Epidemiological, immunological and virological characteristics, and disease progression of HIV-1/HCV-co-infected patients from a southern Brazilian population

EDNA MARIA VISSOCI REICHE[1], ANA MARIA BONAMETTI[1], HELENA KAMINAMI MORIMOTO[1], ARILSON AKIRA MORIMOTO[1,2], SUSANA LILIAN WIECHEMANN[1], TIEMI MATSUO[3], FERNANDO VISSOCI REICHE[1] and INGRIDT HILDEGARD VOGLER[1]

[1]Health Sciences Center, University Hospital of Londrina State University, Av. Robert Koch 60, CEP 86038-440, Londrina; [2]Integrated Center of Infectious Diseases of Londrina, Health State Secretariat of Paraná, Al. Manoel Ribas 1, CEP 86010-140, Londrina; [3]Exact Sciences Center, Londrina State University, Rodovia Celso Garcia Cid, Km 380, s/n, CEP 86051-890, Londrina, Paraná, Brazil

Received October 1, 2007; Accepted November 26, 2007

Abstract. A cross-sectional study was carried out in order to describe the epidemiological, immunological and virological characteristics, and the disease progression of hepatitis C virus (HCV)/human immunodeficiency virus type 1 (HIV-1)-co-infected patients from a southern Brazilian population. Of 778 HIV-1-infected individuals enrolled in the study from September 2001 to December 2003, and followed up until June 2004, 757 were tested for anti-HCV antibodies. Of these, 159 (21.0%) showed positive results for anti-HCV. Males, individuals in the 25 to 34 year age range, and individuals of lower economic levels were more likely to be seropositive for both viruses [prevalence rate (PR), 2.04; 95% confidence interval (95% CI), 1.43-2.92; p<0.001]. The anti-HCV reactivity was also associated with blood routes of transmission (PR, 2.20; 95% CI, 1.28-3.77; p<0.001), intravenous drug use (PR, 5.79; 95% CI, 4.74-7.07; p<0.001), self-reported previous sexually transmitted diseases (PR, 1.55; 95% CI, 1.18-2.04; p=0.002), VDRL positivity (PR, 2.87; 95% CI, 2.40-3.43; p<0.001), and anti-HTLV I/II reactivity (PR, 5.09; 95% CI, 4.16-6.23; p<0.001). In the follow-up period, the HCV/HIV-1-co-infected patients showed a trend toward lower CD4$^+$ T-cell counts, higher HIV-1 RNA plasma viral load and faster disease progression than patients infected only with HIV-1, but significant differences were not observed. Although there were proportionately more deaths in the HCV/HIV-1-co-infected group, the use of highly active antiretroviral therapy (HAART) was a string predictor of increased CD4$^+$ T-cell counts and decreased HIV-1 RNA plasma levels, suggesting that HAART is more important to the immunological and virological outcomes in HIV-1 infection than is HCV co-infection status.

Correspondence to: Dr Edna Maria Vissoci Reiche, Health Sciences Center, Department of Pathology, Clinical Analysis and Toxicology, University Hospital of Londrina State University, Av. Robert Koch 60, Vila Operária, CEP 86038-440, Londrina, Paraná, Brazil
E-mail: reiche@sercomtel.com.br

Key words: AIDS, Brazil, hepatitis C, human immunodeficiency virus type 1, hepatitis C virus, co-infection

Introduction

Hepatitis C virus (HCV) and human immunodeficiency virus type 1 (HIV-1) infection are global public health problems, affecting 170 million and 40 million people worldwide, respectively, and the increasing morbidity and mortality of patients co-infected with both viruses have become a major challenge in the management of such patients (1,2).

HCV infection also occurs throughout Brazil and the use of serological screening assays for HCV in blood banks has uncovered a large population of asymptomatic carriers. The prevalence of anti-HCV among blood donors varies from 0.84 to 3.4%, in different parts of the country (3). Population surveys have shown rates ranging from 0 to 3.0% (4,5). In Londrina, in the state of Paraná in southern Brazil, the frequency of anti-HCV initially reported was 1.2% among blood donors (6), and 0.8% among pregnant patients (7).

Due to sharing the same risk factors for transmission, HCV and HIV-1 co-infection is a very common event (1,8). The prevalence of HCV in HIV-1-infected individuals was investigated in different populations worldwide and varies from as low as 4.0% to greater than 50.0% (9-12). In the US and Europe the prevalence averages ~35.0%, but in clinical populations where there is a prevalence of intravenous drug use (IVDU) as a risk factor for acquiring HIV-1, this value may be as high as 80.0-90.0% (13,14). The high sero-positivity to anti-HCV in HIV-1 patients was also reported in previous studies carried out in Brazil and varied from 17.7 to 25.6% (3,15-17).

It is unclear whether co-infection with HCV can influence the natural history of HIV-1 infection and HIV-1-related morbidity or mortality, either by accelerating HIV-related disease progression, or by contributing to end-stage liver disease. While several studies demonstrated no impact of HCV on HIV-1 infection (18-21), studies both *in vitro* and in patients support a role for HCV in HIV-1 progression (9,13,22,23). Studies have also reported a more rapid progression of HIV-1 in hemophilic patients infected specifically with HCV genotype 1a or 1b (24). A longitudinal analysis of $CD8^+$ T-cell responses to HIV-1 and HCV in a cohort of co-infected hemophiliacs demonstrated that HCV infection had an influence on the phenotype of HIV-specific $CD8^+$ T cells, with a reduction in relative perforin and CD57 expression, and the failure to maintain effective $CD8^+$ T-cell responses against HIV-1 may contribute to disease progression (25). These results could be explained by the fact that HCV is not strictly hepatotropic; it can also replicate in peripheral blood mononuclear cells, and some membrane receptors can be used by both HCV and HIV-1 for entry into cells. In HIV-1/HCV-co-infected individuals, these two different viruses can share cell targets and interact either directly or indirectly (26). On the other hand, Brazilian AIDS patients with HCV infection have a shorter survival period than those without, but this seems to be mainly a result of their receiving less antiretroviral treatment (27).

