Journal of Medical Virology 88:2106–2114 (2016)

# Prevalence of Human Pegivirus (HPgV) Infection in Patients Carrying HIV-1C or Non-C in Southern Brazil

**Luísa Dias Da Mota,[1]* Anna S. Nishiya,[2] Fabiana Finger-Jardim,[1] Maria F.M. Barral,[1] Cláudio M. Silva,[1] Maiba M. Nader,[1] Carla V. Gonçalves, Vanusa P. Da Hora,[1] Jussara Silveira,[1] Rossana P. Basso,[1] Marcelo A. Soares,[3] José E. Levi,[2] and Ana M.B. Martínez[1]**

[1]*Medical School of the Federal University of Rio Grande, Rio Grande do sul, Brazil*
[2]*Foundation Pró-Sangue, São Paulo, Brazil*
[3]*Genetics Program, National Cancer Institute (INCA), Rio de Janeiro, Brazil*

Previous studies have demonstrated that coinfection with HPgV is a protective factor for human immunodeficiency virus (HIV)-infected patients, leading to slower disease progression, and longer survival after established disease. The present study sought to estimate the prevalence of HPgV infection and associated risk factors in patients harboring C or non-C HIV-1 subtypes followed-up at HU-FURG, southern Brazil. Samples from 347 HIV–1-infected subjects were subjected to plasma RNA extraction, cDNA synthesis, HPgV RNA detection, and HIV-1 genotyping. The overall prevalence of HPgV RNA was 34%. Individuals aged 18–30 years had higher chances of infection compared with those 50 years or older (95%CI 1.18–52.36, $P = 0.03$). The number of sexual partner between one and three was a risk factor for HPgV infection (95%CI 1.54–10.23; $P < 0.01$), as well as the time since diagnosis of HIV-1 $\geq$ 11 years (95%CI 1.01–2.89; $P = 0.04$). Patients infected with HIV non-C subtypes had six times more chance of being HPgV-infected when compared to subtype C-infected subjects (95%CI 2.28–14.78; $P < 0.01$). This was the first study conducted in southern Brazil to find the circulation of HPgV. HIV/HPgV coinfection was associated with a longer survival among HIV$^+$ patients. Of novelty, individuals infected by HIV non-C subtypes were more susceptible to HPgV infection. However, additional studies are needed to correlate the HIV-1 subtypes with HPgV infection and to clarify cellular and molecular pathways through which such associations are ruled. ***J. Med. Virol. 88: 2106–2114, 2016***. © 2016 Wiley Periodicals, Inc.

**KEY WORDS:** HPgV; human immunodeficiency virus; HPgV/HIV coinfection; HIV-1 subtype

## INTRODUCTION

Human pegivirus (HPgV), previously named GB virus type C (GBV-C), is an RNA virus of the *Flaviviridae* family, recently assigned to the Pegivirus genus [Stapleton et al., 2012a; Adams et al., 2013]. So far, seven HPgV genotypes have been described (one through seven) which are directly related to their geographic region of origin [Pavesi, 2001; Berzsenyi and Roberts, 2006; Feng et al., 2011]. In Brazil, genotypes one and two were found [Oliveira et al., 2002; Nishiya et al., 2003; Alcalde et al., 2010; Campos et al., 2011; Giret et al., 2011; Souza et al., 2012].

The HPgV was firstly identified in a patient with acute hepatitis of unknown cause at that time. It was thought to be the new causative agent of viral hepatitis non-A–E [Deinhardt et al., 1967]. It has been later found that HPgV did not cause hepatitis and it replicated in peripheral blood mononuclear cells ex vivo, but the main cell targets in which HPgV replicates in vivo have not been fully elucidated. In individuals with chronic HPgV infection, viral RNA is found mainly in natural killer (NK) cells, T and B lymphocytes and monocytes, suggesting a broad cellular tropism [Chivero and Stapleton, 2015]. HPgV infection is common worldwide, and can be transmitted by parenteral, sexual, and mother-to-childroutes [Feucht et al., 1997; Abe, 2001;

Grant sponsor: CAPES; Grant sponsor: CNPq

*Correspondence to: Luisa Dias da Mota, Laboratório de Biologia Molecular, FAMED/FURG, Rua Visconde de Paranaguá, 102, Centro, Rio Grande, RS 96200-190, Brazil.
E-mail: luisadias_sixcp@hotmail.com

Accepted 10 May 2016

DOI 10.1002/jmv.24574
Published online 25 May 2016 in Wiley Online Library (wileyonlinelibrary.com).

Ramia et al., 2004]. In healthy populations, the prevalence of HPgV is approximately 1–4% in Europe and North America, and 5–19% in South America, Africa, and Asia [Mohr and Stapleton, 2009]. Due to the similar modes of transmission, its prevalence increases in populations infected with HIV, HCV and those exposed to transfused blood products, transplanted patients, injecting drug users, hemophiliacs, and hemodialysis users [Mohr and Stapleton, 2009]. The worldwide prevalence of HPgV in HIV-positive individuals varies between 17% and 89%, and it varies according to the region studied [Heringlake et al., 1998; Alcalde et al., 2010; Campos et al., 2011; Giret et al., 2011; Souza et al., 2012; Anggorowati et al., 2013; Ernst et al., 2013; Lee et al., 2014; Sahni et al., 2014]. In Brazil, the prevalence of HPgV RNA in HIV-1-positive patients has been estimated between 21% and 30% [Alcalde et al., 2010; Campos et al., 2011; Giret et al., 2011; Souza et al., 2012].

Several studies have found a positive association between persistent HPgV infection and increased survival in HIV-infected individuals [Heringlake et al., 1998; Toyoda et al., 1998; Tillmann et al., 2001; Xiang et al., 2001; Williams et al., 2004; Zhang et al., 2006; Vahidnia et al., 2012]. Progression to AIDS occurs more slowly and, in patients with AIDS it can result in longer survival and lower mortality rates [Xiang et al., 2001; Stapleton et al., 2013]. It is thought that persistent infection HPgV reduces the activation of T-cells, impairing the efficient replication of HIV-1, and thus contributing to increased survival in coinfection [Schwarze-Zander et al., 2006; Giret et al., 2009; Stapleton et al., 2012b]. In this context, the possibility of developing an alternative therapy to improve survival of HIV-positive patients has been suggested. Gretch [2012] suggests we should consider the development of a biovaccine derived from HPgV for people living with HIV, especially in resource-poor countries where AIDS mortality rates are still high. Some analogous synthetic peptides of the HPgV structural protein E1 interact with HIV in a membrane model, exerting reductive activity in the fusion of gp41 to target cells [Sánchez-Martín et al., 2011], whereas in similar studies, E2 proteins NS3, and NS5A were able to inhibit HIV replication in vitro [George et al., 2007; Jung et al., 2007; Xiang et al., 2012].

