

# Human parvovirus B19 and Epstein-Barr virus Co-infection in a child with hereditary spherocytosis

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**Abstract. – Background:** In patients with chronic congenital haemolytic disorders, human Parvovirus B19 (HPV B19) is frequently involved in pure red-cell aplastic crises. Furthermore, it may inhibit three-lineage haematopoiesis in the bone marrow, causing severe pancytopenia. In such patients, Epstein Barr virus (EBV) infection also seems to share the same mechanism as HPV B19 in inducing bone marrow aplasia, but at present the clinical effect of an infection sustained by both viruses is unknown.

**Clinical Report:** We present a 7-year-old boy affected by hereditary spherocytosis (HS) who suffered from transient aplastic crisis, in whom laboratory findings revealed a double HPV B19 and EBV infection.

**Conclusions:** To our knowledge, this is the first report of a case of HPV B19 and EBV co-infection diagnosis in a paediatric patient. Despite underlying HS, no signs of haemolytic anaemia were detected, but the infection only produced transient pancytopenia. Nevertheless, the reason why there was no additive effect of the two viruses on the aplastic crisis is still unclear.

*Key Words:*

Hereditary Spherocytosis, Human Parvovirus B19, Epstein Barr virus, Children.

## Introduction

Human Parvovirus (HPV) B19 is known to determine a wide range of clinical presentations<sup>1,2</sup>.

Human erythroid progenitor cells are a direct target for the virus: its cytopathic effect is due to its binding to the P antigen on the erythroid cell membrane<sup>3,4</sup>. In patients with chronic congenital haemolytic disorders, HPV B19 is frequently in-

involved in pure red-cell aplastic crises, in consideration of virus-induced destruction of red cell precursors: this form of aplasia usually lasts for 7-10 days, and in normal individuals can go by unnoticed<sup>5,6</sup>. Furthermore, in immune-compromised patients, as well as in patients with haematological disease, the virus can persist and inhibits three-lineage haematopoiesis, causing continuous bone marrow suppression<sup>7-10</sup>.

As HPV B19 infection, Epstein-Barr virus (EBV) infection may lead to bone marrow failure, mainly in susceptible patients, but the pathogenesis of this phenomenon is still unknown, being probably connected to molecular mimicry<sup>11</sup>. In our report we describe a young boy affected by hereditary spherocytosis (HS), developing a eight-day pancytopenia and bone marrow failure after a rare HPV B19 and EBV co-infection. Since the episode was self-limiting and the aplasia had a short duration, the patient benefited from supportive therapy only.

## Case Report

A 7-year-old boy affected by HS, diagnosed at our Paediatric Oncology Division, was admitted to the Emergency Department on account of the following full blood count: haemoglobin 2.5 g/dl, platelets count 42.000/mmc, white cells count 1290/mmc, (lymphocytes 800/mmc, neutrophils 320/mmc, monocytes 170/mmc) and reticulocytes 0.8%. Liver function studies showed increased serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT). Other laboratory values were as follows: serum ferritin 178 ng/ml, triglyceride 85 mg/dl, serum fibrinogen 340 mg/dl, C-reactive protein 120 mg/l. Though lactate dehydrogenase was elevated, other laboratory features of haemoly-

sis were absent. Serum levels of creatinine, albumin, urea nitrogen, prothrombin time and activated partial thromboplastin time were inside the normal range. All laboratory data have been reassumed in Table I. Neither hematuria nor faecal occult blood was found. Normal levels of both total and subclass serum immunoglobulins were found. Lymphocyte-subset evaluation demonstrated a low CD4+/CD8+ cell ratio (0.20) with prevalence of activated CD8+ lymphocytes, suggesting a viral infection. On the peripheral blood smear, atypical activated lymphocytes were present. There were no auto-antibodies and levels of circulating immuno-complexes were inside the normal range.

During the three days before admission, the patient had a high body temperature (BT 39°C), vomiting and worsening fatigue. Physical examination showed pale-greyish skin, but no jaundice or skin rash was detected. The patient had no evidence of pharyngitis or cervical adenopathy. Tachycardia, systolic murmur and polypnoea were also documented. Finally, spleen was palpable five centimetres below the costal margin, whereas hepatomegaly was not appreciable. Abdominal ultrasounds confirmed spleen enlargement (maximum spleen diameter: 16 cm), and that did not show any volume increase compared with previous test. Echocardiography and chest X-ray were normal.

