

Human parvovirus B19 infection in HIV-positive patients

Infecção por parvovirus humano B19 em pacientes HIV-positivos

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Abstract Parvovirus B19 infects predominantly erythroid cells, leading to transient inhibition of erythropoiesis. Immunocompromised patients may be unable to produce neutralizing antibodies and may develop severe chronic anemia. Epidemiological studies done on Niterói population showed that B19 infection occurs periodically in late spring and summer. We report a study from 55 HIV infected patients attending an infectious diseases outpatient clinic in this city during a 5-month period in which B19 circulation was well documented. All patients were under anti-retroviral therapy. No anti-B19 IgM was found, but a high prevalence of IgG anti-B19 (91%) was observed. In six patients, B19 DNA was found by dot-blot hybridization techniques, but this was not confirmed by PCR. None of these 6 patients manifested anemia and only one had CD4 cell count below $200 \times 10^7/L$. We conclude that persistent infection causing anemia is an infrequent finding in our HIV positive patients under drug therapy.

Key-words: Parvovirus B19. AIDS. Chronic anemia. Opportunistic infection.

Resumo O parvovírus B19 infecta predominantemente células eritróides, causando inibição transitória da eritropoiese. Pacientes imunocomprometidos podem ser incapazes de produzir anticorpos neutralizantes, evoluindo com grave anemia crônica. Estudos epidemiológicos da população de Niterói mostraram que a infecção ocorre periodicamente no final da primavera e no verão. Descrevem-se 55 pacientes infectados pelo HIV atendidos num ambulatório de doenças infecciosas nesta cidade num período de cinco meses, no qual a circulação do parvovírus B19 foi documentada. Todos os pacientes estavam sob terapia anti-retroviral. Não se encontrou IgM anti-B19, mas notou-se uma prevalência alta de IgG anti-B19 (91%). Em seis pacientes verificou-se a presença de DNA do B19 por hibridização em dot-blot, o que não se confirmou por PCR. Nenhum destes seis pacientes tinha anemia, e apenas um tinha células CD4 abaixo de $200 \times 10^7/L$. Conclui-se que infecção persistente causando anemia é um achado infrequente em nossos pacientes HIV positivos sob terapia medicamentosa.

Palavras-chaves: Parvovirus B19. AIDS. Anemia Crônica. Infecção Oportunista.

Human Parvovirus B19 is a member of the *Parvoviridae* family and infects predominantly erythroid stem cells. This results in transient inhibition of erythropoiesis⁵. The infection occurs normally in childhood and has been linked to a long known exanthematic disease, erythema infectiosum. It may also cause arthritis and arthralgia in women, through deposition of immunocomplexes in the joints⁸. However, the infection is often asymptomatic. In immunocompromised patients, B19 infection causes severe chronic anemia due to inability to produce neutralizing antibodies, and consequent persistence

of virus replication¹⁴. The virus has a worldwide distribution. Epidemiological studies done from 1994 to 1998 in Niterói, a medium-sized city in Rio de Janeiro State, Brazil, showed that B19 activity occurs periodically each year, commonly in the form of outbreaks in late spring and summer²⁰.

In this study we examined the prevalence of anti-B19 IgG antibodies, and sought evidence of persistent B19 infection, in HIV infected individuals attending the outpatient clinic of the Infectious Diseases Department of Hospital Universitário Antônio Pedro, a public tertiary care university hospital in Niterói.

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MATERIAL AND METHODS

Patients - Between September 1997 and January 1998 clinical data were collected and serum samples from 55 HIV-infected outpatients. A second specimen was collected one year later from three of the six patients suspected to have persistent B19 infection by DNA detection in the first serum sample. The patients were divided into categories A, B and C according to the CDC HIV classification, 1992 (Table 1)². The male-female ratio

was 1.48. The age range was 21 to 50 years, with a median of 36 years. The median time elapsed since the diagnosis of HIV infection was 5.3 years. All patients were under some kind of anti-retroviral drug therapy.

Serologic assays - All sera were examined by a μ -capture enzyme immunoassay (MACEIA) to detect anti-B19 IgM as described by Cubel et al⁹ and Nascimento et al¹⁹. An indirect immunofluorescence test (IFA) was

Table 1 - Patients classification in CDC categories^{2*}.

Categories (CDC, 1992)			
A	B	C	Not classified
4.2%	29.8%	40.5%	25.5%

*CD4 cell count not available for all patients
N = 55

used to detect anti-B19 IgG²¹. In order to detect B19 DNA a dot-blot test was used as described by Mori et al¹⁵. Three different samples taken from a 32-year-old HIV positive male with chronic anemia due to persistent B19 infection were used as a positive control. Positive B19 DNA samples were sent to the Virus Reference

Division of the Central Public Health Laboratory, in the United Kingdom, for confirmation of the diagnosis by nested PCR⁷, and by dot-blot using chemiluminescent detection^{11 16}. All sera, including the positive and negative controls, were stored at -20^o C and heated to 60^o C during 20 minutes before testing.

RESULTS

Table 2 shows that 91% (50/55) of the patients had anti-B19 IgG. None of the patients had anti-B19 IgM. B19 DNA was found in six (11 %) patients, albeit in lower amounts than in our positive control. These six patients (one female and five males) were classified as class C (n = 4) and B (n = 2), according to the CDC criteria. None of them showed laboratory evidence of anemia (all had hematocrit > 40% and

reticulocytes \cong 1 %) and only one had a CD4 cell count lower than 200 x 10⁷/ L. The laboratory findings for these six DNA positive patients are listed in Table 3. For three of them, DNA detection was confirmed using a dot blot hybridization with chemiluminescent detection method, but not by PCR. Three patients had a second sample taken one year later. No B19 DNA was found in these samples.

