

Major Article

Profile of HIV subtypes in HIV/HBV- and HIV/HCV-coinfected patients in Southern Brazil

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Abstract

Introduction: HIV and viral hepatitis infections are major causes of chronic disease worldwide and have some similarities with regard to routes of transmission, epidemiology, front barriers faced during access of treatment, and strategies for a global public health response. The objective was to describe the HIV-1 subtypes, viral tropism and single-nucleotide polymorphisms (SNPs) of interleukin 28B (*IL28B*) from a case series of HIV/viral hepatitis coinfected patients from southern Brazil. **Methods:** Clinical and epidemiological data were evaluated by a review of medical records. Periodic blood draws were taken to determine the viral and host characteristics. **Results:** This study included 38 patients with HIV/HBV or HIV/HCV coinfection; the median age was 49 years. Thirty-seven (97.4%) were on antiretroviral therapy, 32 (84.2%) had an undetectable viral load, a median CD4⁺ T-cell count of 452 cells/mm³. HIV-1 subtyping showed 47.4 and 31.6% of patients with subtypes C and B, respectively. Analysis of viral co-receptor usage showed a predominance of the R5 variant (64.7%), with no significant difference between the subtypes. Twenty patients with HIV/HCV coinfection were eligible to receive HCV therapy with pegylated-interferon-alpha plus ribavirin, and 10/20 (50%) of them achieved sustained virological response. SNPs of *IL28B* were evaluated in 93.3% of patients with HIV/HCV coinfection, and 17 (60.7%) presented the CC genotype. **Conclusions:** In the present case series, a higher frequency of HIV subtype C was found in coinfected patients. However such findings need to be prospectively evaluated with the inclusion of data from regional multicenter analyses.

Keywords: HIV. hepatitis C virus. hepatitis B virus. Coinfection. CCR5. *IL28B*.

INTRODUCTION

It has been estimated that 30% of people living with human immunodeficiency virus/acquired immunodeficiency syndrome (PLWHA) are coinfected with hepatitis C virus (HCV) or hepatitis B virus (HBV)¹ However, the prevalence of these coinfections is variable, reflecting differences in the regional sociodemographic profile and hepatitis B immunization coverage². In Brazil, the prevalence of HIV/HCV coinfection ranged from 3.3% to 82.4%, with an average of around 20.3%³. The introduction of highly active antiretroviral therapy (HAART) for PLWHA has increased their life expectancy, and chronic liver disease has become common among coinfected individuals, being the most frequent cause of hospitalization and mortality in these patients⁴. Meanwhile, the effect of coinfection

on HIV evolution is not completely understood; these patients present with frequent hepatotoxicity after HAART, and drug resistance, which can lead to treatment failure and limitations in alternative treatment regimens⁵.

In Brazil, HIV diversity is driven by the co-circulation of HIV-1 subtypes B, C, and F1. In recent years, several HIV-1 recombinant forms, mainly BF1 and BC subtypes, have frequently been reported⁶. This viral diversity, albeit controversial, may have some effect on transmission patterns, pathogenicity, therapy, and immune response. In addition, it is associated with various host cells and viral mechanisms. In the context of HIV/viral hepatitis coinfection, there are no reports on the genetic profile of HIV infection and the host immunological profile of these coinfected individuals in Brazil, as well as its effect on hepatic and HIV infection progression. This study aimed to describe the HIV subtypes and the epidemiological, immunological, and clinical profiles of HIV/viral hepatitis-coinfected patients from a reference center in the State of Paraná, Southern Brazil.

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METHODS

Study participants

A cross-sectional study was conducted, in which coinfecting outpatients identified in the referral clinics of *Hospital de Clínicas - Universidade Federal do Paraná* (HC/UFPR) from March 2011 to December 2015 were prospectively included. This tertiary-care academic hospital is the largest HIV referral center in the State of Paraná, southern Brazil, where 1,400 PLWH are currently being followed up, with HIV/HBV and HIV/HCV coinfection rates of approximately 1.3% and 4%, respectively⁷. HIV/HBV- or HIV/HCV-coinfecting patients were enrolled in the study according to the following inclusion criteria: age >18 years; positive serology for HIV (anti-HIV), positive serology for HBV (HBV-surface antigen or DNA), and/or polymerase chain reaction (PCR)-positive HCV; and voluntary participation by providing written informed consent. Patients with incomplete medical records, children, and those who did not agree to participate in the study were excluded. All information related to clinical, demographic and epidemiological data were obtained through a structured questionnaire and review of medical records. Venous blood was collected from the enrolled patients. The Institutional Review Board of HC/UFPR approved the study (IRB# 2304.198/2010/08).

