

Infrequent Replication of Parvovirus B19 and Erythrovirus Genotypes 2 and 3 among HIV-Infected Patients with Chronic Anemia

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We investigated the role that erythroviruses (parvovirus B19 and erythrovirus genotypes 2 and 3) play in the lives of immunosuppressed HIV-infected patients with chronic anemia. We screened the serum samples of 428 patients by specific ultrasensitive real-time polymerase chain reaction assay. Sixteen patients had circulating DNA, with no apparent clinical impact. Erythrovirus-associated anemia is an extremely rare event in HIV-infected patients.

Parvovirus B19 (PVB19), by targeting human erythroid progenitors, is responsible for acute anemia among patients with hemolytic disorders [1]. For severely immunocompromised patients, including HIV-infected patients with low CD4 cell counts, the case reports and case series of chronic anemia due to high levels of PVB19 replication associated with pure red cell aplasia have been reported [2–5]. Recently, 2 variants of PVB19 belonging to erythroviruses in the Parvoviridae family (erythrovirus genotypes 2 and 3, formerly called erythrovirus variants A6 and V9, respectively) have been described. However, their pathogenic role has not been established, even among immunocompromised patients [6, 7].

Chronic anemia is common among HIV-infected patients and is a predictor of shorter survival [8]. PVB19 is considered to be a rare cause of acute or chronic anemia in the HIV-infected population, despite a high seroprevalence [9]. However, in the study performed by Naides et al [10] in 1993, many patients who were intolerant to azidothymidine (AZT) had PVB19 infection. Recent data revealed that, by using a highly sensitive PVB19 polymerase chain reaction (PCR) detection assay, low titers of PVB19 DNA were associated with mild to severe chronic anemia for a selection of patients who received a renal transplant [11] and that the newly described erythrovirus genotype 3 has been detected in 3 HIV-infected patients with chronic anemia [6]. We aimed to assess the prevalence of circulating erythrovirus DNA in immunocompromised HIV-infected patients with chronic anemia.

Methods. HIV-infected patients included in the Swiss HIV Cohort Study during the period from January 1998 through April 2008 were screened for erythrovirus DNA by use of a highly sensitive real-time PCR method. Inclusion criteria were (1) Agence Nationale de Recherches sur le SIDA et les Hépatites Virales grade 1 (or higher) persistent anemia (with a hemoglobin level <10.5 g/dL [2 consecutive results with at least 3 months between each sampling]), (2) immunosuppression (CD4 cell count ≤500 cells/μL) during the episode of anemia, and (3) availability of at least 1 serum or plasma sample at the time of the determination of hemoglobin level or between the 2 determinations of hemoglobin level. Patients with ribavirin exposure at any time between the 2 determinations of hemoglobin level were excluded. Patients exposed to myelosuppressive drugs were not excluded, because they might be associated with erythrovirus reactivation.

DNA was extracted from frozen serum or plasma with the Qiagen DNA mini kit (Qiagen). PVB19 and erythrovirus genotype 2 or 3 DNA were detected and quantified by an ultrasensitive real-time PCR targeting the VP1 gene, a well-conserved region of the erythrovirus genomes, using an extract volume corresponding to 10 μL of plasma in duplicate, with an extra reaction spiked with 1000 copies of PVB19 DNA as an inhibition control. Two forward primers (B19_2785_F1: 5'-CTTACACAAGCCTGGGCAAGTTAG-3'; and B19_2785_F2: 5'-CTTACACAAGCCTGGGCGAGTTAG-3'; 50 nM each) and 3 reverse primers (B19_2867_R1: 5'-GGGGCCAGCTTGTA-GCTCATT-3'; B19_2867_R2: 5'-GAGGCCAGCTTGTA-GCTCATT-3'; and B19_2867_R3: 5'-GAGGCCAGCTTGTA-ACTCATT-3'; 33 nM each) were used. The analytical sensitivity, using a 17-base minor-groove binder probe (B19_2817_P FAM-

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Table 1. Baseline Characteristics of Patients With or Without Erythrovirus Replication