HIV comprises two types (HIV-1 and HIV-2). HIV-1 is more virulent and more widespread worldwide. HIV-2 appears to be less virulent and found almost exclusively in West Africa. HIV-1 presents four distinct groups (M through P), being group M the one responsible for the current AIDS pandemic [Simon et al., 1998]. Group M is subdivided into nine pure genetic subtypes (A–D, F–H, J and K), in addition to recombinant circulating forms (CRFs), consisting of more than one subtype, and that reached an epidemic spread [Robertson et al., 2000]. The worldwide distribution of subtypes is heterogeneous, with different prevalence depending on the region. The most prevalent is subtype C, which is most common in sub-Saharan Africa, East Africa, India, and Southern Brazil [Hemelaar et al., 2011]. Subtype B is the most widespread worldwide, predominantly in developed countries like the US, Western European countries, Japan, and Australia [Hemelaar et al., 2011]. Subtype B is predominant in Brazil, but subtypes C, F, and circulating CRFs also circulate significantly in the country [Silveira et al., 2012]. Studies have shown an increased prevalence of subtype C in Brazil, mainly in Rio Grande do Sul (RS), and Paraná states. In the city of Rio Grande/RS, this is shown by the rise in the prevalence of subtype C from 22% in 2002 [Martinez et al., 2002] to 56 % in 2010 [Silveira et al., 2012].

In Brazil, most HPgV studies were performed in the city of São Paulo where HIV-1 subtype B is the most prevalent. Due to the absence of data on HPgV epidemiology from southern Brazil, to the fact that this region has a great diversity of HIV-1 subtypes and a high prevalence of HIV-infected individuals, this study aimed to evaluate the prevalence of HPgV infection, and associated risk factors in HIV-1-infected patients treated at the Hospital Universitário Dr. Miguel Riet Corrêa Jr. (HU/FURG), in Rio Grande, southern Brazil.

## MATERIALS AND METHODS

### Ethical Aspects

The study has been approved by the Health Research Ethics Committee (CEPAS) of Universidade Federal do Rio Grande (FURG) (n° 113/2012). All participants provided written informed consent to participate in the study.

### Study Design and Patients

This was a cross-sectional study designed to assess the prevalence of HPgV infection and associated risk factors in patients with different HIV-1 subtypes. All patients aged over 18 years, seen at the HIV/AIDS service of HU-FURG, a University Hospital in Rio Grande, RS, Brazil, were invited to participate in the study. Plasma samples were obtained from November 2011 to April 2012. Behavioral, clinical, and laboratory data were collected through a questionnaire and analysis of medical records. Sample size was calculated in the Epi-Info software environment, on the basis of a presumed HPgV prevalence of 21–30% in patients with HIV-1 in Brazil [Alcalde et al., 2010; Campos et al., 2011; Giret et al., 2011; Souza et al., 2012]. The series consisted of 347 patients seen at the mentioned service.

### RNA Extraction

Viral RNA was extracted from 140 μL of plasma using the QIAamp® Viral RNA extraction kit (Qiagen) according to the manufacturer's protocol. RNA was eluted in 60 μL of AVE buffer and stored at −70°C until the synthesis of complementary DNA (cDNA).

### Synthesis of cDNA

After RNA elution, 10 μL were added to 300 ng of random oligonucleotides (2 μL of a 150 ng/μL solution, $N_6$, Life Technologies, Carlsbad, CA), and denatured at 70°C for 10 min. For cDNA synthesis, a reaction using 200 U of reverse transcriptase (Superscript; Gibco), 0.1 M of DTT, 5 U of RNaseOUT ® (Life Technologies), and 0.5 mM of each desoxinucleotide cDNA reaction was conducted at 42°C for 1 hr and 30 min in a final volume of 20 μL. From the cDNA, HPgV non-coding genomic region 5'NCR was amplified, using real-time PCR as below.

### Real-Time PCR for HPgV

A fragment of HPgV 5'NCR non-coding genomic region was amplified using the following primers: RTG1 (GTGGTGGATGGGTGATGACA; sense); RTG2 (GACCCACCTATAGTGGCTACCA; antisense) and NFQ (5'-FAM- CCGGGATTTACGACCTACC3′; probe) [Campos et al., 2011]. Five microliters of cDNA were added to 10 μL of 2× TaqMan universal mix and 1 μL of 20× concentrated mix containing the sense and antisense primers at 360 and 100 μmol/L probe to a final concentration of 0.9 pmol/uL of primers, and 0.25 pmol/μL of probe. An ABI Prism 7500 platform (Applied Biosystems, Foster City, CA) was used with an initial cycle at 95°C for 10 min followed by 40 cycles of 95°C for 15 sec and 60°C for 1 min [Campos et al., 2011].

### Detection, Quantification, and Genotyping of HIV-1

HIV-1 detection and quantification assays were performed by the viral load laboratory unit, which provides services to patients seen at the HU-FURG HIV/AIDS clinic, in association with the Brazilian Ministry of Health. The Real-Time Abbott HIV-1 in vitro assay was used in accordance with the manufacturer's protocol.

Genotype information was obtained in two ways: by use of previously genotyped samples through data obtained from medical records and tests carried out in partnership with the Ministry of Health, or data from previous studies by the same research group [Silveira et al., 2012]; and, during this study, by genotyping samples that had not been previously subtyped (n = 53). The latter have been submitted to the GenBank nucleotide database and assigned accession numbers KT958939–KT958991.