Proviral DNA extraction, PCR amplification, and HIV-1 sequencing

A concentration of 10^7 peripheral blood mononuclear cells was separated by density gradient centrifugation (Histopaque® 1077 – Sigma-Aldrich, St. Louis, MO, USA). Proviral HIV-1 DNA was extracted using a commercial QIAmp DNA Blood Kit® (Qiagen, Hilden, Germany) according to the manufacturer's protocol.

Fragments of the *pol* (HXB2 clone positions 2077-3574) and *env* (HXB2 clone positions 7001-7667) genes were generated by nested PCR according to a previously reported method^{8,9}. The DNA fragments were purified and sequenced with the BigDye® Terminator v3.1 Cycle Sequencing Kit and the automated DNA sequencer (ABI 3500xL Genetic Analyzer; Applied Biosystems, Waltham, MA, USA) according to the manufacturer's instructions.

Analysis of the genetic diversity of HIV-1

Nucleotide sequences were edited using DNASTAR Lasergene SeqMan software v7.0 (DNASTAR Inc., Madison, WI, USA). For performing maximum likelihood phylogenetic analyses, HIV-1 group M subtype reference sequences of HIV-1 subtypes were retrieved from the Los Alamos database, (<http://www.hiv.lanl.gov/components/sequence/HIV/search/search.html>), and the tree was constructed using the best model with the algorithm implemented in the MEGA 6.0 package¹⁰. Recombination analysis was performed by bootscanning analysis using Simplot 3.5.1 software¹¹. The subtype assignment of sequences was confirmed using the REGA HIV Subtyping Tool v.3.0 (<http://hivdb.stanford.edu/RegaSubtyping/stanford-hiv/typingtool/http://hivdb.stanford.edu/RegaSubtyping/stanford-hiv/typingtool/http://hivdb.stanford.edu/RegaSubtyping/stanford-hiv/typingtool/http://hivdb.stanford.edu/RegaSubtyping/stanford-hiv/typingtool/>).

Resistance analysis

The resistance-associated mutation profile and antiretroviral (ARV) susceptibility were inferred by submitting the PR/RT sequences to the Calibrated Population Resistance Tool (v5.0 beta; <http://cpr.stanford.edu/cpr/servlet/CPR>) and the INT sequences to the Stanford HIV Resistance Database (<http://hivdb.stanford.edu/>).

Characterization of the CCR5 polymorphism and determination of HIV-1 co-receptor tropism phenotype

CCR5 receptor polymorphisms were analyzed in blood samples by PCR as previously described¹². Genotypic tropism test was performed using HIV envelope V3 loop sequences. Viral co-receptor usage predictions based on V3 sequences were performed by 11/25 rules, using Geno2pheno (<http://coreceptor.bioinf.mpg.de/cgi-bin/coreceptor.pl>) with a false-positive rate of 0.1, and using the Phenoseq platform (https://www.burnet.edu.au/facilities/9_phenoseq).

Testing for SNPs of IL28B

The single-nucleotide polymorphism (SNP) rs12979860 in the region of the interleukin 28B (IL28B) gene was analyzed using confronting two-pair primers as previously described¹³.

Statistical analyses

Demographic, epidemiological, clinical, and genetic data were compiled and analyzed using GraphPad Prism® software version 5.03 (Graph Pad Software Inc., La Jolla, CA, USA). Baseline demographic and clinical characteristics were presented as medians and interquartile ranges (IQRs). Univariate analysis using the Fisher's exact test was performed for each variable. The level of significance was set at 0.05.

Nucleotide sequence accession numbers

The sequences reported in this study were deposited in GenBank under accession numbers KU820990 to KU821019 and KU8823632 to KU883661.

