Clinical features and laboratory findings of human parvovirus B19 in human immunodeficiency virus-infected patients

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Immunocompromised patients may develop severe chronic anaemia when infected by human parvovirus B19 (B19V). However, this is not the case in human immunodeficiency virus (HIV)-infected patients with good adherence to highly active antiretroviral treatment (HAART). In this study, we investigated the clinical evolution of five HIV-infected patients receiving HAART who had B19V infections confirmed by serum polymerase chain reaction. Four of the patients were infected with genotype 1a strains and the remaining patient was infected with a genotype 3b strain. Anaemia was detected in three of the patients, but all patients recovered without requiring immunoglobulin and/or blood transfusions. In all cases, the attending physicians did not suspect the B19V infections. There was no apparent relationship between the infecting genotype and the clinical course. In the HAART era, B19V infections in HIV-positive patients may be limited, subtle or unapparent.

Key words: HIV - human parvovirus B19 - seroconversion

Parvovirus B19 (B19V), a member of the genus *Erythrovirus* within the Parvoviridae family, has been grouped into three distinct genotypes: (i) genotype 1, with subtypes 1a (the prototypic virus) and 1b, (ii) genotype 2 (A6 and LaLi strains) and (iii) genotype 3, with subtypes 3a (V9 strains) and 3b (D91.1 strains) (Nguyen et al. 1999, 2002, Servant et al. 2002, Fauquet et al. 2005).

B19V primarily infects erythroid cells, leading to transient inhibition of erythropoiesis. In immunocompetent individuals, it usually causes an acute and self-limited childhood disease known as erythema infectiosum (EI) ("slapped cheek" rash or 5th disease). Adults with EI (particularly females) frequently present with joint symptoms. However, most patients are asymptomatic (Woolf et al. 1989). Immunocompromised patients who are unable to produce neutralising antibodies may develop severe chronic anaemia. In human immunodeficiency virus (HIV)-infected patients with residual immunity, the clinical manifestations, if any, are those of fifth disease (Frickhofen & Young 1990). With the advent of highly active antiretroviral treatment (HAART), several studies, including one from our group (de Azevedo et al. 2012), have shown a decrease in cases of anaemia caused by B19V (Mylonakis et al. 1999, Ware & Moore 2001).

To better understand the importance of B19V infection in the HAART era, a study to estimate the frequency of B19V seroconversion in a cohort of HIV-infected patients was conducted by our group during an eight-year period (2001-2008) at the Infectious Diseases Department of Antonio Pedro University Hospital (HUAP) at the Fluminense Federal University (Niterói, state of Rio de Janeiro, Brazil) (de Azevedo et al. 2009). Seroconversions were detected in approximately 30% (28 of 88) of our anti-B19 IgG-negative patients, a similar proportion to that found by others (Chernak et al. 1995, Mylonakis et al. 1999). Most seroconversions occurred during incidence peaks of a B19V infection in Niterói (Oliveira et al. 2005, de Azevedo et al. 2012). All sera from the 88 patients were tested by polymerase chain reactions (PCRs) and B19 DNA was detected in five of the patients, four of whom also exhibited seroconversion. This paper describes the clinical and laboratory findings of B19V infections in these five HIV-infected patients.

PATIENTS, MATERIALS AND METHODS

Data, such as haemoglobin (Hb) concentration, CD4+ T cell counts and B19V IgG and IgM serology, were retrieved from a previous study by our group (de Azevedo et al. 2012). ELISAs (Biotrin International™, Dublin, Ireland) were performed, according to the manufacturer's instructions, to detect IgG and IgM antibodies to B19 in all sera. The following definitions were used in this study: (i) anaemia was defined according to the World Health Organization (WHO 2001) criteria as a Hb concentration below 13 g/dL in men and below 12 g/dL in women and (ii) severe anaemia was defined as a Hb concentration below 7 g/dL.

PCRs were performed to detect and genotype B19V. Briefly, viral DNA was extracted from serum samples of 88 HIV-positive patients using a QIAamp DNA Blood Mini Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. For the screening of B19V DNA, PCR was performed using the primers E1905F (nt 1905-1923) and E1987R (nt 2007-1987), which amplify a 102-bp fragment of the NS1 gene as previously described by Nguyen et al. (2002), with some modifications (de Azevedo et al. 2012). This PCR can routinely detect as few as 20 copies of the B19V DNA (Nguyen et al. 2012).