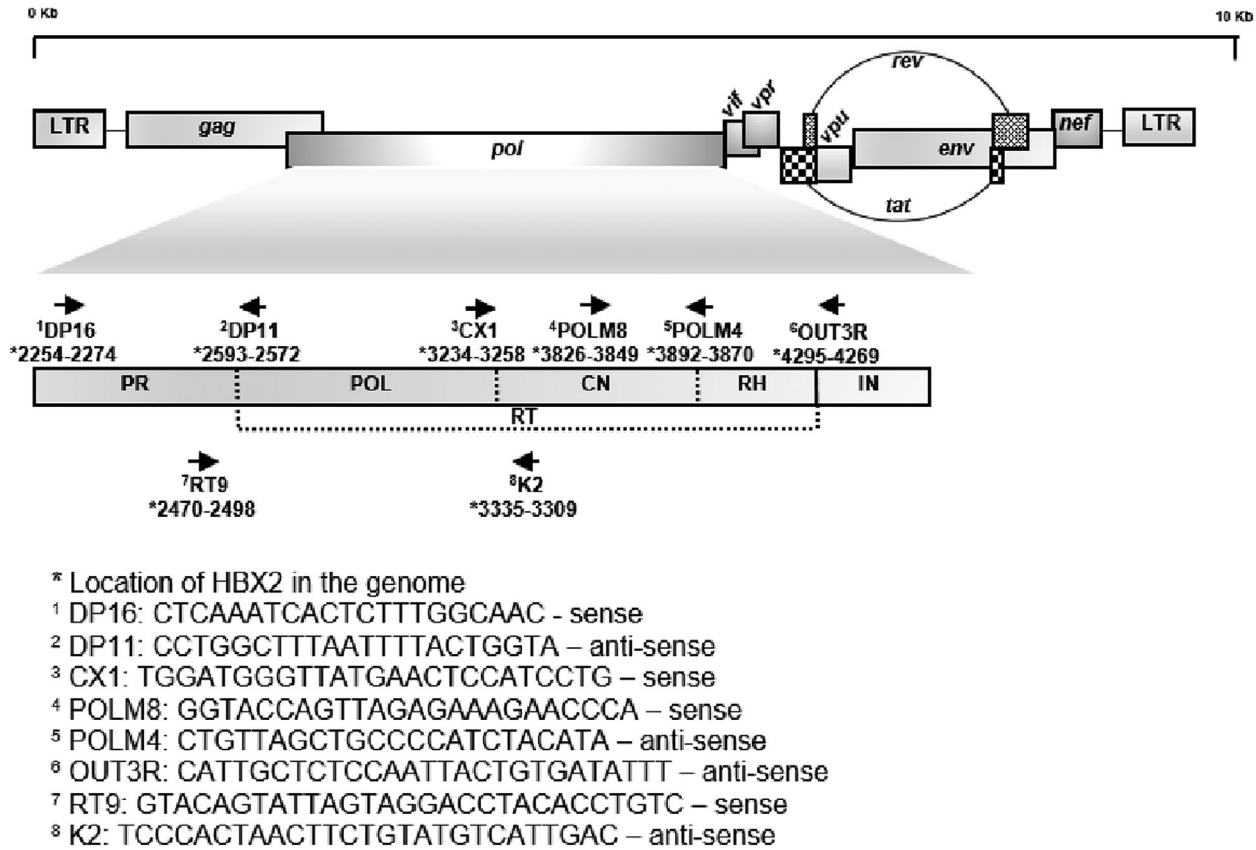


Fig. 1. Genomic map of HIV-1, providing a schematic overview of the position of the designed primersfor PCR, and sequencing according to the relative position of the HBX2 clone (GenBank accession number K03455).

First, the 1,081 bp polymerase domain region of HIV-1 reverse transcriptase (RT) (protease [PR], partial RT) was amplified and sequenced using the primers DP16 (sense), and K2 (antisense) (Fig. 1). If subtyping failed, sequencing was then performed using primers CX1 (sense) and OUT3R (antisense) to amplify the C-terminal portion of the RT containing the subdomain connection (CN), and the RNase H (RH) domain, with approximately 1,061 bp. If the subtype still could not be obtained, we attempted to amplify each viral region separately: CN (658 bp, primers CX1 [sense] and POLM4 [antisense]), RH (469 bp, primers POLM8 [sense] and OUT3R [antisense]), and PR (339 bp, primers DP16 [sense] and DP11 [antisense]) (Fig. 1). This method was used to improve sequencing and obtain the largest possible number of subtypes. The POL region is often used to subtype HIV [Nie et al., 2011]. Nevertheless, samples used in this study were from patients with low viral loads. Thus, the decision was made to sequence the C-terminal portion (CN and RH) of the virus, as it is conserved, and easily amplified and sequenced [Snoeck et al., 2005]. Figure 1 shows a genomic map of HIV-1, providing a schematic overview of the position of the designed primers for PCR, and sequencing according to the relative position of the HBX2 clone (GenBank accession number K03455). PCR products were purified with the GFX PCR DNA and TM-Gel Band Purification kit (GE Healthcare, São Paulo, Brazil) and sequenced using the ABI PRISM ® BigDye™ Terminator Cycle Sequencing Ready Reaction kit (Life Technologies, Foster City, CA). After precipitation, samples were sequenced on an ABI 3130xl Genetic Analyzer (Life Technologies). Chromatograms obtained were manually edited using the SeqMan program (DNAStar, Madison, WI). Sequence alignment was performed using the CLUSTALW algorithm implemented in the BioEdit package. Sequences obtained were then converted into a FASTA format file-consensus for the remaining analyses. To determine HIV subtypes, sequences in FASTA format were aligned with representative sequences of each subtype of HIV-1 (obtained from the database at Los Alamos National Laboratory; http://hiv-web.lanl.gov). Aligned sequences were subjected to phylogenetic analysis using the neighbor-joining method, applying the Kimura two-parameter model, and 100 bootstrap replicates using the software MEGA. Samples suspected of recombination were analyzed using SIMPLOT via plot similarity analysis and bootscanning.