Characteristic	Patients with erythrovirus replication (n = 16)	Patients without erythrovirus replication (n = 412)	P
Age, ^a years	43.5 (40–46)	44 (40–50)	.422
Male sex	5 (31.3)	160 (38.8)	.541
White	12 (75.0)	260 (63.1)	.332
European ancestry	13 (81.3)	290 (70.4)	.416
Intravenous drug user	8 (50.0)	145 (35.2)	.225
Men who have sex with men	3 (18.8)	59 (14.3)	.714
CD4 cell count, ^a cells/ μ L	191.0 (156.5–322.0)	184.5 (77.0–290.0)	.223
Median log ₁₀ HIV-1 RNA level, ^a copies/mL	4.13 (3.66–11.04)	6.68 (3.66–11.06)	.819
Patients without HIV-1 replication ^{a,b}	6 (40.0)	150 (36.7)	.793
Tested positive for HBsAg	2 (13.3)	28 (7.3)	.313
Tested positive for hepatitis C antibody	7 (43.8)	173 (42.6)	.928
Duration of AIDS, years	10.1 (4.1–15.7)	8.2 (3.1–13.6)	.332
Diagnosis of AIDS ^{a,b}	8 (57.1)	214 (67.1)	.563
Received ART ^a	11 (68.8)	281 (68.4)	.910
Received ART with AZT ^a	6 (37.5)	133 (32.3)	.651
Hemoglobin level, ^a g/dL	9.6 (9.3–9.9)	9.4 (8.5–10.0)	.574
Hemoglobin level <9.5 g/dL	7 (43.8)	206 (50.1)	.617

NOTE. Data are no. (%) of patients or median value (interquartile range [IQR]). HBsAg, hepatitis B surface antigen; ART, antiretroviral therapy; AZT, azidothymidine; IQR, interquartile range.

^a During episode of anemia.

^b The calculation of the percentages was done after considering missing values.

TACCCGGTACTA ACTAT-MBG-DQ; 100 nM), an ABgene QPCR Rox mix AB-1139b, and a 15-s, 95°C, 1-min, 60°C cycle on a taqMan 7900 machine, was close to 1 input copy of viral DNA (the World Health Organization International Standard for parvovirus B19 DNA was used for calibration—National Institute for Biological Standards code 99/800) [12]. In each run, the linearity of the Ct/input DNA relationship was verified from 10 to 100,000 copies/reaction using appropriate standards.

From the entire patient population, we compared the characteristics of patients with erythrovirus replication with those without erythrovirus replication, and from that patient population, we subsequently compared the number of patients with AZT exposure. We used the χ^2 test to compare qualitative variables, and we used the Fisher exact test (as appropriate) and the nonparametric Mann-Whitney test to compare continuous variables. All statistical tests were 2 sided, and a threshold of 5% was chosen as a cutoff for declaring statistical significance. We finally went back to the medical files of patients with erythrovirus replication to determine the characteristics of anemia by collecting additional biological data (eg, the mean corpuscular volume, the reticulocyte cell count, the iron and ferritin serum levels).

Results. There were 428 patients who fulfilled the inclusion criteria. At study entry, 165 (39%) were male, the median age was 44 years (interquartile range [IQR], 40–50 years), and 153 (36%) reported intravenous drug use. The median CD4 cell count was 185 cells/ μ L (range, 77–290 cells/ μ L), with 150 pa-

tients having a CD4 cell count of <50 cells/ μ L. The median hemoglobin level was 9.4 g/dL (range, 8.5–10.0 g/dL), and 138 patients (32%) were exposed to AZT at the time blood samples were obtained.

Erythrovirus DNA was detected in 16 patients (4%). The erythrovirus viral load ranged from 18 to 6820 copies/mL and was detectable at a very low level (<500 copies/mL) in 13 (81%) of the 16 patients. No statistically significant differences in characteristics at the time of sampling were noticed after comparison of patients with erythrovirus replication with those without erythrovirus replication (Table 1). Erythrovirus replication was not more frequently detected in patients with a CD4 cell count of <200 cells/ μ L (9 [56%] vs 7 [44%] of 16 patients with erythrovirus replication). Of note, the prevalence of intravenous drug users seemed to be higher among patients with erythrovirus replication than among patients without erythrovirus replication (8 [50%] of 16 patients vs 145 [35%] of 412 patients; $P = .225$). However, both groups of patients had similar median hemoglobin levels (hemoglobin level of 9.6 g/dL [IQR, 9.3–9.9 g/dL] for patients with erythrovirus replication vs 9.4 g/dL [IQR, 8.5–10.0 g/dL] for patients without erythrovirus replication; $P = .574$) and a similar prevalence of severe anemia (7 [44%] of 16 patients with erythrovirus replication vs 206 [50%] of 412 patients with erythrovirus replication; $P = .617$). Circulating erythrovirus DNA was detected in only 6 (4%) of the 139 patients on AZT, and no statistically significant difference was noticed between patients with circulating erythrovirus