Based on his clinical findings and blood tests, a viral infection was supposed and serologies for Leishmania, Measles, Rubella, Herpes Simplex Virus, Paramyxovirus, HIV, EBV, HPV B19, Cytomegalovirus (CMV), Toxoplasma, Varicella-Zoster Virus were performed.

On serological tests, negative serum IgG and positive anti-HPV-B19 IgM (80.28 UA) were detected (cut off value: 10 UA). At the same time, anti-VCA IgM levels were 90 UA (cut off: 20 UA), whereas serology for anti-VCA and anti-EBNA IgG was negative.

The patient received a packed red cell transfusion and empirical antibiotic therapy with amoxicillin-clavulanic-acid combination. Body temperature decreased on the third day of hospitalization. On the fifth day the patient has been discharged in good clinical conditions. Laboratory investigations showed a reduction of both amino-transferase levels (ST 175U/L ALT 29U/L) and a partial recovery of blood count, as documented by haemoglobin 8.2 g/dL, white cell count 2180/mm<sup>3</sup> (neutrophils 870/mm<sup>3</sup>, lymphocytes 1000/mm<sup>3</sup>), platelets count

Table I. Summary of patient's laboratory data.

	Hb (g/dl)	Reticulocytes (%)	Haptoglobin (mg/dl)	LDH (U/L)	AST (U/L)	ALT (U/L)	Ferritin (ng/ml)	CRP (mg/L)	Bilirubin (mg/dl)
Before aplastic crisis	10.7	3.1	240	580	27	30	64	0.7	0.40
During aplastic crisis (admission)	2.5	0.8	211	1254	194	280	108	120	0.70
5 days after aplastic crisis	8.2	3.7	220	874	29	175	84	27	0.64
8 days after aplastic crisis	9.8	5.3	218	862	31	43	82	1.6	0.60
1 month after aplastic crisis	11	2.8	232	624	25	28	62	1.2	0.42
2 months after aplastic crisis	10.8	3.2	235	587	26	30	63	0.8	0.40

Hb: haemoglobin; LDH: lactate dehydrogenase; AST: aspartate amino-transferase; ALT: alanine amino-transferase; CRP: C-reactive protein.

112,000/mmc. Three days later the patient came back to our Department to be reevaluated. As observed on laboratory tests, amino-transferase levels returned inside the normal range and a full blood count showed: haemoglobin 9.8 g/dL, white cell count 3630/mmc (neutrophils 1940/mmc, lymphocytes 1400/mmc, monocytes 290/mmc), platelets 232,000/mmc, and the reticulocyte count rose to 5.3%. Abdominal ultrasounds showed persistence of mild spleen enlargement. On repeating serologic tests, the diagnosis of acute HPV B19 and EBV co-infection was confirmed by sero-conversion of HPV B19 antibody titers (IgG: 30 UA, IgM 40 UA) and detection of EBV viral genome on Polymerase Chain Reaction (PCR), with a doubling of anti-VCA IgM titers to 160 UA.

The patient did not require any more transfusions and haemoglobin levels finally returned to their steady state levels of 11 g/dl a month after the admission. Complete blood cells count trend is shown in Figure 1.

The follow-up monitoring for the next 10 months documented a progressive increase of anti-VCA IgG titers (with a peak at the sixth month after the onset of symptoms, reaching a six-fold increase of antibody titers), a gradual lowering of

IgM levels and appearance of anti-EBNA IgG about six weeks after the earliest serum assay, which was all confirmed by later serology testing. At present, no further episodes of pancytopenia were documented.