While previous studies of the effects of HCV infection on HIV-1 infection showed conflicting results, there is no doubt that HIV-1 accelerates HCV-related liver disease, especially when immunodeficiency progresses (1). Several studies have confirmed that HIV-1 co-infection accelerates the natural course of chronic hepatitis C, and is associated with a reduced likelihood of HCV clearance, higher HCV RNA viral load, and a more rapid progression of HCV-related liver disease, leading to an increased risk of cirrhosis (8,14,28). HCV infection may also affect the course and management of HIV-1 disease, particularly by increasing the risk of antiretroviral drug-induced hepatotoxicity (29). The $CD4^+$ depletion caused by HIV-1 infection was associated with lower HCV-specific $CD8^+$ T cells, indicating that the frequency of circulating HCV-specific $CD8^+$ T-cell responses is sensitive to absolute $CD4^+$ T-cell counts and provides a possible explanation for the accelerated HCV disease course in persons co-infected with HIV-1 and HCV (30).

Brazil, the most populous country in Latin America, has 620,000 (370,000 to 1 million) people living with HIV-1 (31). Up to June 2006, a total of 433,067 cases of AIDS had been reported in Brazil, and the southern state of Paraná has the fifth highest incidence of AIDS in the country, with 20,176 cases (32). The municipality of Londrina ranked second in the number of AIDS cases in Paraná, with a total of 1,070 cases reported between 1984 and 2002 (33). The pattern of the HIV/AIDS epidemic in Brazil is heterogeneous and has changed according to time, geographic region, and the subpopulations affected. The scant data about the risk factors associated with HCV/HIV-1 co-infection in Paraná state, was obtained in a limited number of HIV-1-infected-individuals (17). To address this issue, the present cross-sectional study was carried out on a large cohort of HIV-1-infected patients to describe the frequency and certain epidemiological, immunological, and virological characteristics of HCV/HIV-1 co-infection, and also to evaluate the impact of HCV infection in the HIV-1 clinical and laboratory outcome.

Materials and methods

Data and laboratory procedures. The protocol was approved by the institutional Research Ethics Committees of Londrina State University and of the Health State Secretariat of Paraná, in southern Brazil. Individuals were invited to participate and informed in detail about the research, and their written consent was obtained.

Study subjects. A total of 778 patients infected with HIV-1 attending various specialized, public, and nonprofit centers for sexually transmitted diseases (STD) from Londrina and region, were enrolled from September 2001 to December 2003 and tracked until June 2004. Details of methods, including sampling strategy, data collection, AIDS diagnostic criteria and clinical and laboratory evaluation of disease progression have been described previously (16,34,35). The patients were divided into two groups. The first group consisted of 161 asymptomatic patients infected with HIV-1 with $CD4^+$ T-cell counts $\geq$350 cells/mm^3. At the time of enrollment, 102 of the subjects were receiving no highly active antiretroviral therapy (HAART) (36), and 59 were on HAART. During the follow-up period (n=157), 89 subjects were receiving no HAART, and 68 were on HAART. The second group consisted of 617 outpatients and inpatients infected with HIV-1 with the symptoms of the disease and/or CD4 counts <350 cells/mm^3, according to the Brazilian criteria for the definition of AIDS that consider a $CD4^+$ T-cell count of 350/mm^3 as the cut-off value and/or the presence of the opportunist infections for the AIDS diagnosis (37). At the time of enrollment (n=617), 128 subjects were receiving no HAART, and 489 were on HAART. During the follow-up period (n=611), 45 subjects were receiving no HAART, and 566 were on HAART.

Serological, immunological, and virological tests. The screening and confirmatory serological tests for anti-HIV-1 were performed in serum samples according to standard government procedures (38). The screening tests used were the enzyme immunosorbent assay (ELISA, Murex™ HIV-1.2.0, Murex Biotech Limited, Dartford, Kent, UK), and the microparticle enzyme-immunoassay (MEIA, Abbott Axsym™ System, HIV-1/2 gO, Abbott GmbH, Weisbaden, Delkenheim, Germany). The confirmatory tests used were indirect immunofluorescence (slides from Fiocruz Institute, Rio de Janeiro, Brazil) and Western blot analysis (Genelabs Diagnostics, Singapore). The serological tests for syphilis used were VDRL (RPR Bras®, Laborclin, Curitiba, Paraná) and ELISA (ICE Syphilis™ Murex Biotech Limited, UK). The serological markers for HBsAg, anti-HCV antibodies were also determined by MEIA (Abbott Axsym™ System, Abbott Laboratórios, Brazil). The infection with human T-lymphotropic virus type I and II (HTLV I/II) was investigated by screening serological tests using two ELISA with different antigens, one consisting of purified viral

Table I. Sociodemographic and epidemiological characteristics associated with seropositivity for anti-hepatitis C virus observed in HIV-1 infected individuals from southern Brazil, 2001-2004.