To genotype the B19V strains detected in HIV-positive patients, a semi-nested PCR using the primer pairs P12/P16 (4127-4689) and P13/P16 (4214-4689) for partial amplification of the VP1/VP2 capsid gene was performed as described by Durigon et al. (1993). The 476-bp amplicons were purified using GFX™ PCR DNA and the Gel Band Purification kit (GE, Healthcare, UK) and were then subjected to direct sequencing using the BigDye terminator v. 1.1 cycle sequencing kit (Applied Biosystems, CA, USA) (Otto et al. 2008).

Sequences were retrieved and analysed by the Bio-Edit sequence alignment editor, v. 7.0.1 (mbio.ncsu.edu/BioEdit/bioedit.html) and they were compared with the following sequences available in GenBank (Hall 1999): genotype 1a: M24682-Wi (UK); genotype 1b: DQ35706 and DQ35705 (Ghana); genotype 2: AY06477-A6 (Denmark) and AY04416-LaLi; genotype 3a: NC0049-V9 (Italy); genotype 3b: Z68146 (UK), AF162273 (Italy), AY083234-D01.1 (France), DQ234778, DQ234779.2 (Ghana), EF089227, EF089229.1 (Brazil/PA) and AY647977.1 (Brazil/SP).

Ethics - Written informed consent was obtained from all volunteers and the project was approved by the Hospital Review Board (CMM/HUAP 134/05).

RESULTS

Case 1 - A male patient diagnosed with acquired immune deficiency syndrome (AIDS) in 1996 at 41 years of age, with poor adherence to HAART. The observation period started in December 2001 (CD4$^+$ T cell count: 158 cells/mm³; anti B19V IgG: negative). There was a gradual decrease in CD4$^+$ T cell counts, which reached 3 cells/mm³ in August 2004. In February 2005, the patient was hospitalised because of fever, hepatosplenomegaly, severe anaemia (Hb: 5.9 g/dL) and multiple opportunistic infections (oro-oesophageal candidiasis, perianal condylomata and herpes). The patient underwent a bone marrow biopsy (February 2005) and was started on empirical treatment for atypical mycobacteriosis. Clinical improvement was observed and the patient did well afterward, which was attributed to a better adherence to HAART (lamivudine, stavudine, efavirenz and lopinavir/ritonavir). The biopsy report described *"overall cellularity of 60%, with presence of the three series. The myeloid/erythroid ratio was 2:1, with predominance of immature forms, numerous myeloblasts and moderate increase in the number of red blood cells with megaloblastic maturation. Megakaryocytes were observed in the usual number, but with frequent atypical forms. There were rare erythroblasts with nuclear viral inclusions consistent with B19V. Conclusion: myelodysplastic changes secondary to HIV infection, compatible with B19V infection".* The patient received neither immunoglobulin therapy nor a blood transfusion. There was a gradual recovery from anaemia during 2005. In November 2005, stavudine was replaced by zidovudine to improve adherence. In December 2005, seroconversion (anti-B19V IgG-positive and anti-B19V IgM-positive) and the presence of B19V DNA (strain RJ 011/2005, genotype 1a) were both detected in association with an increase in the CD4$^+$ T cell count (34 cells/mm³) and resolution of anaemia (Hb: 13.9 g/dL) (Table I). A subsequent serum sample (June 2006) was negative for B19V DNA.

Case 2 - A female patient diagnosed with AIDS in 1997 at 54 years of age, when she was started on HAART with good adherence to therapy and immune recovery. The observation period began in December 2001 (CD4$^+$ T cell count: 598 cells/mm³). Seroconversion to B19V (anti-B19 IgG) was observed in June 2005. There is no record of blood counts during the period of seroconversion (November 2004-June 2005). During this period, there were no complaints or changes in physical examination suggestive of anaemia. A serum sample collected in June 2005 (strain RJ 026/2005, genotype 1a) was B19V DNA-positive by PCR. The next available blood sample (September 2005) remained B19V DNA-positive (Table II), but the subsequent sample (January 2006) was negative for B19V DNA.