### Discussion

In paediatric patients, episodes of co-infection with EBV or HPV B19 with other viruses have frequently been reported. Double infections or co-infections of EBV and CMV are shown to be occasional in children<sup>12</sup>, whereas episodes of infection conjunctly produced by HPV-B19 and HIV-1 are reported more often<sup>7,13,14</sup>. The only case of HPV B19 and EBV co-infection documented to date concerns a previously healthy adult without underlying disease, who developed severe bone marrow failure in the absence of clinical evidence of viral infections, and required bone marrow transplantation for persistent pancytopenia<sup>15</sup>. At our knowledge, we report on the first case of HPV B19 and concomitant EBV infection in a paediatric patient with haemolytic disorder as HS. Furthermore, in our report the

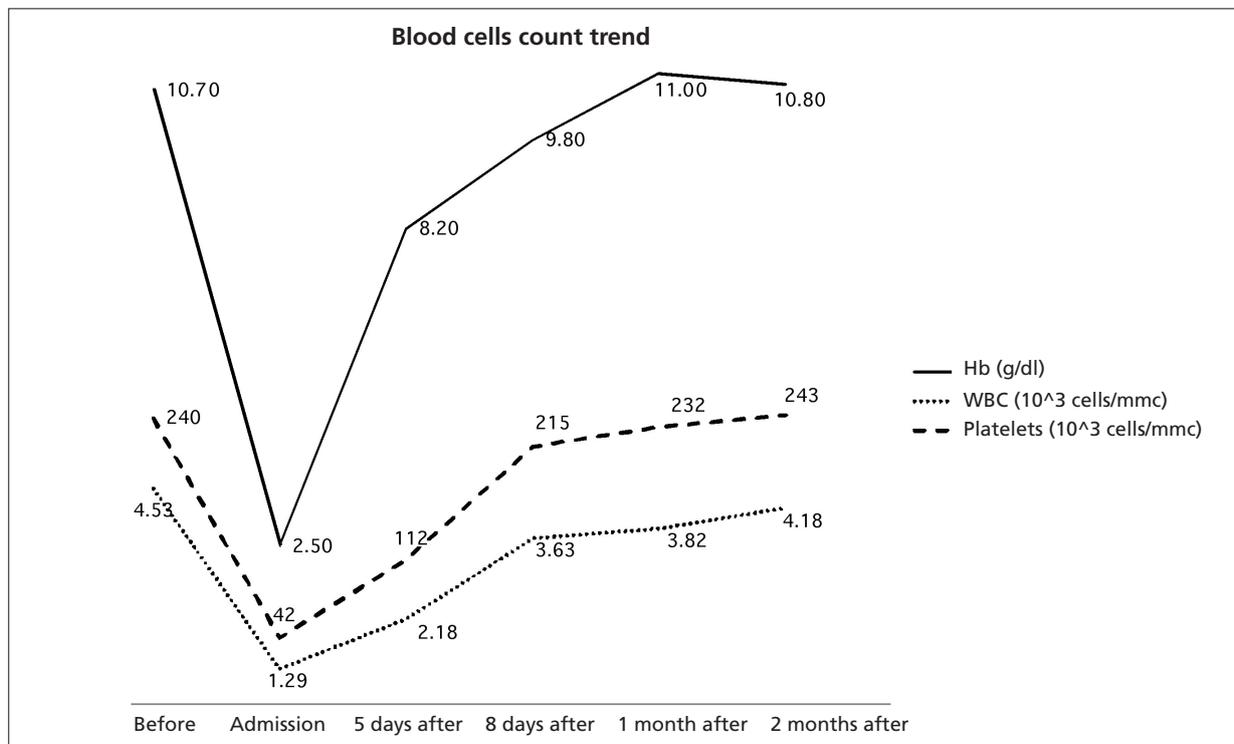


Figure 1. Summary of blood cells count trend.

patient suffered from transient aplastic crisis and obtained a prompt recovery with transfusional support only.

In the literature a wide range of clinical expressions due to HPV B19 or EBV infections is reported, ranging from asymptomatic infections to complete bone marrow suppression, in healthy patients as well as in patients with blood disorders<sup>16-21</sup>: especially in the latter group, several viral insults may result in complete bone marrow failure, as observed by Serjeant et al<sup>5</sup>.