Characteristics	Total n (%)	Tested n (%)	Anti-HCV+[a] n (%)	PR	95% CI	p-value[b]
Sex						
Female	345 (44.3)	335 (97.1)	46 (13.7)	1.00		
Male	433 (55.7)	422 (97.4)	113 (26.8)	1.95	1.43-2.66	<0.001
Total	778 (100.0)	757 (93.7)	159 (21.0)			
Age (years)						
<25	64 (8.2)	63 (96.9)	6 (9.5)	1.00		
25-34	302 (38.8)	291 (96.3)	68 (23.4)	2.45	1.11-5.40	0.056
>35	412 (52.9)	403 (97.8)	85 (21.1)	2.21	1.01-4.85	
Total	778 (100.0)	757 (97.3)	159 (21.0)			
Education						
Illiterate	30 (3.8)	29 (96.7)	6 (20.7)	1.00		
≤8 years	482 (61.9)	470 (97.5)	59 (12.5)	1.65	0.78-3.49	0.4506
>8 years	266 (34.2)	258 (97.0)	34 (13.2)	1.57	0.72-3.42	
Total	778 (100.0)	757 (97.3)	159 (21.0)			
Economic level						
A, B, and C	346 (44.5)	343 (99.1)	55 (15.9)	1.00		
D	314 (40.4)	300 (95.5)	67 (22.3)	1.39	1.01-1.92	<0.001
E	117 (15.0)	113 (96.6)	37 (32.7)	2.04	1.43-2.92	
Total[c]	777 (100.0)	756 (97.3)	159 (21.0)			
Transmission						
Sexual	693 (83.5)	674 (97.2)	99 (14.7)	1.00		
Blood	34 (4.1)	31 (91.2)	10 (32.2)	2.20	1.28-3.77	<0.001
IVDU	102 (12.3)	100 (98.0)	85 (85.5)	5.79	4.74-7.07	
Total[d]	829 (100.0)	805 (97.1)	194 (24.0)			
Previous STD[e]						
No	486 (62.5)	474 (97.5)	83 (17.5)	1.00		0.002
Yes	285 (36.6)	276 (96.8)	75 (27.2)	1.55	1.18-2.04	
Total[c]	771 (100.0)	750 (97.3)	158 (21.1)			

PR, prevalence rate; CI, confidence interval; IVDU, intravenous drug use. [a]Antibodies against hepatitis C virus assayed by microparticle enzyme-linked immunosorbent assay; [b]Chi-square test, $p<0.05$; [c]totals vary because of missing observations; [d]totals vary because certain individuals presented more than one route of transmission; [e]self-reported sexual transmitted disease (gonorrhoea, syphilis, genital herpes condyloma).

lysates of HTVL-I and HTLV-II with recombinant antigen p21E of HTLV-I (Vironostika HTLV-I/II, Bio-Meriéux, Brazil), and the other of a synthetic peptide from the envelope protein of HTLV I/II and a recombinant transmembrane protein of HTLV-II (Murex HTLV-I+II, Abbott Laboratórios, Brazil). The results of reactive samples were confirmed by Western blot analysis and/or the polymerase chain reaction (PCR), as described elsewhere (39).

Baseline and follow-up $CD4^+$ T-cell counts were determined in peripheral blood samples collected with EDTA anticoagulant and by flow cytofluorimetry (FACSCount™, Becton Dickinson, San Jose, CA). The results were recorded as number of cells/mm^3 and were used as one of the criteria for AIDS definition (37). Baseline and follow-up plasma HIV-1 RNA levels were obtained using the Cobas Amplicor™ Monitor HIV-1 version 1.5 (Roche Diagnostic Systems, Brachburg, NJ, USA), with a lower limit of detection of 400 copies/ml and a linear dynamic range of up to 750,000 copies/ml. The viral load was recorded as the number of HIV-1 RNA copies/ml and was $\log_{10}$ copies/ml transformed.

The endpoints considered for analysis of the clinical disease progression were the development of AIDS, according to the Brazilian criteria for the definition of AIDS (37), and death.

Statistical analysis. All data were recorded with Epi Info software v.6.04d. The variables analyzed included the following factors: sociodemographic (sex, age, educational and economic levels), epidemiological (transmission category, previous STD), serological (VDRL, hepatitis B, HTLV I/II

infection), immunological ($CD4^+$ T-cell counts) and virological (HIV-1 RNA plasma levels), and the clinical evolution of HIV-1 infection. Median, mean, standard deviation and percentage were used to describe the results. The results are presented as prevalence rate (PR) with 95% confidence interval (95% CI). To compare the rates of $CD4^+$ T-cell and viral-load changes among HCV/HIV-1-co-infected individuals with those of individuals infected only with HIV-1, we selected those individuals for whom at least two $CD4^+$ T-cell counts and viral-load measurements were available from September 2001 to June 2004. The rates of $CD4^+$ T-cell and viral-load changes were assessed by using the Mann-Whitney non-parametric test. To evaluate the potential confounding effects of HAART, the slopes were also calculated and compared between the individuals receiving HAART and the individuals not receiving HAART. The slopes were calculated for yearly time intervals. To examine bivariate associations, the Chi-square or, when appropriate, Fisher's exact test was used. P value <0.05 was considered statistically significant.

Results

Sociodemographic, epidemiological, and serological characteristics. As shown in Table I, of the 778 HIV-1-infected individuals studied, 345 (44.3%) were female and 433 (55.7%) were male, and 757 (97.3%) were tested for anti-HCV antibodies. Of these, 159 (21.0%) showed positive results for anti-HCV. The univariate analysis showed that among the HIV-1-infected subjects, males, individuals in the 25 to 34 year age range, and individuals with lower economic levels were more likely to be seropositive for anti-HCV, with PRs of 1.95 (95% CI, 1.43-2.6), 2.45 (95% CI, 1.11-5.40) and 2.04 (95%CI, 1.43-2.92), respectively ($p<0.05$). Although individuals of lower education presented a higher rate of seropositivity for anti-HCV, the differences observed among the individuals evaluated did not differ (p=0.4506). The prevalence of anti-HCV seropositivity in HIV-1-infected individuals was associated with blood routes of transmission (PR, 2.2; 95% CI, 1.28-3.77; $p<0.001$), IVDU (PR, 5.79; 95% CI, 4.74-7.07; $p<0.001$) and with self-reported previous STD (PR, 1.55; 95% CI, 1.18-2.04; p=0.002). Fifty one (51/775, 6.5%) HIV-1-infected patients presented both sexual and blood routes of transmission of HIV-1 infection. As shown in Table II, the prevalence of anti-HCV in HIV-1-infected individuals was also associated with seropositivity for the VDRL (PR, 2.87; 95% CI, 2.40-3.43; $p<0.001$), and seropositivity for anti-HTLV I/II tests with lysate viral antigen (PR, 5.09; 95% CI, 4.16-6.23; $p<0.001$).