RESULTS

A total of 38 HIV-positive patients were included in the study. Of these, 30 (79%) had HIV/HCV coinfection, while 8 (21%) had HIV/HBV coinfection. Most patients were male ($n = 27$; 71%), and the median age was 49 years (IQR: 44-75 years). With regard to the risk factors for HIV/HCV/HBV acquisition, 22 (58%) patients were drug users, with 14 (42.1%) being injection drug users. In general, the patients included in the study had a long duration of HIV infection, with a median duration of 17 years (IQR: 12-25 years); 32 (84.2%) patients had an undetectable viral load and showed an improvement in cell-mediated immune function, with a median nadir of 147 cells/mm³ and a CD4⁺ T-cell count of 452 cells/mm³. Thirty-seven (97.4%) patients had been treated with ART for a median duration of 14 years (IQR: 9-25 years).

The HIV *pol* and/or *env* genes of all patients were sequenced, with 47.4% ($n = 18$) of patients belonging to subtype C, 31.6% ($n = 12$) to subtype B, and 21% ($n = 8$) to circulating recombinant

forms (CRFs) (**Figure 1**). Genetic polymorphisms of the CCR5 co-receptor were evaluated in 35 individuals, and no case of partial deletion was found. Regarding viral receptor usage, there was a predominance of R5 tropism between B and C subtypes with no significant difference.

With regard to ARV resistance, six (15.8%) patients showed mutations related to resistance to nucleoside reverse transcriptase inhibitors (NRTI) and one (2.6%) showed mutations related to resistance to non-NRTI; however, no mutation to protease inhibitors was found. Three (7.9%) patients had died. The epidemiological and clinical characteristics according to the HIV subtypes of all participants are summarized in **Table 1**.

Among the HIV/HBV-coinfected patients, seven (87.5%) were on ART. Three (43%) patients received HBV-specific therapy, which comprised a dual therapy of lamivudine and tenofovir, and one (14.3%) patient received a combination therapy of conventional interferon and lamivudine. There were no records of laboratory investigations of HBV treatment response.

Among the HIV/HCV-coinfected individuals, 20/30 (66.7%) were eligible for HCV therapy based on pegylated interferon alpha and ribavirin (PEG-IFN α /RBV); of these, 10/20 (50%) achieved sustained virological response (SVR), 9/20 (45%) showed no response, and 1/20 (5%) had relapsed. Regarding the SNPs of *IL28B* (rs12979860), 28/30 (93.3%) of patients were

examined, of which 16 (57.1%) presented the CC genotype (**Table 2**).

DISCUSSION

Most studies on the association of viral and host genetic profiles with the outcome and therapy response in HIV/viral hepatitis-coinfected patients have been conducted in populations infected with HIV-1 subtype B. This is the commonest variant in the USA and Western Europe but represents less than 15% of HIV-1 infections worldwide. This knowledge should not be directly extrapolated to all subtypes given the significant sequential differences observed in both the structural and regulatory genes of HIV-1 subtypes, which can influence the biological properties of the virus¹⁴.

A previous study conducted in southern Brazil have reported that subtype C is more prevalent in the heterosexual population, whereas subtype B is more prevalent in men who have sex with men (MSM) and men who have sex with men and women (MSMW)⁶. This greater predisposition of heterosexual transmission of subtype C compared with other subtypes seems to be related to the stronger preference for the female genital mucosa, which may also enable vertical transmission¹⁵.

With regard to the distribution by gender, age, the risk of contamination, and sexual behavior, HIV subtype prevalence in