In patients with erythrocyte membrane disease, such as HS, viral infection may indeed trigger haemolysis, causing a rapid fall of haemoglobin levels<sup>22</sup>. In our case, anaemia was secondary to the bone marrow failure and no sign of underlying haemolytic state being evident. Moreover, the reported case showed features of an atypical infection, for the complete absence – except for fever – of signs suggestive of a viral aetiology. The spleen enlargement was already a feature of this patient, due to his chronic haemolytic disease. In our opinion the absence of a skin rash, resulting from immune-complex deposits, was probably caused by EBV co-infection, that could induce a disordered antibody response to HPV B19. Aspecific increased amino-transferase levels were the only abnormality resulted from blood chemistry tests, possibly sustained by both HPV B19 and EBV infection.

Currently, viral infections are detected by serologic tests and viral genome retrieval. In our case, we documented the co-infection by means of serologic tests for both HPV B19 and EBV and through genome search for EBV alone. HPV B19 neutralising antibodies (IgM) appear after 10 to 12 days and may be found in serum samples for several months after exposure, whereas IgG are life-persisting and appear 2 weeks after inoculation<sup>23</sup>. Regarding EBV infection, anti-VCA IgM appear after 1-2 weeks from exposure persisting for 2-3 months, whereas life persisting anti-VCA IgG usually become detectable later. Finally, anti-EBNA IgG have a late onset and are a well-known marker of past exposure to virus<sup>24</sup>.

The specific role of viruses EBV and HPV B19 in the development of aplastic anaemia in this patient remains unclear; notwithstanding a double hit to the bone marrow, the self-limitation of aplasia excluded an additive effect of both viruses to the marrow injury. In fact, it is not even possible to establish which of the two viruses mainly induced transient bone marrow failure.

## Conclusions

To our knowledge, this is the first report described in the literature of a case of HPV B19 and EBV co-infection diagnosis in a paediatric patient. Despite underlying HS, no signs of haemolytic anaemia were detected, but the infection only produced transient pancytopenia. Nevertheless, at the present time, the reason why there was no additive effect of the two viruses on the aplastic crisis is still unclear.

## References

- 1) BROWN KE, YOUNG NS. Parvoviruses and bone marrow failure. *Stem Cells* 1996; 14: 151-163.
- 2) BARANSKI B, YOUNGH N. Hematologic consequences of viral infections. *Hematol Oncol Clin North Am* 1987; 1: 167-183.
- 3) BROWN KE, ANDERSON SM, YOUNG NS. Erythrocyte P antigen: cellular receptor for B19 parvovirus. *Science* 1993; 262: 114-117.
- 4) OZAWA K, KURTZMAN G, YOUNG N. Productive infection by B19 parvovirus of human erythroid bone marrow cells in vitro. *Blood* 1987; 70: 384-391.
- 5) SERJEANT BE, HAMBLETON IR, KERR S, KILTY CG, SERJEANT GR. Haematological response to parvovirus B19 infection in homozygous sickle-cell disease. *Lancet* 2001; 358(9295): 1779-1780.
- 6) KONDO Y, KAKAMI M, KAWAGUCHI H, MIYAKE F, URISU A, ASANO Y, KOJIMA S. Transient pancytopenia associated with parvovirus infection in a healthy child. *Pediatrics Int* 2002; 44: 695-697.
- 7) CROOK TW, ROGERS BB, MCFARLAND RD, KROFT SH, MURETTO P, HERNANDEZ JA, LATIMER MJ, MCKENNA RW. Unusual bone marrow manifestations of parvovirus B19 infection in immunocompromised patients. *Hum Pathol* 2000; 31: 161-168.
- 8) MYERS B, DOLAN G. Parvovirus-induced pancytopenia in a child with acquired haemolytic anaemia. *Clin Lab Haematol* 1997; 19: 277-278.
- 9) KELLEHER JF, LUBAN NLC, MORTIMER PP, KAMIMURA T. Human serum "parvovirus": a specific cause of aplastic crisis in children with hereditary spherocytosis. *J Pediatr* 1983; 102: 720-722.
- 10) YARALI N, DURU F, SIPAHI T, KARA A, TEZIC T. Parvovirus B19 infection reminiscent of myelodysplastic syndrome in three children with chronic hemolytic anemia. *Pediatr Hematol Oncol* 2000; 17: 475-482.
- 11) AHRONHEIM GA, AUGER F, JONCAS JH, GHIBU F, RIVARD GE, RAAB-TRAUB N. Primary infection by Epstein-Barr virus presenting with aplastic anaemia. *N Engl J Med* 1983; 309: 313-314.
- 12) KOBAYASHI S, MARUTA A, YAMAMOTO T, KATAYAMA N, HIGUCHI R, SAKANO Y, FUJITA H, KOHARAZAWA H, TOMITA N, TAGUCHI J, KODAMA F, NAKAMURA Y, SHIMIZU A.