Immunological and virological assays. In a cross-sectional analysis performed with baseline measurements of $CD4^+$ T-cell count and HIV-1 RNA viral load from the HIV-1-infected individuals enrolled, the median $CD4^+$ T-cell count was lower and the median HIV-1 RNA was higher among anti-HCV-positive than anti-HCV-negative individuals, independent of use of HAART, and the clinical phase of the disease (Table III). However, these differences were not statistically significant ($p>0.05$). Undetectable plasma viral RNA HIV-1 (<400 copies/ml) was observed in 36.3% (41/113) and in 38.9% (168/432) of anti-HCV-negative and anti-HCV-positive individuals, respectively (Chi-square test, p=0.612). Among the individuals who were not using HAART, undetectable viral load was recognized in 13.3% (2/15) of anti-HCV-positive individuals versus 24.3% (17/70) of anti-HCV-negative individuals (Fisher's exact test, p=0.503). Among the individuals who were using HAART, undetectable viral load was recognized in 39.8% (39/98) of anti-HCV-positive individuals versus 41.2% (151/362) of anti-HCV-negative individuals (Chi-square test, p=0.732).

Slopes of $CD4^+$ T-cell counts and HIV-1 viral-load determinations. During the follow-up study from September 2001 to June 2004, a mean of 4.4 $CD4^+$ T-cell counts (range, 1-9) and 4.9 viral-load determinations (range, 1-9) were performed for each patient. Considering that at least two evaluations were required to calculate the slopes of the $CD4^+$ T-cell counts and of plasma HIV-1 RNA levels, 692 HIV-1-infected patients (557 anti-HCV-negative and 135 anti-HCV-positive) and 626 HIV-1-infected patients (509 anti-HCV-negative and 117 anti-HCV-positive) had sufficient data to estimate the $CD4^+$ T-cell and viral-load slopes, respectively.

The immunological and virological responses of the HIV-1 patients were heterogeneous during the follow up period (Table IV). While the $CD4^+$ T-cell count increased an average of 31.72 cells/mm^3/year in anti-HCV-negative individuals, a decrease of 5.47 cells/mm^3/year in anti-HCV-positive individuals was seen. However, due to the heterogeneity of the immune response observed, the changes observed in the $CD4^+$ T-cell counts per year did not differ among the HIV-1/HCV-co-infected patients and those infected only with HIV-1 (p=0.2168). Similar slopes were also observed when stratified by HAART use (p=0.7677) and when both anti-HCV serological status and HAART conditions were considered (p=0.6731). In the same period, the anti-HCV-positive patients showed an average decrease of 16,400 RNA HIV-1 copies/ml/year versus an average increase of 18,459 RNA HIV-1 copies/ml/year observed among the anti-HCV-positive individuals. These results demonstrated that the changes in RNA HIV-1 levels per year were different in the groups of HIV-1/HCV-co-infected patients and those infected only with HIV-1 (p=0.004). However, similar slopes were observed when HAART use was considered (p=0.1416). Patients who were receiving HAART presented rates of -45,931 copies/ml/year versus 1,474 copies/ml/year observed among patients who were not receiving HAART. When both anti-HCV-serological status and HAART conditions were considered, the slopes were also similar (p=0.0659).

Clinical disease progression. During the follow-up, the clinical disease progression and survival of the HIV-1-infected individuals were evaluated considering the serological status for anti-HCV. When the clinical baseline HIV-1 infection was not considered, the progression to AIDS was similar between anti-HCV-positive and anti-HCV-negative individuals (PR, 0.96; 95% CI, 0.61-1.49; p=0.8322). However, the death rate was higher among the anti-HCV-positive individuals than the anti-HCV-negative

Table II. Serological characteristics associated with the seropositivity for anti-hepatitis C virus (HCV) observed in HIV-1 infected individuals from southern Brazil, 2001-2004.

Characteristics	Total n (%)	Tested n (%)	Anti-HCV[+a] n (%)	PR	95% CI	p-value[b]
VDRL						
Negative	712 (94.3)	712 (100.0)	142 (19.9)	1.00		<0.001
Positive	43 (5.7)	43 (100.0)	170 (39.5)	2.87	2.40-3.43	
Total[c]	755 (100.0)	755 (100.0)	312 (41.3)			
Anti-HTLV I/II[d]						
Negative	713 (94.2)	712 (99.8)	122 (17.1)	1.00		
Positive	44 (5.8)	43 (97.7)	37 (84.7)	5.09	4.16-6.23	<0.001
Total[c]	757 (100.0)	755 (99.7)	159 (21.0)			
HBsAg[e]						
Negative	730 (96.6)	730 (100.0)	153 (21.0)	1.00		0.795
Positive	26 (3.4)	26 (100.0)	6 (23.1)	1.10	0.54-2.25	
Total[c]	756 (100.0)	756 (100.0)	159 (21.0)			

PR, prevalence rate; CI, confidence interval. [a]Antibodies against hepatitis C virus assayed by microparticle enzyme-linked immunosorbent assay; [b]Chi-square test, $p<0.05$; [c]totals vary because of missing observations; [d]enzyme-linked immunosorbent assay for specific antibodies against human T lymphotropic virus type I and type II; [e]microparticle enzyme-linked immunosorbent assay for hepatitis B surface antigens.