### Statistical Analysis

Data on sociodemographic, behavioral, clinical, and laboratory variables were analyzed. The chi-square test was used for comparing categorical variables. Prevalence ratios, potential risk factors and protective factors were calculated, and frequency distributions and percentages were determined. Differences were considered statistically significant when $P < 0.05$. Multivariate analysis with Poisson regression was also performed, followed by construction of a hierarchical linear model, which incorporated variables with $P \leq 0.20$ on crude analysis. In the first level, socioeconomic variables were inserted; in the second level, behavioral variables were included and in the third level features of HIV infection were evaluated. In a second step, statistical analysis was performed to evaluate the clinical profile of patients with HPgV, and HIV-1. All analyses were carried out in SPSS for Windows v.12 and Epi-info v.3.5.2.

## RESULTS

Plasma samples were obtained from 347 patients harboring different HIV-1 subtypes. The prevalence of HPgV RNA in the samples was 34% (n = 118). Socioeconomic, behavioral, clinical, and laboratory characteristics are shown in Table I. After bivariate analysis (Table I), the only sociodemographic variable significantly associated with HPgV RNA infection was age. HIV-1-infected patients aged <50 years were almost twice as likely to be infected with HPgV (Table I). With respect to behavioral risk factors, sexual activity as measured by the number of sexual partners was a risk factor; subjects with sexual partners were more than twice as likely to be infected with HPgV as subjects without sexual partners. Regarding clinical and laboratory variables, 39 (11.2%) patients had $CD4^+$ T-cell counts below $200/mm^3$, but only seven of those were infected with HPgV (95%CI 0.25–0.99, $P = 0.02$, Table I). HIV viral load was undetectable in 244 (70.3%) of the studied patients, of whom 87 (35.7%) had HIV/HPgV coinfection (95%CI 0.60–1.18, $P = 0.31$). A time elapsed since HIV-1 diagnosis ≥11 years was also a risk factor for HPgV infection (95%CI 1.01–1.84, $P = 0.05$). HIV-1 subtype was determined in 117 subjects (33.7%) (Table II). On univariate analysis, patients infected with HIV-1C were less likely to be infected with HPgV than those with non-C subtypes (95%CI 1.96–5.27, $P < 0.01$; Table I).

On multivariate analysis (Table III), the following variables were identified as independent risk factors for HPgV infection: age <50 years, number of sexual partners ≠ 0, time since HIV diagnosis ≥11 years, and non-C HIV subtype. The remaining variables included in the model had no significant effect.

## DISCUSSION

The present study was the first to evaluate the prevalence of HPgV in $HIV^+$ patients in Southern Brazil, where a distinct HIV-1 clade (HIV-1C) circulates with high frequency. The prevalence of active HPgV infection in coinfected patients was 34% (118). This result was expected when observing the prevalence of HPgV RNA in HIV-infected patients in different areas of the world, which ranges from 16.6% to 88.8% [Heringlake et al., 1998; Souza et al., 2012; Anggorowati et al., 2013;

TABLE I. Sociodemographic, Behavioral, Clinical, and Laboratory Profiles of HIV-1-Infected Patients Analyzed in the Study, Stratified by **HPgV** Positivity

| Variable/category | n (%) | HPgV$^+$ n (%) | Prevalence ratio | 95%CI | $P^a$ |
|---|---|---|---|---|---|
| Age | | | | | |
| ≥51 years | 83 (23.9) | 18 (15.2) | 1.0 | | 0.02 |
| 31–50 years | 211 (60.8) | 81 (68.7) | 1.77 | 1.14–2.76 | |
| 18–30 years | 53 (15.3) | 19 (16.1) | 1.65 | 0.96–2.85 | |
| Skin color | | | | | |
| White | 239 (68.9) | 81 (68.7) | 1.0 | | 0.94 |
| Non-white | 108 (31.1) | 37 (31.3) | 1.01 | 0.74–1.39 | |
| Gender | | | | | |
| Female | 169 (48.7) | 55 (46.6) | 1.0 | | 0.57 |
| Male | 178 (51.3) | 63 (53.4) | 1.09 | 0.81–1.46 | |
| Education | | | | | |
| ≥9 years | 110 (31.7) | 38 (32.2) | 1.0 | | 0.62 |
| ≤4 years | 82 (23.6) | 31 (26.2) | 1.09 | 0.75–1.60 | |
| 5–8 years | 155 (44.7) | 49 (41.6) | 0.92 | 0.65–1.29 | |
| Income[b] | | | | | |
| >2 MW | 48 (13.8) | 21 (17.8) | 1.0 | | 0.26 |
| <1 minimum wage (MW) | 225 (64.9) | 75 (63.6) | 0.76 | 0.53–1.10 | |
| 1–2 MW | 74 (21.3) | 22 (18.6) | 0.68 | 0.42–1.09 | |
| Injectings drug user | | | | | |
| No | 301 (86.7) | 103 (87.3) | 1.0 | | 0.83 |
| Yes | 46 (13.3) | 15 (12.7) | 0.95 | 0.61–1.49 | |
| Inhaled drug user | | | | | |
| No | 234 (67.4) | 73 (61.9) | 1.0 | | 0.11 |
| Yes | 113 (32.6) | 45 (38.1) | 1.28 | 0.95–1.72 | |
| Shared syringes or needles | | | | | |
| No | 278 (80.1) | 92 (78) | 1.0 | | 0.47 |
| Yes | 69 (19.9) | 26 (22) | 1.14 | 0.81–1.61 | |
| Tattoo | | | | | |
| No | 225 (64.8) | 77 (65.3) | 1.0 | | 0.90 |
| Yes | 122 (35.2) | 41 (34.7) | 0.98 | 0.72–1.34 | |
| Blood transfusion | | | | | |
| No | 260 (74.9) | 90 (76.3) | 1.0 | | 0.67 |
| Yes | 87 (25.1) | 28 (23.7) | 0.93 | 0.66–1.32 | |
| Men who had sex with men | | | | | |
| No | 298 (85.9) | 104 (34.9) | 1.0 | | 0.38 |
| Yes | 49 (14.1) | 14 (28.6) | 0.82 | 0.51–1.31 | |
| Number of sexual partners per year | | | | | |
| None | 61 (17.6) | 10 (8.5) | 1.0 | | <0.01 |
| 1–3 partners | 246 (70.9) | 90 (76.3) | 2.23 | 1.24–4.03 | |
| Condom use with regular partner | | | | | |
| Yes | 223 (72.9) | 83 (76.9) | 1.0 | | 0.20 |
| No | 39 (12.7) | 9 (8.3) | 0.62 | 0.34–1.13 | |
| Sometimes | 44 (14.4) | 16 (14.8) | 0.98 | 0.64–1.50 | |
| Preservative use with a casual partner | | | | | |
| Yes | 204 (73.6) | 76 (79.2) | 1.0 | | 0.25 |
| No | 34 (12.3) | 8 (8.3) | 0.63 | 0.34–1.19 | |
| Sometimes | 39 (14.1) | 12 (12.5) | 0.83 | 0.50–1.37 | |
| STD[c] in the past year | | | | | |
| No | 301 (86.7) | 99 (83.9) | 1.0 | | 0.26 |
| Yes | 46 (13.3) | 19 (16.1) | 1.26 | 0.86–1.84 | |
| Hepatitis | | | | | |
| No | 280 (80.7) | 98 (83) | 1.0 | | 0.42 |
| Yes | 67 (19.3) | 20 (17) | 0.85 | 0.57–1.27 | |
| Anti-HCV[d] | | | | | |
| No | 317 (91.4) | 107 (90.7) | 1.0 | | 0.74 |
| Yes | 30 (8.6) | 11 (9.3) | 1.09 | 0.66–1.78 | |
| HbsAg[e] | | | | | |
| No | 337 (97.1) | 116 (98.3) | 1.0 | | 0.34 |
| Yes | 10 (2.9) | 2 (1.7) | 0.58 | 0.17–2.02 | |
| On HAART[f] | | | | | |
| Yes | 309 (89) | 106 (89.8) | 1.0 | | 0.73 |
| No | 38 (11) | 12 (10.2) | 0.92 | 0.56–1.51 | |
| Years since diagnosis of HIV-1 infection | | | | | |
| ≤10 years | 252 (72.6) | 78 (66.1) | 1.0 | | 0.05 |
| ≥11 years | 95 (27.4) | 40 (33.9) | 1.36 | 1.01–1.84 | |