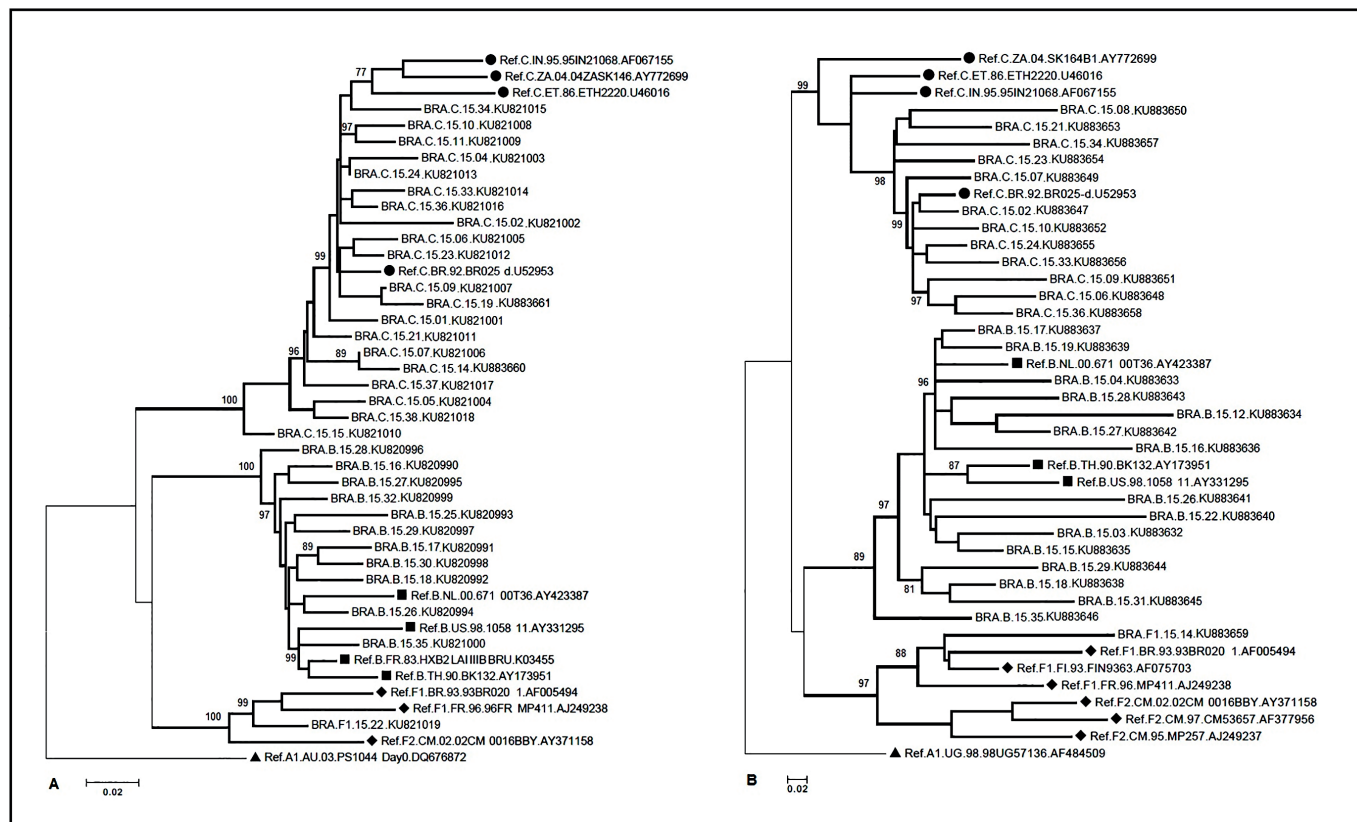


FIGURE 1 - Maximum likelihood phylogenetic trees for the *pol* (A) and *env* (B) HIV genes from the HIV/HCV- or HIV/HBV-coinfected Brazilian patients. Reference sequences (REF) were obtained from the Los Alamos National Laboratory database (<http://www.hiv.lanl.gov>) and are indicated by arbitrary symbols (■ = Subtype B; ◆ = Subtype F; ● = Subtype C; ▲ = Subtype A). Numbers at branch nodes refer to the bootstrap support (only values greater than 75% are shown). Scale bar, 0.2 nucleotide substitutions per site. **HIV**: human immunodeficiency virus; **HCV**: hepatitis C virus; **HBV**: hepatitis B virus; **BRA**: Brazil.

TABLE 1
Epidemiological, clinical and viral characteristics of coinfecting patients according to the HIV subtype.