Table III. Immunological and virological characteristics of 752 HIV-1-infected patients from southern Brazil (asymptomatic and with AIDS) according to HAART[a] and anti-HCV[b] serological status, evaluated from September 2001 to June 2004.

Characteristics	Anti-HCV negative (n=595) median (IQR) baseline	Anti-HCV positive (n=157) median (IQR) baseline	p-value[c]
HIV-1 infected asymptomatic			
Without HAART (n=84)			
CD4+ T (cell/mm^3)[d]	582 (483-731)	539 (426-711)	0.5488
HIV-1 RNA (copies/ml)[e]	7,900 (614-29,900)	8,160 (4,160-109,000)	0.8468
log HIV-1 RNA (copies/ml)	3.89 (2.78-5.23)	3.90 (3.62-4.35)	0.3331
With HAART (n=70)			
CD4+ T (cell/mm^3)	494 (426-652)	457 (419-516)	0.3497
HIV-1 RNA (copies/ml)	1,070 (<400-610,000)	24,300 (<400-145,000)	0.4009
log HIV-1 RNA (copies/ml)	3.09 (2.60-4.15)	4.39 (2.60-5.16)	0.2589
HIV-1/AIDS			
Without HAART (n=43)			
CD4+ T (cell/mm^3)	337 (306-485)	271 (57-522)	0.2651
HIV-1 RNA (copies/ml)	3,180 (<400-17,200)	9,399 (<400-132,700)	0.0764
log HIV-1 RNA (copies/ml)	3.50 (<2.60-4.24)	3.43 (<2.60-4.83)	0.6343
With HAART (n=555)			
CD4+ T (cell/mm^3)	279 (151-417)	236 (161-396)	0.3955
HIV-1 RNA (copies/ml)	1,810 (<400-55,700)	3,180 (<400-48,900)	0.8897
log HIV-1 RNA (copies/ml)	3.26 (<2.60-4.75)	3.70 (<2.60-4.69)	0.5120

[a]Highly active antiretroviral therapy according to the Brazilian guidelines (36); [b]antibodies against hepatitis C virus (HCV) assayed by microparticle enzyme-linked immunosorbent assay; [c]Kruskal-Wallis Test ($p<0.05$); [d]determined by cytofluorimetry (FACSCount™); [e]determined by Cobas Amplicor™ Monitor HIV-1 version 1.5.

individuals, and anti-HCV seropositivity was associated with a higher death rate in HIV-1-infected individuals (PR, 2.12; 95% CI, 1.02-4.36; p=0.026). However, when the clinical baseline of the HIV-1 infection presented by individuals at the time of enrollment in the study (asymptomatic or with AIDS) was considered in the analysis, the rates of AIDS

Table IV. Slope of the CD4+ T-cell counts and HIV-1 RNA plasma levels observed among HIV-1-infected patients from southern Brazil (asymptomatic and with AIDS), followed up from September 2001 to June 2004, considering anti-HCV[a] serological status and HAART[b] condition.

Condition	CD4+ T cell[c] cells/mm^3/year (mean ± SD)	p value[d]	HIV-1 RNA[e] copies/ml/year (mean ± SD)	p-value[d]
Anti-HCV-	31.72±330.70	0.2168	-16,400±131,703	
Anti-HCV+	-5.47±330.80		18,459±218,982	0.0040
HAART-	23.07±368.80	0.7677	-45,931±206,171	
HAART+	24.98±316.00		1,474±128,963	0.1416
Anti-HCV-/HAART-	35.01±286.60	0.6731	-61,877±185,308	
Anti-HCV-/HAART+	30.53±345.42		-2,064±105,848	
Anti-HCV+/HAART-	-23.74±591.53		23,544±273,000	0.0659
Anti-HCV+/HAART+	1.68±133.20		16,859±200,831	

[a]Antibodies against hepatitis C virus assayed by microparticle enzyme-linked immunosorbent assay; [b]highly active antiretroviral therapy according to the Brazilian guidelines (36); [c]determined by cytofluorimetry (FACSCount™); [d]Mann-Whitney test, $p<0.05$; [e] the RNA HIV-1 level was determined by Cobas Amplicor™ Monitor HIV-1 version 1.5. SD, standard deviation; anti-HCV+, anti-HCV-positive; anti-HCV-, anti-HCV-negative; HAART+, using highly active antiretroviral therapy; HAART-, not using highly active antiretroviral therapy.

Table V. Clinical disease progression and survival evaluated in 757 HIV-1-infected patients from a southern Brazilian population (asymptomatic and with AIDS) according to anti-hepatitis C virus serological status, followed up from September 2001 to June 2004.

Disease progression	Anti-HCV[a] negative n (%)	Anti-HCV[a] positive n (%)	Total n	p-value[b]
HIV-1 infected asymptomatic and with AIDS[c]				
Remained asymptomatic	96 (16.0)	16 (10.0)	112 (14.8)	
Progressed to AIDS[d]	471 (78.8)	124 (78.0)	595 (78.6)	0.8322
Death	26 (4.3)	14 (8.8)	40 (5.3)	
Without follow-up	5 (0.08)	5 (0.03)	10 (1.3)	
Total	598 (100.0)	159 (100.0)	757 (100)	
HIV-1 infected asymptomatic[e]				
Remained asymptomatic	96 (74.4)	16 (64.0)	112 (72.7)	
Progressed to AIDS[d]	31 (24.0)	6 (24.0)	37 (24.0)	
Death	1 (0.08)	1 (4.0)	2 (1.3)	0.0550
Without follow-up	1 (0.08)	2 (8.0)	3 (1.9)	
Total	129 (100.0)	25 (100.0)	154 (100.0)	
HIV-1 infected with AIDS[e]				
Remained with AIDS	438 (94.0)	117 (88.6)	555 (92.8)	
Death	25 (5.4)	13 (9.8)	38 (6.3)	0.1051
Without follow-up	3 (0.06)	2 (1.5)	5 (0.08)	
Total	466 (100.0)	132 (100.0)	598 (100.0)	