TABLE I. (*Continued*)

| Variable/category | n (%) | HPgV$^+$ n (%) | Prevalence ratio | 95%CI | $P^a$ |
|---|---|---|---|---|---|
| CD4$^+$ cell count (cells/mm$^3$) | | | | | |
| >200 | 308 (88.8) | 111 (94) (36) | 1.0 | | 0.02 |
| ≤200 | 39 (11.2) | 7 (6) (17.9) | 0.50 | 0.25–0.99 | |
| HIV load copies/ml | | | | | |
| Undetectable | 244 (70.3) | 87 (73.7) | 1.0 | | 0.31 |
| >50 copies/ml | 103 (29.7) | 31 (26.3) | 0.84 | 0.60–1.18 | |
| HIV-1 subtype | | | | | |
| C | 68 (58.1) | 14(20.6) (19.1) | 1.0 | | <0.01 |
| Non-C | 49 (41.9) | 24(49) | 3.22 | 1.96–5.27 | |

[a]Chi-square test.
[b]MW at the time of the study: approximately US$ 300.00.
[c]Sexually transmitted disease.
[d]As positivity for antibodies against the hepatitis C virus.
[e]As marker of active hepatitis B virus infection.
[f]Antiretroviral therapy.

Lee et al., 2014; Sahni et al., 2014]. Other Brazilian studies found a similar prevalence of HPgV RNA in HIV-1-positive patients, estimated between 21% and 30% [Alcalde et al., 2010; Campos et al., 2011; Giret et al., 2011; Souza et al., 2012], but Southern Brazil may have slightly higher rates of coinfection when compared to the rest of the country.

Of the sociodemographic variables of interest, age between 18–30 and 31–50 years were the only ones to reach statistical significance. Patients aged 18–30 were almost eight times more likely to be coinfected, and those aged 31–50 years were almost six times more likely to have such infection when compared to those aged >50 years. The present study suggests that the risk of HPgV infection declines as age advances, unlike in study performed by Ribeiro-dos-Santos et al. [2002], where advancing age was shown to be a risk factor for HPgV infection. Younger people are generally more sexually active, which can increase the odds of HPgV infection. Sexual activity tends to decrease with age, thereby decreasing the risk of infection [Barbosa and Koyama, 2005]. Viral clearance must also be considered, because it likely decreases the frequency of active HPgV infection in previously infected individuals aged >50, an estimate we were unable to perform in the present study.

It should be noted that 32.6% of injecting drug users, 39.8% of inhalant users, 37.7% of participants who had ever shared hypodermic needles, 33.6% of those with tattoos, and 32.2% of those who had ever received blood transfusion were coinfected with HPgV/HIV RNA. Despite this factor, the parenteral route of transmission was not significantly associated as a risk factor for HPgV infection in this study. Although the parenteral route is the one most documented in the literature and is considered very effective, mainly through blood and blood products [Abe, 2001], sexual transmission of HPgV is also well documented [Ramezani et al., 2008]. In a study carried out in São Paulo, sexual transmission of HPgV was found to be very efficient [Ribeiro-dos-Santos et al., 2002]. The virus was more prevalent in sexually active individuals, especially those engaging in high-risk sexual practices, such as men who have sex with men [Ribeiro-dos-Santos et al., 2002].

CD4$^+$ T cell counts lost statistical significance after the adjusted analysis, but it should be stressed that 94% of participants with HPgV/HIV-1 coinfection had values >200 cells/mm$^3$. Likewise for the variable HIV-1viral load, even though 73.7% of coinfected individuals had undetectable HIV viral loads, there was no significant association. These findings are similar to those observed a Brazilian cohort of 233 homosexual men with recent HIV infection in which there was no significant difference in age, sex, HIV viral load, or CD4$^+$ T cell count [Giret et al., 2011].

