein-Barr virus (EBV) were not checked because the patient did not present symptoms and signs of infectious mononucleosis or lymphoproliferative disorders with which most cases of EBV associated hemolytic anemia were reported.”


3. How can the authors say that the patient acquired B19V infection during second trimester of pregnancy? Was B19V infection checked earlier?

Answer:
It is true that we didn’t check B19V infection earlier and persistent infections may be observed in immunocompromised patients unable to produce neutralizing antibodies and to clear the virus, leading to chronic carriage of B19V. So far as we know, our patient seemed to be an immunocompetent healthy woman before she got pregnant. Studies in the past showed
that the virus is transmitted to the human host by inhalation of virus containing aerosol droplets. Viremia occurs one week after exposure, leading to infection of cells through the P antigen or globoside expressed on erythroid cells and on other cells (Clinica Chimica Acta 2006;372:14–23). The nadir of reticulocyte levels in patients with transient aplastic crisis appeared 10 days after infection (N Engl J Med 2004;350:586-597). That’s why we believe our patient got B19V infection about one to two weeks earlier before her aplastic crisis appeared in her second trimester of pregnancy despite that B19V infection was only checked after presentation of her disease. However, even though the immune response is able to clear infection in healthy individuals and to provide lifelong protection against B19V, persistence of infection in the bone marrow has been reported in immunocompetent individuals with or without symptoms, and recently, persisting low levels of B19V DNA has been evidenced in the blood of immunocompetent individuals several years after primary infection (J Virol 2010;84:9658–9665). Since this probability can’t be exclusively ruled out, we like to adjust our description based on this possibility in our manuscript. Thank you very much for reminding us of that.

The change of manuscript regarding this point in red color:

*** Abstract section. Now it reads “We present here a female with autoimmune hemolytic anemia who got parvovirus B19 induced transient aplastic crisis during her second-trimester-pregnancy and faced the high risk of both fetal and maternal complications related to this specific viral infection.”

*** Discussion section. Now it reads “Whether our patient’s parvovirus B19 disease in her second trimester pregnancy is a fresh one or a reactivation of persisting previous infection cannot be surely answered since persistence of infection in the bone marrow has been reported in immunocompetent individuals several years after primary infection [3].”
Also changed are:

Reference 3 is replaced with a new one. The order of reference 4 to 6 has been rearranged. Red “[ ]” brackets mark where reference numbers changed in Discussion section on page 5 and 6.