Characteristics	Subtype			p-value [#]
	B	C	Other*	
	(n = 12)	(n = 18)	(n = 8)	
	N (%)	N (%)	N (%)	
Gender				
male	9 (75.0)	11 (61.1)	7 (87.5)	0.10
female	3 (25.0)	7 (38.9)	1 (12.5)	
Age, years (median [IQR])				
	47 (44-75)	49 (44-75)	49 (44-75)	
Drug use				
yes	5 (41.6)	14 (77.8)	3 (37.5)	0.04
IVDU	3 (60.0)	9 (64.3)	2 (66.7)	
other	2 (40.0)	5 (35.7)	1 (33.3)	
no	7 (58.4)	3 (16.7)	3 (37.5)	
NI	0	1 (5.5)	2 (25.0)	
Sexual behavior				
MSM	2 (16.7)	1 (5.6)	0	NA
heterosexual	7 (58.3)	16 (88.8)	6 (75)	
bisexual	3 (25.0)	0	1 (12.5)	
NI	0	1 (5.6)	1 (12.5)	
Sexual partners per year (n)				
<2	7 (58.3)	15 (83.3)	3 (37.5)	NA
2-5	3 (25.0)	1 (5.6)	0	
>5	2 (16.7)	0	3 (37.5)	
NI	0	2 (11.1)	2 (25.0)	
Hepatitis coinfection				
HBV	6 (50.0)	0	2 (25.0)	NA
HCV	6 (50.0)	18 (100.0)	6 (75.0)	
Number of years since diagnosed with hepatitis (median [IQR])				
	10 (7-23)	10 (9-23)	10 (8-23)	
Hepatitis therapy				
yes	8 (66.7)	11 (61.1)	5 (62.5)	NA
no	4 (33.3)	8 (38.9)	3 (37.5)	
HIV diagnosis length y (median/IQR)				
	17 (12-24)	17 (12-25)	16 (12-25)	
HIV viral load				
detectable (≥ 40 copies/mL)	0	5 (27.7)	1 (12.5)	NA
undetectable (< 40 copies/mL)	12 (100.0)	13 (72.3)	7 (87.5)	

Continue...

TABLE 1 - Continuation.

CD4 ⁺ T lymphocyte value cells/mm ³ (median [IQR])				
nadir	188 (89-445)	193 (91-445)	133 (83-445)	
current	397 (283-967)	485 (285-1,038)	413 (283-1,038)	
ART use				
yes	12 (100.0)	18 (100.0)	7 (87.5)	NA
no	0	0	1 (12.5)	
CCR5 polymorphism				
wild	12 (100.0)	18 (100.0)	5 (62.5)	NA
NS	0	0	3 (37.5)	
HIV tropism				
CCR5	9 (75.0)	10 (55.6)	3 (37.5)	1.00
CCR5/CXCR4	3 (25.0)	4 (22.2)	5 (62.5)	
NS	0	4 (22.2)	0	
Death				
	1 (8.3)	1 (5.6)	0	NA

HIV: human immunodeficiency virus; **IQR:** interquartile range; **IVDU:** intravenous drug user; **NI:** not informed; **MSM:** men who have sex with men; **NA:** not applicable; **HBV:** hepatitis B virus; **HCV:** hepatitis C virus; **CD4⁺:** cluster of differentiation 4⁺; **ART:** antiretroviral therapy; **CCR5:** C-C chemokine receptor type 5; **R5:** R5-receptor tropic virus; **R5/X4:** R5/CXCR4-receptor tropic virus; **NS:** not subtyped; **CRF:** circulating recombinant forms; *Other: CRF subtypes: BC (6 cases), BF1 (1 case), CF1 (1 case). #P-value calculated between B and C subtypes. **Bold values:** statistically significant.

Curitiba shows a higher frequency of subtype B (55%), followed by subtype C (30%), subtype F (7.5%), and CRFs (7.5%)⁶. However, in the present study, the distribution of HIV-1 subtypes found in coinfecting patients was different from that reported, with a higher predominance of subtype C among these patients, and determining the effect of HIV-1 subtypes in the context of coinfection remains a challenge. In this study, some viral and host characteristics of HIV/viral hepatitis-coinfecting patients were evaluated in order to gain further knowledge about this issue. Furthermore, the results of the present study are in accord with previous investigations, that most HIV-1 subclade F1 strains circulating in Brazil may contain recombinant genomes¹⁶.