Table 2 - Anti-B19 antibodies and B19 DNA results for the 55 HIV infected patients.

Patients	Anti-B19 IgG (IFA)		Anti-B19 IgM (MACEIA)		B19 DNA (Dot-Blot)	
	Positive	Negative	Positive	Negative	Positive	Negative
N = 55	50	5	0	55	6	49
Percentages	91	9	0	100	11	89

Table 3 - Laboratory findings for the 6 patients suspected of having persistent B19 infection.

Patient n ^o	Sex	CDC Class	Sample collection Month/Year	Anti-B19 Antibodies		B19 DNA		PCR
				IgG (IFA)	IgM MACEIA	Dot Blot A	B	
10	M	ND	S1 11/97	Neg VP1 and VP2	Neg	+	+	-
38	M	C	S1 12/97	Pos VP1 and VP2	Neg	+	+	-
50	F	C	S1 01/98	Pos VP1 and VP2	Neg	+	+	-
			S2 04/99	Pos WB VP1	Neg	ND	-	-
53	M	C1	S1 01/98	Neg VP1 and VP2	Neg	+	-	-
			S2 02/99	ND		ND	-	ND
58	M	ND	S1 02/98	Pos VP1 and VP2	Neg	+	-	ND
			S2 04/99	ND			-	
60	M	ND	S1 02/98	Neg VP1 pos VP2	Neg	+	-	ND

A - B19 DNA detected by dot-blot using color detection¹⁵
PCR - Nested PCR as described by Clewley, 1993⁷,

B - B19 DNA detected by dot-blot using chemiluminescent detection^{11 16}
ND - Not done

DISCUSSION

Anemia is a common finding in the HIV infected patient¹². Up to 80% of these patients are anemic, a percentage that increases as the infection advances¹³. Several causes of anemia have been described in HIV positive patients¹³, such as the drugs used in the course of treatment (zidovudine, trimethoprim-sulfamethoxazole), lymphomas, the direct effect of HIV on bone marrow cells and coinfection with mycobacteria or human parvovirus B19. The diagnosis of B19 infection in anemic HIV patients is important, since chronic B19 viremia is a treatable cause of anemia in this population^{10 13}.

IgG antibodies to B19 are usually found in immunocompetent adults in rates that varies from 60% to 80%. Among HIV infected groups, however, the prevalence has shown a large variation. The initial studies, involving a small number of patients, showed a higher prevalence of IgG antibodies to B19, when compared with immunocompetent individuals^{3 17 24}. Recent studies involving larger groups of patients confirmed this higher seroprevalence among anemic HIV-infected patients²³. On the other hand, van Elsacker-Niele et al²², studying 317 consecutive HIV infected patients searching for B19 DNA and antibodies, have found no difference in seroprevalence rates between the anemic and non-anemic HIV groups, as well as between HIV patients and normal immunocompetent individuals. B19 DNA was not detected among the patients and the authors concluded that parvovirus B19 infection has a limited role in the anemia of HIV infected patients.

Patients infected with HIV may be incapable of producing IgG antibodies against parvovirus B19, but even among the more immunocompetent individuals such antibodies may be unable to neutralize the virus, leading to persistent infections¹⁴. Patients with a CD4 count of over 300×10^6 cells/L are usually capable of producing neutralizing antibodies, and persistent infections are more common among patients with advanced immunodeficiency. The degree of immunodeficiency and other confounding factors could explain the difference found in the IgG seroprevalence in different studies.

Controversial findings have also been reported in the literature regarding the persistence of B19 infection

in HIV positive patients. Naides et al¹⁷ found a high rate of B19 persistence in a highly selected AIDS population. On the other hand, Bremner et al⁴, studying 39 HIV positive patients with anemia (B19 viremia-negative by nested-PCR), concluded that B19 infection is an uncommon cause of anemia in the HIV positive population, especially in non epidemic years. Vernazza et al²³ described persistent B19 infection as an uncommon cause of anemia in these patients, due to preservation of an adequate humoral immune response until the late stages of HIV infection. Since persistent parvovirus B19 chronic infection is typically associated with absence of an antibody response to B19⁶, the continued ability to synthesize antibodies would render B19 chronic infection with anemia uncommon.

In the present paper we studied a non-anemic population of HIV positive patients for anti-B19 antibodies (IgM and IgG) and B19 DNA. We did not detect IgM antibodies in any of the samples, but we found a significantly higher prevalence of IgG antibodies to B19 in this group (91%) than among general population matched by age, as inferred by the previous population studied by Nascimento et al¹⁸.

B19 DNA was found in the sera of six (10.9%) patients by dot-blot hybridization, although in lower amount than in our viremic control samples. Chemiluminescent detection was positive for 3 of the 6 samples. These findings were not confirmed by a more sensitive PCR technique, which was subsequently performed on 5 of the 9 samples. These 6 patients (5 male and 1 female) were classified accordingly to the CDC 1992 criteria as belonging to class C (four) and B (two). None of them had anemia. Only one had a CD4 cell count lower than 200×10^7 /L. Five of these patients were positive for anti-B19 IgG antibodies, and only one was IgG negative.

We conclude that B19 IgG antibody prevalence was high in our patients and, although six of them were positive by a DNA dot-blot, we were unable to confirm persistent B19 infection by PCR. Chronic anemia due to B19 infection was not found in our HIV positive patients under drug therapy.

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