[a]Antibodies against hepatitis C virus assayed by microparticle enzyme-linked immunosorbent assay; [b]Chi-square test, $p<0.05$; [c]without considering the clinical condition of the HIV-1 infection of the individuals at the time of enrollment in the study; [d]according to the Brazilian criteria for the definition of AIDS (37); [e]considering the clinical condition of the HIV-1 infection of the individuals at the time of enrollment in the study.

diagnosis and death did not differ between the anti-HCV-positive and anti-HCV-negative individuals ($p>0.05$), although individuals infected only with HIV-1 who were asymptomatic at the time of enrollment, showed a trend to lower progression to death than individuals infected with both viruses ($p=0.055$) (Table V).

Discussion

Brazil has one of the greatest number of AIDS cases in the world and accounts for one third of all persons living with the virus in Latin America, with a national adult HIV prevalence of 0.5% since 2000 (31). The major features of the HIV/AIDS epidemic in Brazil are the steady decrease in the male to female ratio as a result of an increasing proportion of female patients fueled by heterosexual transmission, and the fact that patients are from the poorer part of society and live farther away from urban centers (16,32,33,40).

The seroprevalence of anti-HCV among individuals infected with HIV-1 is considerable and the rate obtained in this study is much higher than previously reported among either healthy blood donors, or through populational studies of HIV-1-infected patients from Brazil (3,13,15,16,41,42). However, it is much lower than that found in HIV-1-infected patients from other countries (10,12,13). The rates obtained were similar to those found in other HIV-1-infected populations. Previous studies carried out among blood donors from Londrina in southern Brazil, detected a frequency of 1.2% in 1990 (6), and 29.2% in 2002 (17). Anti-HCV seropositivity of 0.5 and 25.6% were obtained among HIV-1-seronegative and HIV-1-seropositive blood donors from this same region, respectively, during the period from July 1994 to April 2001 (15). In another cohort from the Londrina region, consisting of 161 HIV-1-infected asymptomatic individuals and 617 individuals with AIDS, the rates of seropositivity for anti-HCV were 16.0 and 22.3%, respectively, higher than the rate of 4.1% observed among HIV-1 exposed but uninfected individuals (16).

The rate of anti-HCV in HIV-1-infected individuals varies according to the risk factors for HCV and HIV-1 infection. Among HIV-1-infected hemophiliacs the frequency of anti-HCV varies between 60.0 and 95.0%, and among individuals infected with HIV-1 through IVDU it may be approximately 70.0% (1,2). Anti-HCV antibodies were detected in 51.4% of Italian HIV-1-positive, AIDS-free persons infected through IVDU or sexual activity (9). In Argentina, the results showed rates of 58.5%, significantly higher in HIV-1-seropositive individuals than in HIV-1-seronegative individuals, confirming the high prevalence of co-infection due to the occurrence of a common epidemiologic factor for the transmission of these viruses (10).

Although the HIV-1 infection levels among individuals infected through IVDU are declining in Brazil (40), they are still high and the level of seropositivity for HCV infection (21.0%) observed in the HIV-1-infected patients enrolled in this study was expected, confirming the overlap of the routes of transmission of these microorganisms and perhaps reflecting the lifestyle of the population assessed. The results obtained are consistent with a previous report that found a high (52.1%) frequency of HCV/HIV-1 co-infection among individuals from the same region infected through IVDU (17).

Regarding the impact of HCV infection in the HIV-1-clinical course, until recently, the negative interaction between HCV and HIV-1 was thought to be due to the accelerated course of HCV-related liver disease in the context of immunosuppression caused by HIV-1. It was believed that HCV did not impact negatively on HIV-1 disease progression (43). However, independent studies (9,22,23) have proven that chronic HCV infection can act as a co-factor for HIV-1-disease progression. This deleterious effect is not due to a greater risk of hepatotoxicity in co-infected patients, which in turn may compromise the benefit of antiretroviral drugs. In fact, HCV infection seems to accelerate HIV-1-disease progression by itself (43).

The HCV/HIV-1-co-infected patients enrolled in this study showed a trend toward lower $CD4^+$ T-cell counts, higher HIV-1 RNA plasma viral load and faster disease progression than patients infected only with HIV-1. However, the difference was not statistically significant, which underscores that HCV infection does not increase either the risk of acquiring an AIDS-defining illness or the risk of death when comparing HCV-infected and HCV-uninfected HIV-1 patients, as reported previously (18-21,44). Although there were proportionately more deaths in the HCV/HIV-1-co-infected group, as reported previously (45), the use of HAART was a string predictor of increased $CD4^+$ T-cell counts and decreased HIV-1 RNA plasma levels, suggesting that HAART is more important to the immunological and virological outcomes than is HCV co-infection status. A previous study of a Brazilian cohort of AIDS patients also suggested that the shorter survival period observed among HCV/HIV-1-co-infected individuals seems to be mainly due to their receiving less antiretroviral treatment (27).

Studies of the natural history of persons co-infected with HCV and HIV-1 reported previously are limited in the extent to which their findings can be attributed to co-infection *per se*, since co-infected persons may differ from those with only one of these infections in important respects such as the route of transmission (43), age, gender, the immunocompromised status, and the co-morbidities. These host differences, in addition to the possibility of infection with different HCV genotypes (24), may bias observational studies and may explain the contradictory results.

The present study was conducted on a large cohort with relative homogeneity in demographic characteristics such as gender, age, risk factors for HIV-1 infection, access to free health care and relatively uniform clinical, diagnostic procedures, and treatment patterns for the HIV-1 infection. This relative homogeneity may contribute to the validation of the results obtained and to underscore the earlier observations that HCV infection, by itself, may not be a co-factor in the progression of HIV-1 infection.