TABLE II. Results of HIV-1 Genotyping

| Study | HIV B n (%) | HPgV+ n (%) | HIV C n (%) | HPgV+ n (%) | HIV F n (%) | HPgV+ n(%) | HIV CRF[a] n (%) | HPgV+ n(%) | Total[b] n (117) (%) |
|---|---|---|---|---|---|---|---|---|---|
| This study [2016] | 12 (10.3) | 6 (50) | 34 (29) | 8 (25.5) | 0 (0) | 0 (0) | 7 (6) | 3 (42.8) | 53 (45.3) |
| Silveira et al. [2012] | 8 (6.8) | 5 (62.5) | 25 (21.3) | 2 (8) | 6 (5.1) | 2 (33.3) | 3 (2.6) | 2 (40) | 42 (35.9) |
| Network of the Brazilian Ministry of Health | 7 (6) | 4 (57.1) | 9 (7.8) | 4 (44.4) | 1 (0.9) | 0 (0) | 5 (4.3) | 2 (40) | 22 (18.8) |
| Total | **27 (23.1)** | **15 (55.5)** | **68 (58.1)** | **14 (20.6)** | **7 (6)** | **2 (28.6)** | **15 (12.8)** | **7 (46.6)** | **117 (100)** |

[a]Circulating recombinant forms include strains consisting of more than one subtype reached an epidemic spreading [Robertson et al., 2000].
[b]Total of samples HIV-1 in the study with known genotype.

TABLE III. Multivariate Analysis of Characteristics Associated With HPgV Infection in a Sample of HIV-1-Infected Patients in Southern Brazil

| Variables | Prevalence ratio | 95%CI | *P* |
|---|---|---|---|
| Age | | | |
| ≥51 years | 1.0 | | |
| 31–50 years | 5.85 | 1.05–32.44 | **0.04** |
| 18–30 years | 7.87 | 1.18–52.36 | **0.03** |
| Inhaled drug user | | | |
| No | 1.0 | | |
| Yes | 1.01 | 0.50–2.04 | 0.96 |
| Number of sexual partners per year | | | |
| None | 1.0 | | |
| 1–3 | 3.97 | 1.54–10.23 | <**0.01** |
| ≥4 | 2.69 | 1.27–5.68 | <**0.01** |
| Years since the diagnosis of HIV- | | | |
| ≤10 years | 1.0 | | |
| ≥11 years | 1.71 | 1.01–2.89 | **0.04** |
| $CD4^+$ T cell count (cell /$mm^3$) | | | |
| >200 | 1.0 | | |
| ≤200 | 2.29 | 0.94–5.58 | 0.06 |
| HIV-1 subtype | | | |
| C | 1.0 | | |
| Non-C | 5.80 | 2.28–14.78 | <**0.01** |

The above variables were adjusted for age and duration of HIV infection.
Bold indicates statistically significant results.

In another cohort in Brazil, with 248 $HIV^+$ women, the prevalence of HPgV/HIV-1 RNA coinfection was 23%, and there was no significant difference in HIV viral load or $CD4^+$ cell count between groups exposed and not exposed to HPgV [Campos et al., 2011].

In this study, the majority of individuals coinfected with HPgV/HIV-1 had lived with HIV for more than 11 years. Coinfection was associated with increased survival in HIV-1-positive patients. Similar results were found in a study of 271 $HIV^+$patients who were evaluated from seroconversion for a period of 11 years [Williams et al., 2004], in which active HPgV infection was found to lead to greater survival. Another study evaluated a cohort of 197 patients with HIV/AIDS under highly active antiretroviral therapy (HAART) and found that HPgV RNA-positive patients had lower HIV viral load, slower disease progression, improvement in survival, and increased $CD4^+$ T-cell counts compared to HPgV-uninfected patients [Tillmann et al., 2001]. Coinfection has been associated with reduced mortality rates in $HIV^+$ patients even after introduction of HAART [Tillmann et al., 2001]. Our data are consistent with these findings, but additional studies are needed to evaluate whether there are other confounders or unstudied factors involved, as some reports have failed to show slower progression of HIV infection in coinfected individuals [Quiros-Roldan et al., 2002; Kaye et al., 2005].

Despite several studies assessing the genotypic diversity of HPgV in HIV/HPgV coinfection [Nishiya et al., 2003; Schwarze-Zander et al., 2006; Xiang et al., 2012; Timmons et al., 2013], there is little information correlating HIV genotypic diversity in such coinfection. In a study of 210 $HIV^+$ patients in São Paulo, Brazil, circulation of HIV-1C was very low (0.5%), and only 1.8% of HIV/HPgV-coinfected individuals carried the HIV-1C subtype [Alcalde et al., 2010]. Conversely, in the population assessed herein, we found high rates of HIV-1C circulation, and a significant association between HIV-1 non-C subtypes and HPgV infection. Unlike in other regions of Brazil, where the predominant HIV-1 subtype is B, incidence of HIV-1C is increasing in Southern Brazil [Martinez et al., 2002; Silveira et al., 2012]. Whether HIV-1C-infected subjects are less prone to contracting HPgV cannot be stated based on current data; as this was a cross-sectional study of prevalence, we were unable to identify which infection (HIV or HPgV) occurred first.

In conclusion, younger individuals with more sexual partners were more susceptible to HPgV infection in our study. HIV/HPgV coinfection was associated with longer survival and higher $CD4^+$ T-cell counts among $HIV^+$ patients. In a novel finding, individuals infected with non-C HIV subtypes were more susceptible to HPgV infection. However, additional studies are needed to ascertain whether specific HIV-1 subtypes correlate with HPgV infection, and to elucidate the cellular and molecular pathways that underlie these potential associations.

## ACKNOWLEDGMENTS

The authors would like to thank the Fundação Pró-Sangue de São Paulo, the HIV/AIDS HU-FURG Service, and the Oncovirology Program at the Brazilian National Cancer Institute for making this study possible with laboratory support.

## REFERENCES

Abe K. 2001. GB virus C/hepatitis G virus. Jpn J Infect 54:55–63.

Adams MJ, King AMQ, Carstens EB. 2013. Ratification vote on taxonomic proposals to the International Committee on Taxonomy of Viruses (2013). Arch Virol 58:2023–2030.

Alcalde R, Nishiya AS, Casseb J, Inocêncio L, Fonseca LA, Duarte AJ. 2010. Prevalence and distribution of the GBV-C/HGV among HIV-1 infected patients under antirretroviral terapy. Virus Res 151:148–152.

Anggorowati N, Yano Y, Subronto W, Utsumi T, Heriyanto DS, Mulya DP, Rinonce HT, Widasari DI, Lusida MI, Soetjipto, Hayashi Y. 2013. GB virus C infection in Indonesian HIV-positive patients. Microbiol Immunol 57:298–308.