Case 3 - A woman diagnosed with AIDS in 1996 at 36 years of age, when she was started on HAART, followed by immunological recovery. The observation period began in January 2002 (CD4$^+$ T count: 520 cells/mm³). B19V seroconversion (anti-B19 IgM and anti-B19 IgG) was observed in September 2005 (CD4$^+$ T count: 649 cells/mm³). At that time, there were no complaints or physical examination changes suggestive of anaemia. A serum sample collected in September 2005 (strain RJ 071/2005, genotype 3b) was B19V DNA-positive (Table III). Two subsequent samples collected in June 2006 and September 2006 were, respectively, positive and negative for B19V DNA.

Case 4 - A man diagnosed with AIDS in November 2003 at 33 years of age. At that time, he presented with mild anaemia (Hb = 12.8 g/dL) attributed to HIV infection. He began HAART in September 2004. The observation period started in November 2003 (CD4$^+$ T cell count: 215 cells/mm³). B19V seroconversion (anti-B19 IgG) was observed in April 2007 (CD4$^+$ T cell count: 504 cells/mm³). Hb levels below 13.0 g/dL, suggestive of mild anaemia, were observed from November 2003-July 2006, before the period of seroconversion (anti-B19 IgG) (July 2006-April 2007). A sample collected in July 2006 (strain RJ 350/2006, genotype 1a) was B19V DNA-positive (Table IV). A subsequent sample (April 2007) was negative for B19V DNA.

Case 5 - A man diagnosed with AIDS in November 2001 at 35 years of age. The observation period began in January 2002 (CD4$^+$ T cell count: 80 cells/mm³),

TABLE I
Laboratory data of Case 1

Month/year	Dec/2001	Mar/2002	Aug/2002	Jul/2003	Feb/2004	Aug/2004	Feb/2005	Mar/2005	Jul/2005	Sep/2005	Dec/2005
IgG anti-B19V	NEG	-	NEG	NEG	NEG	NEG	-	-	-	-	POS
IgM anti-B19V	NEG	-	NEG	NEG	NEG	NEG	-	-	-	-	POS
PCR-B19	-	-	-	-	-	NEG	-	-	-	-	POS
CD4$^+$ (cells/mm^3)	158	-	118	45	9	3	-	-	1	-	34
Hb (g/dL)	-	13.1	-	-	-	-	5.9	6.9	10	10.5	13.9

B19V: parvovirus B19; Hb: haemoglobin; NEG: negative; PCR: polymerase chain reaction; POS: positive; -: lack of data in the medical records.

TABLE II
Laboratory data of Case 2

Month/year	Dec/2001	May/2002	Oct/2002	Jun/2003	Oct/2003	Jul/2004	Nov/2004	Jun/2005	Sep/2005
IgG anti-B19V	NEG	-	NEG	NEG	NEG	NEG	NEG	POS	POS
IgM anti-B19V	NEG	-	NEG	NEG	NEG	NEG	NEG	NEG	NEG
PCR-B19	-	-	-	-	-	-	NEG	POS	POS
CD4$^+$ (cells/mm^3)	589	-	241	702	474	712	769	613	702
Hb (g/dL)	-	12.6	13.5	-	-	-	-	-	13

B19V: parvovirus B19; Hb: haemoglobin; NEG: negative; PCR: polymerase chain reaction; POS: positive; -: lack of data in the medical records.

TABLE III
Laboratory data of Case 3

Month/year	Jan/2002	Oct/2002	Dec/2002	Apr/2003	Sep/2003	May/2004	Aug/2004	Feb/2005	Sep/2005
IgG anti-B19V	NEG	NEG	-	-	-	-	NEG	NEG	POS
IgM anti-B19V	NEG	NEG	-	-	-	-	NEG	NEG	POS
PCR-B19	-	-	-	-	-	-	-	NEG	POS
CD4$^+$ (cells/mm^3)	520	681	-	-	-	-	483	610	649
Hb (g/dL)	-	-	13.7	13.2	13.9	13.1	-	-	-