In addition, other host co-factors such as the genetic polymorphisms of cytokines, chemokines and their receptors (46,47), HLA system (48), antiviral immune response mediated by the $CD8^+$ T cells and the levels of ß-chemokines (49-51), alcohol use (44,52), co-infection with other microorganisms and HAART adhesion (53), may exert additional or synergic effects with HIV-1 viral factors such as the genotype profiles of HCV (24), subtype of HIV-1 and the presence of mutations that confer resistance to HAART in HIV-1 disease progression in the Brazilian population (54-56).

Taken together, knowledge of all these factors that can exert interactions with HIV-1 is required for the design of effective antiviral therapies and the optimization of the evaluation and management of HCV/HIV-1-co-infected individuals.

Acknowledgements

We thank the Health Ministry, Brazilian National Sexually Transmitted Disease/AIDS program (CN-DST/AIDS), for their financial support with the cooperative agreement of the State University of Londrina (Grants UNESCO-UEL CFA 667/01), the Londrina University Hospital for technical and administrative support, and the PIBIC CNPq program for student grants.

References

1. Rockstroh JK and Spengler U: HIV and hepatitis C virus co-infection. Lancet Infect Dis 4: 437-444, 2004.
2. Shepard CW, Lyn F and Alter MJ: Global epidemiology of hepatitis C virus infection. Lancet Infect Dis 5: 558-567, 2005.
3. Mendes-Corrêa MC, Barone AA and Guastini C: Hepatitis C virus seroprevalence and risk factors among patients with HIV infection. Rev Inst Med Trop Sao Paulo 43: 15-19, 2001.
4 Martins RMB, Porto SOB, Vanderborght BOM, *et al*: Short report: prevalence of hepatitis C viral antibody among Brazilian children, adolescents, and street youths. Am J Trop Med Hyg 53: 654-655, 1995.
5. Silva L, Paraná R, Mota E, *et al*: Prevalence of hepatitis C virus in urban and rural population of Northeast Brazil: pilot study. Arq Gastroenterol 32: 168-171, 1995.
6. Pontello R, Reiche EMV, Inouye MMZ and Souza JLK: Freqüência de positividade dos marcadores sorológicos dos vírus de hepatite A, B e C em 500 candidatos a doadores de sangue do Hemocentro do Hospital Universitário Regional Norte do Paraná, Londrina, PR. Rev Bras Anal Clin 25: 7-10, 1993.
7. Reiche EMV, Morimoto HK, Farias GN, *et al*: Prevalência de tripanossomíase americana, sífilis, toxoplasmose, rubéola, hepatite B, hepatite C e da infecção pelo vírus da imunodeficiência humana, avaliada por intermédio de testes sorológicos, em gestantes atendidas no período de 1996 a 1998, no Hospital Universitário Regional Norte do Paraná. UEL, Londrina, PR, Brasil. Rev Soc Bras Med Trop 33: 519-527, 2000.
8. Haydon GH and Mutimer DJ: Hepatitis B and C virus infections in the immune compromised. Curr Opin Infect Dis 16: 473-479, 2003.
9. Dorrucci M, Pezzotti P, Phillips AN, Lepri AC and Rezza G: Co-infection of hepatitis C virus with human immunodeficiency virus and progression to AIDS. Italian Seroconversion Study. J Infect Dis 172: 1503-1508, 1995.
10. Fainboim H, Gonzáles J, Fassio E, *et al*: Prevalence of hepatitis viruses in an anti-human immunodeficiency virus-positive population from Argentina. A multicentre study. J Viral Hepat 6: 53-57, 1999.
11. Moorman JP: Issues in HIV/Hepatitis C co-infection. Curr Infect Dis Rep 3: 131-136, 2001.
12. Rodríguez L, Collado-Mesa F, Aragón U, Díaz B and Rivero J: Hepatitis B exposure in human immunodeficiency virus seropositive Cuban patients. Mem Inst Oswaldo Cruz 95: 243-245, 2000.
13. Carlos Martin J, Castilla J, Lopez M, Arranz R, Gonzalez-Lahoz J and Soriano V: Impact of chronic hepatitis C on HIV-1 disease progression. HIV Clin Trials 5: 125-131, 2004.
14. Verucchi G, Calza L, Manfredi R and Chiodo F: Human immunodeficiency virus and hepatitis C virus coinfection: epidemiology, natural history, therapeutic options and clinical management. Infection 32: 33-46, 2004.
15. Reiche EMV, Vogler IH, Morimoto HK, *et al*: Evaluation of surrogate markers for human immunodeficiency virus infection among blood donors at the blood bank of Hospital Universitário Regional Norte do Paraná, Londrina, PR, Brazil. Rev Inst Med Trop Sao Paulo 45: 23-27, 2003.
16. Reiche EMV, Bonametti AM, Watanabe MAE, *et al*: Sociodemographic and epidemiological characteristics associated with human immunodeficiency virus type 1 (HIV-1) infection in HIV-1-exposed but uninfected individuals, and in HIV-1-infected patients from a southern Brazilian population. Rev Inst Med Trop Sao Paulo 47: 239-246, 2005.
17. Vogler IH, Nishiys A, Morimoto HK, *et al*: Serological, epidemiological and molecular aspects of hepatitis C virus infection in a population from Londrina, PR, Brazil, 2001-2002. Rev Inst Med Trop Sao Paulo 46: 303-308, 2004.
18. Quan CM, Krajden M, Grigoriew GA and Salit IE: Hepatitis C virus infection in patients infected with the human immunodeficiency virus. Clin Infec Dis 17: 117-119, 1993.