Barbosa RM, Koyama MAH. 2008. Sexual behavior and practices among men and women, Brazil 1998 and 2005. Rev Saúde Pública 42:1–12.

Berzsenyi MD, Roberts SK. 2006. What is the role of GB virus C infection in hepatitis C virus/HIV coinfection? J Infect Dis 194:407–409.

Campos AF, Tengan FM, Silva SAA, Levi JE. 2011. Influence of hepatitis G virus (GB virus C) on the prognosis of HIV-infected women. Int J STD AIDS 22:209–213.

Chivero ET, Stapleton JT. 2015. Tropism of human pegivirus (formerly known as GB virus C/Hepatitis G virus) and host immunomodulation: Insights into a highly successful viral infection. J Gen Virol 96:1531–1532.

Deinhardt F, Homes AW, Capps RB, Popper H. 1967. Studies on the transmission of human viral hepatitis to marmoset monkeys. Transmission of disease, serial passages, and description of liver lesions. J Exp Med 125:673–688.

Ernst D, Greer M, Akmatova R, Pischke S, Wedemeyer H, Akmatova R, Pischke S, Wedemeyer H, Heiken H, Tillmann HL, Schmidt RE, Stoll M. 2013. Impact of GB virus C viraemia on clinical outcome in HIV-1-infected patients: A 20-year follow-up study. HIV Med 15:245–250.

Feng Y, Zhao W, Daí J, Li Z, Zhang X, Liu L, Bai J, Zhang H, Lu L, Xia X. 2011. A novel genotype of GB virus C: Its identification and predominance among injecting drug users in Yunnan, China. PLoS ONE 6:1–10.

Feucht HH, Zollner B, Polywka S, Knodler B. 1997. Prevalence of hepatitis G viremia among healthy subjetcs, individuals with liver disease, and persons at risk of parenteral transmission. J Clin Microbiol 35:767–768.

George SL, Varmaz D, Tavis JE, Chowdhury A. 2007. The GB virus C (GBV-C) NS3 serine protease inhibits HIV-1 replication in a CD4+ T lymphocyte cell line without decreasing HIV receptor expression. PLoS ONE 7:1–8.

Giret MTM, Silva TM, Sauer MM, Tomiyama H, Levi JE, Bassichetto KC, Nishiya A, Diaz RS, Sabino EC, Palacios R, Kallas EG. 2009. GB virus type C infection modulates T-cell activation independently of HIV-1 viral load. AIDS 23:2277–2287.

Giret MTM, Miraglia JL, Sucupira MCA, Nishiya A, Levi JE, Diaz RS, Sabino EC, Kallas EG. 2011. Prevalence, incidence density, and genotype distribution of GB virus C infection in a cohort of recently HIV-1-infected subjects in Sao Paulo, Brazil. PLoS ONE 6:1–9.

Gretch D. 2012. Advocating the concept of GB virus C biotherapy against AIDS. Ckin Infec Dis 55:1020–1021.

Hemelaar J, Gouws E, Ghys P, Osmanov S. 2011. Global trends in molecular epidemiology of HIV-1 during 2000–2007. AIDS 25:679–689.

Heringlake S, Ockenga J, Tillmann HL, Trautwein C, Stool M, Hunt J, Jou C, Solomon N, Schmidt RE, Manns MP. 1998. GB virus C/hepatitis G virus infection: A favorable prognostic factor in human immunodeficiency virus-infected patients? J Infect Dis 177:1723–1726.

Jung S, Eichenmüller M, Donhauser N, Neipel F, Engel AMB, Hess G, Fleckenstein B, Reil H. 2007. HIV entry inhibition by the envelope 2 glycoprotein of GB virus C. AIDS 21:645–647.

Kaye S, Howard M, Alabi A, Hansmann A, Whittle H, Schim van der Loeff M. 2005. No observed effect of GB virus C coinfection on disease progression in a cohort of African woman infected with HIV-1 or HIV-2. Clin Infect Dis 40:876–878.

Lee CK, Tang JWT, Chiu L, Loh TP, Olszyna D, Chew N, Archuleta S, Koay ESC. 2014. Epidemiology of GB virus Type C among patients infected with HIV in Singapore. J Med Virol 86:737–744.

Martinez AMB, Barbosa EF, Ferreira PC, Cardoso FA, Silveira J, Sassi G, Silva M, Mendonça SV, Antunes CMl. 2002. Molecular epidemiology of HIV-1 in Rio Grande, RS, Brazil. Rev Soc Bras Med Trop 35:471–476.

Mohr EL, Stapleton JT. 2009. GB virus type C interactions with HIV: The role of envelope glycoproteins. J Viral Hepat 16:757–768.

Nie T, Detorio M, Schinazi RF. 2011. Universal profiling oh HIV-1 *pol* for genotypic study and resistance analysis across subtypes. Antivir Ther 16:1267–1275.

Nishiya AS, Ribeiro-Dos-Santos G, Bassit L, Focaccia R, Chamone DF, Sabino EC. 2003. Genotype distribution of the GB virus C in citizens of São Paulo city Brazil. Instituto de Medicina Tropical de São Paulo 45:213–216.

Oliveira LA, Martins RM, Carneiro MA, Teles SA, Silva SA, Cardoso DD, Lampe E, Yoshida CF. 2002. Prevalence and genotypes of GB virus C/hepatitis G virus among blood donors in Central Brazil. Memórias do Instituto Oswaldo Cruz 97:953–957.

Pavesi A. 2001. Origin and evolution of GBV-C/hepatitis G virus and relationships with ancient human migrations. J Mol Evol 53:104–113.

Quiros-Roldan E, Maroto MC, Torti C, Moretti F, Casari S, Pan A, Carosi G. 2002. No evidence of beneficial effect of GB virus type C infection on the course of HIV infection. AIDS 16:1430–1431.

Ramezani A, Mohraz M, Vahabpour R, Jam S, Banifazl M, Eslamifar A, Mahboudi F, Aghakhani A, Edalat R, Hekmat S. 2008. Frequency of hepatitis G virus infection among HIV positive subjects with parenteral and sexual exposure. J Gastrointestin Liver Dis 17:267–272.