- Human parvovirus B19 capsid antigen in granulocytes in parvovirus-B19-induced pancytopenia after bone marrow transplantation. *Acta Haematol* 1998; 100: 195-199.
- 13) SETÚBAL S, DE OLIVEIRA SA, PIRES AR, DA FONSECA EC, CAMACHO LA, SERODIO AC, DO NASCIMENTO JP. Erythrovirus B19 infection in acquired immunodeficiency syndrome: screening by histopathology, immunohistochemistry, and in situ hybridization. *Mem Inst Oswaldo Cruz* 2006; 101: 407-414.
  - 14) LAMONTE AC, PAUL ME, READ JS, FREDERICK MM, ERDMAN DD, HAN LL, ANDERSON LJ. Persistent parvovirus B19 infection without the development of chronic anemia in HIV-infected and -uninfected children: the Women and Infants Transmission Study. *J Infect Dis* 2004; 189: 847-851.
  - 15) KAPTAN K, BEYAN C, URAL AU, USTÜN C, CETIN T, AVCU F, KUBAR A, ALI M, YALÇIN A. Successful treatment of severe aplastic anaemia associated with human Parvovirus B19 and Epstein-Barr virus in healthy subject with allo-BMT. *Am J Hematol* 2001; 67: 252-255.
  - 16) YETGIN S, CETIN M, OZYUREK E, ASLAN D, UCKAN D. Parvovirus B19 infection associated with severe aplastic anaemia in an immunocompetent patient. *Pediatric Hematol Oncol* 2004; 21: 223-226.
  - 17) OSAKI M, MATSUBARA K, IWASAKI T, KURATA T, NIGAMI H, HARIGAYA H, BABA K. Severe aplastic anemia associated with human parvovirus B19 infection in a patient without underlying disease. *Ann Hematol* 1999; 78: 83-86.
  - 18) ANDERLINI P, RIGGS SA, KÖRBLING M, CHAMPLIN R. Syngeneic blood stem cell transplantation for infectious mononucleosis-related aplastic anaemia. *Br J Hematol* 1999; 106: 159-161.
  - 19) GUILLOT M, LEFRERE JJ, RAVENET N, LEVEQUE E, GIROT R. Acute anaemia and aplastic crisis without haemolysis in human parvovirus infection. *J Clin Pathol* 1987; 40: 1264-1265.
  - 20) McLELLAN NJ, RUTTER N. Hereditary spherocytosis in sisters unmasked by parvovirus infection. *J Am Board Fam Pract* 1987; 63: 49-50.
  - 21) SUZUKI Y, SHICHISHIMA T, MUKAE M, OHSAKA M, HAYAMA M, HORIE R, TOGANO T, MIYAZAKI K, ICHINOE M, IWABUCHI K, FUJII H, HIGASHIHARA M. Splenic infarction after Epstein-Barr virus infection in a patient with hereditary spherocytosis. *Int J Hematol* 2007; 85: 380-383.
  - 22) SKINNIDER LF, MCSHEFFREY BJ, SHERIDAN D, DENEER H. Congenital spherocytic hemolytic anemia in a family presenting with transient red cell aplasia from parvovirus B19 infection. *Am J Hematol* 1998; 58: 341-342.
  - 23) PETERLANA D, PUCCEITI A, CORROCHER R, LUNARDI C. Serologic and molecular detection of human Parvovirus B19 infection. *Clin Chim Acta* 2006; 372: 14-23.
  - 24) DEBYSER Z, REYNDEERS M, GOUBAU P, DESMYTER J. Comparative evaluation of three ELISA techniques and an indirect immunofluorescence assay for the serological diagnosis of Epstein-Barr virus infection. *Clin Diagn Virol* 1997; 8: 71-81.