HIV-1 subtypes may exhibit differences in rates of transmission, disease progression, neurotoxicity, antiretroviral treatment failure profiles, and accuracy of viral load measurements¹⁵. Several clinical studies have addressed the differences in disease progression between viral subtypes and outcomes. Toulomi et al.¹⁷ revealed that CD4⁺ T lymphocyte decline was significantly slower for subtype A and CRF02 and marginally slower for subtype C compared to subtype B. In addition, a previous study reported that subtype D is associated with faster disease progression, rapid CD4⁺ T cell decline, and a higher rate of treatment failure compared to subtypes A, B, and C¹⁸. *In vitro* studies suggested that the replication of subtype C is less competent than that of subtype B¹⁹. In this study, the mutations related to resistance to ART did not affect clinical features, as most patients (n = 32; 94.7%) showed a good response to HIV treatment.

With regard to the CCR5 polymorphisms, no case of CCR5Δ-32 mutation was found. Previous studies in the Brazilian population showed that less than 10% of the investigated individuals carried the heterozygous CCR5Δ-32 genotype^{20,21}. The small number of participants included in the present study may have affected the detection of this mutation.

The application of bioinformatic tools in co-receptor usage determination has been widely investigated and could provide an alternative approach in clinical practice for screening candidates for CCR5 antagonist therapy, especially when confirmatory assays for co-receptor usage determination are unavailable²². In this study, the Geno2pheno and PhenoSeq analyses showed R5 and R5/X4 variants in 22/34 (64.7%) and 12/34 (35.3%) HIV samples, respectively. However, we found no case of CCR5Δ-32 heterozygosity, and it is possible that other genetic alterations or levels of expression, including specific cellular proteins such as restriction factors, APOBEC3, TRIM5, and Tetherin²³, could be associated with disease progression. In a study of patients with a slower progression to acquired immune deficiency syndrome conducted in the municipality of Curitiba, it was noted that the R5 variant was the most common type for this patient group (95%)²⁴. However, all these studies were conducted in HIV-monoinfected patients; thus, we do not know if the behavior of HIV is different in patients coinfecting with viral hepatitis. Because of the effect of coinfection on the progression of the disease, we expected that a greater number of patients would express the R5/X4 co-receptor.

TABLE 2
HIV, host genetic characteristics, pathological findings, and therapy response according to HCV genotype.

Characteristics	HCV genotypes N = 30*			
	1 n = 20 (%)	2 n = 1 (%)	3 n = 6 (%)	4 n = 1 (%)
HIV subtype				
B	4 (20.0)	0	1 (17.0)	1 (100.0)
C	10 (50.0)	1 (100.0)	5 (83.0)	0
other**	6 (30.0)	0	0	0
Hepatic biopsy*				
yes (N = 25)	18 (90.0)	0	6 (100.0)	1 (100.0)
hepatic steatosis (N = 16)	11 (61.1)	0	4 (66.7)	1 (100.0)
fibrosis (N = 20)	15 (83.3)	0	4 (66.7)	1 (100.0)
cirrhosis (N = 7)	6 (33.3)	0	1 (16.7)	0
normal (N = 1)	1 (5.6)	0	0	0
no (N = 5)	2 (10.0)	1 (100.0)	0	0
HCV therapy				
yes (N = 20)	15 (75)	0	4 (66.7)	1
SVR (N = 10)	5 (33.3)	0	4 (100.0)	1 (100.0)
null response (N = 9)	9 (60.0)	0	0	0
relapse viral (N = 1)	1 (6.7)	0	0	0
no (N = 10)	5 (25.0)	1 (100.0)	2 (33.3)	0
IL28B (rs12979860)				
CC	10 (50.0)	1 (100.0)	5 (83.3)	0
CT	8 (40.0)	0	1 (16.7)	1 (100.0)
NS	2 (10.0)	0	0	0

HIV: human immunodeficiency virus; **HCV:** hepatitis C virus; **SVR:** sustained virological response; **IL28B:** interleukin 28B; **NS:** not subtyped; **CRF:** circulating recombinant forms; * HCV genotyping was not performed in two patients. **Other: CRF subtypes = BC (6 cases), BF1 (1 case), CF1 (1 case).

In Brazil, data on HBV/HIV coinfection are limited, and some studies conducted in the southern, southeastern, and midwestern regions revealed a prevalence ranging from 3.7 to 36.9%²⁵⁻²⁷. In this study, we identified only a small number of HBV/HIV-coinfecting individuals probably because of the low frequency of laboratory investigations for HBV in drug users, as well as the routine vaccination against HBV in HIV-infected patients.