B19V: parvovirus B19; Hb: haemoglobin; NEG: negative; PCR: polymerase chain reaction; POS: positive; -: lack of data in the medical records.

when HAART (zidovudine, lamivudine and efavirenz) was started and it ended in October 2005 (CD4$^+$ T cell count: 244 cells/mm³). The patient had several opportunistic infections and anaemia during the follow-up and a decrease in Hb level (5.9 g/dL) was observed in June 2002. In addition, CD4$^+$ T cell counts less than 100 cells/mm³ were observed until November 2002. As the first test for the presence of IgG antibodies was indeterminate or weakly positive, these samples were tested again and found to be negative. Nevertheless, B19 DNA was detected in one of the samples (November 2002, strain RJ 109/2002, genotype 1a). At this time, the Hb level was 9.14 g/dL and, thus, no longer compatible with severe anaemia (Table V). There is no record of a B19V outbreak during 2002/2003 (Oliveira et al. 2005).

According to sequence analysis of the VP1/VP2 capsid region, the strains RJ 011/2005, RJ 026/2005, RJ 109/2002 and RJ 350/2006 shared high nucleotide (nt) identity (100-99%) with 1a B19V isolates from other countries (EU78564, M2468, M13178, EU478541, EU14278 and JN211184). One strain (RJ 071/2005) displayed 99.3-97.2% nt identity with 3b B19V Brazilian isolates (EF089227 and EF089229). Based on their nt identity with other B19V isolates previously described, four of the B19V strains in this study were characterised as belonging to genotype 1a and one B19V strain was characterised as belonging to genotype 3b.

DISCUSSION

This is the first study to analyse clinical manifestations in HIV-infected patients during an outbreak of B19V in Brazil. Persistent B19 infection has been reported to be associated with HIV infection, possibly due to inability to mount a protective B19-specific antibody response (Chernak et al. 1995, van Elsacker-Niele et al. 1996) and some reports have shown that complete remission of B19-associated pure red cell aplasia can be achieved just by treating patients with HAART (Mylonakis et al. 1999, Ware & Moore 2001).

Our findings show that the impact of restoring the immune response by either starting or continuing HAART may explain the reduction and even the elimination, of severe haematological changes caused by B19V infections, even before a detectable increase in the number of CD4$^+$ T cells in the blood occurs (Cases 1, 4 and 5). Although anaemia had been detected in three patients (2 of whom had severe anaemia), all of the patients recovered and none required immunoglobulin and/or blood transfusions, including the two patients for whom no data existed in the medical records regarding the occurrence of anaemia. Additionally, our results also show that B19 infection may be apparent only in retrospective analyses, as there are many causes of anaemia in HIV-infected patients, including co-infection by mycobacteria, fungus or cytomegalovirus, adverse drug effects, lymphoma or

TABLE IV

Laboratory data of Case 4

Month/year	Nov/2003	Jan/2006	Jul/2006	Apr/2007	Jan/2008	Mar/2008	Jul/2008
IgG anti-B19V	NEG	NEG	NEG	POS	POS	POS	POS
IgM anti-B19V	NEG	NEG	NEG	NEG	NEG	NEG	NEG
PCR-B19	-	NEG	POS	NEG	-	-	-
CD4$^+$ (cells/mm³)	215	364	327	504	539	285	528
Hb (g/dL)	12.8	12.3	12.5	14.2	-	13.9	13.6

B19V: parvovirus B19; Hb: haemoglobin; NEG: negative; PCR: polymerase chain reaction; POS: positive; -: lack of data in the medical records.

TABLE V

Laboratory data of Case 5

Month/year	Jan/2002	Jun/2002	Nov/2002	Jun/2003	Jul/2004	May/2005	Oct/2005
Test 1 IgG anti-B19V	IND	NEG	+/-[a]	IND	NEG	IND	IND
Test 2 IgG antiB19V	NEG	NEG	NEG	NEG	NEG	NEG	NEG
Test 3 IgG anti-B19V	NEG	NEG	NEG	NEG	NEG	NEG	NEG
IgM anti-B19V	NEG	NEG	NEG	NEG	NEG	NEG	NEG
PCR-B19	-	NEG	POS	NEG	-	-	-
CD4$^+$ (cells/mm³)	80	61	36	314	253	344	244
Hb (g/dL)	-	5.92	9.14	11.3	12.3	-	13.7

a: weakly positive; B19V: parvovirus B19; Hb: haemoglobin; IND: indetermined; NEG: negative; PCR: polymerase chain reaction; POS: positive; -: lack of data in the medical records.

the direct effect of HIV infection itself on the bone marrow (Abkowitz et al. 1997, Koduri 2000). As there are usually no clinical clues of a B19V infection in these patients, the presence of anaemia, especially during B19V epidemics, should alert the physician to the possibility of B19 infection.