DNA and patients without circulating erythrovirus DNA (data not shown).

Finally, the origin of anemia for the 16 patients with erythrovirus replication was mainly considered to be carencial (6 patients experienced iron deficiency) or multifactorial (HIV infection with or without AZT therapy, cirrhosis, drug abuse, chronic renal insufficiency, and/or acute non-Hodgkin lymphoma). None of our patients presented with pure red cell aplasia.

Discussion. We found that PVB19 and erythrovirus genotype 2 or 3 DNA detection was infrequent in the blood samples of a large cohort of immunocompromised HIV-infected anemic patients, despite using highly sensitive real-time PCR methods. The typical clinical presentation of PVB19 infection for HIV-infected patients includes transfusion-dependent severe anemia with reticulocytopenia [2–5]. This clinical form of anemia, which requires multiple infusions of intravenous immunoglobulins and the introduction of highly active antiretroviral therapy to recover, is rarely observed, despite a high PVB19 seroprevalence in HIV-infected individuals (eg, 81% of HIV-infected individuals vs 57% of individuals in the general population of Switzerland) [2–5, 8]. Previous studies that tried to detect circulating PVB19 DNA in the blood samples of anemic HIV-infected patients were limited, because the studies (1) included only a small number of patients; (2) were performed before the year 2000, mainly using qualitative dot blot PCR (which may have a low sensitivity) or nested PVB19 PCR; and (3) did not detect the newly described erythroviruses, such as erythrovirus genotypes 2 and 3, that may also be involved in the process of chronic anemia in immunocompromised patients [13–15].

Our study, which targeted both PVB19 and erythrovirus genotypes 2 and 3 DNA with a real-time PCR, demonstrates that the prevalence of erythrovirus replication was low for a large selection of HIV-infected anemic patients, even for patients with a low CD4 cell count. Moreover, the viral load of positive samples was low, probably corresponding to circulating DNA without crude active infection and without any clinical significance. The prevalence of 4% observed in our selected population of HIV-infected patients with chronic anemia was close to the prevalence of 1% observed in US blood donors in 2000–2003 but seemed to be higher than the prevalence of 0.2% observed in German and Austrian blood donors in 2004–2006 [16, 17]. It was noteworthy that, in the latter study, most of the patients with circulating PVB19 DNA exhibited a low viral load, $<10^5$ copies/mL [17]. This range of viral load may correspond to “viral dust,” probably following a symptomatic or not primary PVB19 old infection. Our observation supports the hypothesis that (1) PVB19-induced anemia is an extremely rare event for HIV-infected patients and (2) erythrovirus genotypes 2 and 3 may not have any pathogenic role to play in

this population. Of note, a low CD4 cell count *per se* does not seem to be sufficient to allow reactivation of PVB19 [18]. Patients who were on AZT at the time of the sampling were not more likely to experience erythrovirus replication, compared with patients who were on other antiretroviral drugs.

Our study has some limitations, including the cross-sectional design and the absence of indicators of the circumstances and characteristics of anemia (data on the reticulocyte count, the mean corpuscular volume of red cells, and the level of serum ferritin were not routinely collected in the Swiss HIV Cohort Study). In conclusion, PVB19-associated anemia is a rare event among HIV-infected patients, and erythrovirus genotypes 2 and 3 may not have any pathogenic role to play in the process of chronic anemia in such patients. This study does not support the extensive use of erythrovirus PCR for HIV-infected patients with chronic anemia, except in the previously described specific context of severe anemia with pure red cell aplasia (which was not observed in our study).

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