Vaccination against HBV for high-risk individuals and children younger than 1 year of age was included in the National Immunization Program in Brazil in 1996; in 2001, immunization against HBV was expanded to include young people up to 19 years of age²⁸. Currently, the vaccination program has been extended to individuals up to 49 years of age, in addition to all HIV-infected patients²⁹. However, as HIV+ individuals

respond poorly to the HBV vaccine, regular monitoring of the immunological status in these patients is recommended³⁰.

Previous studies have reported that in the treatment of HCV/HIV-coinfecting patients on dual therapy, SVR was achieved in approximately 20-40% of those with HCV genotype-1/4 (GT1/4) and 50-60% of those with HCV genotype 2/3 (GT2/3)³¹. The baseline predictors for PEG-IFN α /RBV therapy response include age, sex, HCV genotype, HCV viral load, liver fibrosis, and SNPs of the *IL28B* gene³². The efficiency of treatment with PEG-IFN α /RBV was limited in patients with HCV mono-infection, and only 45-55% of the patients infected with HCV genotype-1 (GT1) achieved SVR³³.

The *IL28B* gene encodes interferon- λ 3, which has strong antiviral activity, and this cytokine can activate cell signaling

pathways that control viral replication³⁴. Recent studies have demonstrated the effect of some favorable genotypes (the CC and TT genotypes, and the C and T alleles of polymorphisms rs8099917 and rs12979860, respectively) on the response to the standard treatment for chronic hepatitis C, as well as their relationship with spontaneous HCV clearance in mono- and coinfecting individuals^{29,35}.

Lunge et al.³⁶ reported the correlation between the SNP rs12979860 and the spontaneous clearance of HCV in a population of HIV-coinfecting Brazilian patients. The presence of the CT and TT genotypes increased the risk of developing chronic infection by three times compared to the CC genotype. In this study, 16/30 (53.3%) individuals presented favorable genotypes (CC) for the SNP rs12979860. However, only 8/19 (42.1%) achieved SVR; we believe that other factors are associated with the outcome. Bertol et al.³⁷ suggested that SNPs near the *IL28B* gene exert a significant influence on the establishment of HCV infection in HCV-monoinfected patients, contributing to a better understanding of the susceptibility, natural history of HCV infection, and the high rate of SRV in this patient group. However, Martin et al.³⁸ and Nattermann et al.³⁹ did not observe any correlation between the SNP rs12979860 and HIV-monoinfected patients; there was no significant difference in the susceptibility to HIV infection, the progression of the disease, or the clinical and laboratory parameters such as viral load. Therefore, SNPs of the *IL28B* gene seem to be associated with HIV only when there is coinfection with HCV³⁹.

The small number of participants is the major limitation of this study; therefore, it was not possible to make inferences about the impact of viral characteristics and some host genetic factors on the outcome. However, with the currently high survival rate of HIV+ individuals, HCV-related liver disease in coinfecting patients is becoming a major cause of morbidity and mortality, and the burden of HIV/HCV coinfection is poorly understood⁴⁰. In Brazil, descriptive studies of this nature face several difficulties, including the need for expertise and financial investment to perform laboratory tests, resulting in cohorts with a small number of cases.

Despite these limitations, this study provides insights regarding the clinical and laboratory profile of HIV/viral hepatitis-coinfecting patients in the country. In addition, our findings emphasize the importance of screening for viral hepatitis when individuals are identified as HIV positive and vice versa. Moreover, the management of viral hepatitis should be integrated into HIV treatment programs, including effective treatment for a triple infection (HIV/HCV/HBV), as well as vaccination for HBV-negative individuals with HIV to reduce HIV-related morbidity and mortality.

In conclusion, in the present case series, a higher frequency of HIV-1 subtype C was found in patients with HIV/viral hepatitis coinfection, and there was a predominance of R5 tropism between both B and C subtypes. Genetic polymorphisms for the CCR5 co-receptor were not found, and most patients presented the favorable genotype regarding the SNPs of *IL28B*. Further larger-scale and long-term studies are needed to assess the effect of HIV-1 subtypes on the progression of viral hepatitis,

as well as for the analysis of other markers that may influence the interaction between pathogens and the host response, affecting its outcome.

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Conflict of interest

The authors declare that they have no competing interests.

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