One of our patients (Case 3) had viraemia persisting for at least nine months (from September 2005-June 2006). This propensity to develop protracted B19V viraemias in HIV-infected and other immunosuppressed individuals was described long ago (Kurtzman et al. 1989b, Weiland et al. 1989, Frickhofen et al. 1990, Gyllensten et al. 1994). Prolonged viraemias have also been described in healthy blood donors (Hitzler & Runkel 2002).

Dissociation between the serological and molecular markers was observed in Case 5, most likely due to the inability of this immunocompromised patient to produce neutralising antibodies. According to Kurtzman et al. (1989a), the immune response may be quantitatively and qualitatively altered by immunosuppression or during persistent infections. Consequently, in this clinical context, diagnostic genome detection has been advocated by some authors (Calvet et al. 1999).

It is well known that genotype 1 is the most common B19V genotype detected world-wide (Servant-Delmas et al. 2010). In this study, genotype 1 was detected in four of the five HIV-infected patients. This genotype was also detected in EI patients during outbreaks in Niterói (Pereira et al. 2010). Unexpectedly, a genotype 3b strain was detected in a sample from September 2005.

Ferry et al. (2009) found that the detection of B19V genotype 2 or 3 DNA was infrequent in the blood samples of a large cohort of immunocompromised, HIV-infected anaemic patients, despite the use of highly sensitive real-time PCR methods. All three genotypes have been identified in Brazil, although genotype 1 is the most common (Sanabani et al. 2006, Freitas et al. 2008, Keller et al. 2009, Garcia 2010). Genotype 3b was identified in a serum sample obtained from a patient with systemic lupus erythematosus in 1999 in Belém, state of Pará (Freitas et al. 2008); in a sample from a patient from São Paulo, state of São Paulo, with anaemia after a kidney transplantation in 2004 (Keller et al. 2009) and in children with exanthaematic disease (Freitas et al. 2008).

This study had the limitations inherent in retrospective studies involving the review of medical records. One such limitation was the long interval between blood collections. These long intervals made it difficult to precisely know the time of B19V seroconversion and they prevented complete analysis of the interactions between serological (especially IgM) and molecular markers. Another limitation was not performing quantitative PCR (qPCR), as it is known that the virus may persist for a long time and maintain persistent viraemias in HIV-positive patients (Frickhofen et al. 1990, Miao et al. 2012). However, the following evidence of primary infections in our patients was found: (i) the previous serum samples (in relation to the PCR-positive samples) were negative by PCR, (ii) the subsequent samples (in relation to the PCR-positive samples) were also negative by PCR, (iii)

seroconversions were observed in four of the five cases and (iv) all but one case occurred during an outbreak of B19V infection in Niterói. If these positive PCRs corresponded to long persistent infections in HIV-positive patients who were recovering their immunity, a decrease in the intensity of the viraemia would be expected by the time of seroconversion, together with a switch from positive to negative PCR results. This outcome did not occur, as the PCR results changed from negative to positive at the time of seroconversion and then changed from positive to negative afterward. Considering the high sensitivity of qPCR and the long intervals between the blood collections, the use of this technique would be expected to detect low DNA levels in the blood samples despite the presence of circulating IgG antibodies. However, this finding would not change the interpretation of the obtained results.

In conclusion, our findings show that B19V infections in HIV-infected patients may be limited, subtle and apparent only in retrospective analyses due to the variety of causes of anaemia in these patients and the adequate use of HAART. The finding of four strains of genotype 1 and one strain of genotype 3 is consistent with their relative frequency in Brazil. There were no apparent relationships between the infecting genotype and the clinical course.

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