Ramia S, Mokhbat J, Sibai A, Klayme S, Naman R. 2004. Exposure rates to hepatitis C and G virus infections among HIV-infected patients: Evidence of efficient transmission of HGV by the sexual route. Int J STD AIDS 15:463–466.

Ribeiro-dos-Santos G, Nishiya AS, Nascimento CM, Bassit L, Chamone DF, Focaccia R, Eluf-Neto J, Sabino EC. 2002. Prevalence of GB virus C (hepatitis G virus) and risk factors for infection in São Paulo, Brazil. Eur J Clin Microbiol Infect Dis 21:438–443.

Robertson DL, Anderson JP, Bradac JA, Carr JK, Foley B, Funkhouser RK, Gao F, Hahn BH, Kalish ML, Kuiken C, Learn GH, Leitner T, McCutchan F, Osmanov S, Peeters M, Pieniazek D, Salminen M, Sharp PM, Wolinsky S, Korber B. 2000. HIV-1 Nomenclature Proposal. Science 288:55–57.

Sánchez-Martín MJ, Hristova K, Pujol M, Gómara MJ, Haro I, Alsina MA, Busquets MA. 2011. Analysis of HIV-1 fusion peptide inhibition by synthetic peptides from E1 protein of GB virus C. J Colloid Interface Sci 360:124–131.

Sahni H, Kirkwood K, Kyriakides TC, Stapleton J, Brown ST, Holodniy M. 2014. GBV-C viremia and clinical events in advanced HIV infection. J Med Virol 86:426–432.

Schwarze-Zander C, Blackard JT, Zheng H, Addo MM, Lin W, Robbins GK, Sherman KE, Zdunek D, Hess G, Chung RT. 2006. GB virus C (HPgV) infection in hepatitis C virus (HCV)/ HIVcoinfected patients receiving HCV treatment: Importance of the HPgV genotype. J Infect Dis 194:410–419.

Silveira J, Santos AF, Martinez AM, Goes LR, Mendonsa RMM, Muniz CO, Tupinambas U, Soares MA, Greco DB. 2012. Heterosexual transmission of human immunodeficiency virus type 1 subtype C in southern Brazil. J Clin Virol 54:36–41.

Simon F, Mauclere P, Roques P, Loussert-Ajaka I, Müller-Trutwin MC, Saragosti S, Georges-Courbot MC, Barré-Sinoussi F, Brun-Vézinet F. 1998. Identification of a new human imunodeficiency virus type 1 distinct from group M and O. Nat Med 4:1032–1037.

Snoeck JA, Riva C, Steegen K, Schrooten Y, Maes B, Vergne L, Van Laethema K, Peeters M, Vandamme A-M. 2005. Optimization of a genotypic assay applicable to all human immunodeficiency virus type 1 protease and reverse transcriptase subtypes. J Virol Methods 128:47–53.

Souza AKV, Komninakis VCS, Zappia BLG, Barbosa JÁ, Mantovani PN, Diaz SR, Abrao P, Lanzara AG, Granato HFCl. 2012. Evaluation of GB vírus C/hepatitis G viral load among HIV type 1 co-infected patients in São Paulo, Brazil. AIDS Res Hum Retroviruses 28:289–294.

Stapleton JT, Chaloner K, Martenson JA, Zhang J, Klinzman D, Xiang J, Sauter W, Desai SN, Landay A. 2012a. GB virus C infection is associated with altered lymphocyte subset distribution and reduced T cell activation and proliferation in HIV-infected individuals. PLoS ONE 7:1–9.

Stapleton JT, Muerhoff AS, Foung S, Simmonds P, 2012b Assignment of human, simian and bat pegiviruses (previously described as GBV-A, GBV-C, and GBV-D) as members of a new Genus (Pegivirus) within the Flaviviridae; <http://www.ictvonline.org/proposals/2012.011a-dV.A.v2.Pegivirus.pdf>.

Stapleton JT, Martinson JA, Klinzman D, Xiang J, Desai SN, Landay A. 2013. GB virus C infection and B-cell, natural killer cell, and monocyte activation markers in HIV-infected individuals. AIDS 27:1829–1832.

Tillmann HL, Heiken H, Knapik-Botor A, Heringlake S, Ockenga J, Wilber JC, Goeren B, Detmer J, Mcmorrow M, Stoll M, Schidt RE, Manns MP. 2001. Infection with GB virus C and reducet mortality among HIV-co-infected patients. N Engl J Med 345:715–724.

Timmons CL, Shao Q, Wang C, Liu L, Liu H, Dong X, Liu B. 2013. GB virus type C E2 protein inhibits human immunodeficiency virus type 1 assembly through interference with HIV-1 gag plasma membrane targeting. J Infect Dis 207:1171–1180.

Toyoda H, Fukuda Y, Hayakawa T, Takamatsu J, Saito H. 1998. Effect of GB virus C/hepatitis G virus coinfection on the course of HIV infection in hemophilia patients in Japan. J Acquir Immune Defic Syndr Hum Retrovirol 17:209–213.

Vahidnia F, Petersen M, Stapleton JT, Rutherford GW, Busch M, Custer B. 2012. Acquisition of GB virus type C (HGV) and lower mortality in patients with advanced HIV disease. Clin Infect Dis 55:1012–1019.

Williams CF, Klinzman D, Yamashita TE, Xiang J, Polgreem PM, Rinaldo C, Liu C, Phair J, Margolick JB, Zdunek D, Hess G, Stapleton JT. 2004. Persistent GB virus C infection an survival in HIV infected men. N Engl J Med 350:981–990.

Xiang J, Wünschmann S, Diekema DJ, Klinzman D, Patrick KD, George SL, Stapleton JT. 2001. Effect of co-infection with GB virus C (hepatitis G virus) on survival among patients with HIV infection. N Engl J Med 345:707–714.

Xiang J, McLinden JH, Kaufman TM, Mohr EL, Bhattarai N, Chang Q, Stapleton JT. 2012. Characterization of a peptide domain within the GB virus C envelope glycoprotein (E2) that inhibits HIV replication. Virology 430:53–62.

Zhang W, Chaloner K, Tillmann HL, Williams CF, Stapleton JT. 2006. Effect of earlyand late GB virus C viraemia on survival of HIV-infected individuals: A meta-analysis. HIV Med 7:173–180.