19. Haydon GH, Flegg PJ, Blair CS, Brettle RP, Burns SM and Hayes PC: The impact of chronic hepatitis C virus infection on HIV disease and progression in intravenous drug users. Eur J Gastroenterol Hepatol 10: 485-489, 1998.
20. Hershow RC, O'Driscoll PT, Handelsman E, *et al*: Hepatitis C virus coinfection and HIV load, $CD4^+$ cell percentage, and clinical progression to AIDS or death among HIV-infected women: Women and Infants Transmission Study. Clin Infect Dis 40: 859-867, 2005.
21. Sulkowski MS, Moore RD, Mehta SH, Chaisson RE and Thomas DL: Hepatitis C and progression of HIV disease. JAMA 288: 199-206, 2002.
22. Piroth L, Grappin M, Cuzin L, *et al*: Hepatitis C virus co-infection is a negative prognostic factor for clinical evolution in human immunodeficiency virus-positive patients. J Viral Hep 7: 302-308, 2000.
23. Greub G, Ledergerber B, Battegay M, *et al*: Clinical progression, survival, and immune recovery during antiretroviral therapy in patients with HIV-1 and hepatitis C virus coinfection: the Swiss HIV Cohort Study. Lancet 356: 1800-1805, 2000.
24. Sabin CA, Telfer P, Phillips AN, Bhagani S and Lee CA: The association between hepatitis C virus genotype and human immunodeficiency virus disease progression in a cohort of hemophilic men. J Infect Dis 175: 163-168, 1997.
25. Harcourt GC, Donfield S, Gomperts E, *et al*: Longitudinal analysis of CD8 T-cell responses to HIV and hepatitis C virus in a cohort of co-infected haemophiliacs. AIDS 19: 1135-1143, 2005.
26. Parodi C, Belmonte L, Bare P, De Bracco MM and Ruibal-Ares B: Impact of human immune deficiency virus infection on hepatitis C virus infection and replication. Curr HIV Res 5: 55-67, 2007.
27. Marins JRP, Barros MB de A, Machado H, *et al*: Characteristics and survival of AIDS patients with hepatitis C: Brazilian National Cohort of 1995-1996. AIDS 19: S27-S30, 2005.
28. Daar ES, Lynn H, Donfield S, *et al*: Relation between HIV-1 and hepatitis C viral load in patients with hemophilia. J Acquir Immune Defic Syndr 26: 455-472, 2001.
29. Sulkwoski MS: Hepatitis C virus infection in HIV-1 infected patients. Curr Infect Dis Rep 3: 469-476, 2001.
30. Kim AY, Lauer GM, Ouchi K, *et al*: The magnitude and breadth of hepatitis C virus-specific $CD8^+$ T cells depend on absolute $CD4^+$ T cell count in individuals coinfected with HIV-1. Blood 105: 1170-1178, 2005.
31. UNAIDS - Joint United Nations Programme on HIV/AIDS. AIDS epidemic update: special report on HIV/AIDS: December 2006.
32. Brazil. Ministry of Health. National Coordination of Sexually Transmitted Diseases (STD) and AIDS. 1ª. a 26ª. semanas epidemiológicas January-June, 2006. Boletim Epidemiológico AIDS 1: 3-49, 2006 (In Portuguese).
33. Brazil. Ministry of Health. National Coordination of Sexually Transmitted Diseases (STD) and AIDS. 1ª. a 52ª. semanas epidemiológicas January-December, 2003. Boletim Epidemiológico AIDS 17, 2003 (In Portuguese).
34. Reiche EMV, Watanabe MAE, Bonametti AM, *et al*: Stromal cell-derived factor 1 (SDF1) genetic polymorphism in a sample of healthy individuals, seronegative individuals exposed to human immunodeficiency virus type 1 (HIV-1) and patients infected with HIV-1 from the Brazilian population. Int J Immunogenet 33: 127-133, 2006.
35. Reiche EMV, Watanabe MAE, Bonametti AM, *et al*: The effect of stromal cell-derived factor 1 (SDF1/CXCL12) genetic polymorphism on HIV-1 disease progression. Int J Mol Med 18: 785-793, 2006.
36. Brazil. Ministry of Health. National Coordination of Sexually Transmitted Diseases (STD) and AIDS. Recomendações para Terapia anti-retroviral em adultos e adolescentes infectados pelo HIV 2002/2003, Brasília, 2002. URL: http://www.aidsw.gov.br. Captured in 27/12/2002, 2002 (In Portuguese).
37. Brazil. Ministry of Health. National Coordination of Sexually Transmitted Diseases (STD) and AIDS. Revisão da Definição Nacional de Caso de AIDS em indivíduos com 13 anos ou mais, para fins de vigilância epidemiológica. Brasília: Ministry of Health, 1998 (In Portuguese).
38. Brazil. Ministry of Health. Agência Nacional de Vigilância Sanitária. Portaria no. 488, 17 June, 1998 (In Portuguese).

39. Morimoto HK, Caterino-de-Araújo A, Morimoto AA, *et al*: Seroprevalence and risk factors for human T cell lymphotropic virus type 1 and 2 infection in human immunodeficiency virus-infected patients attending AIDS referral center health units in Londrina and other communities in Paraná, Brazil. AIDS Res Hum Retroviruses 21: 256-262, 2005.
40. Fonseca MG, Szwarcwald CL and Bastos FI: Análise sociodemográfica da epidemia de Aids no Brasil, 1989-1997. Rev Saude Publica 36: 678-685, 2002.
41. Mendes-Corrêa MC, Barone AA, Cavalheiro NP, Tengan FM and Guastini C: Prevalence of hepatitis B and C in the sera of patients with HIV infection in São Paulo, Brazil. Rev Inst Med Trop Sao Paulo 42: 81-85, 2000.
42. Braga WS, Da Costa Castilho M, Dos Santos IC, Moura MA and Segurado AC: Low prevalence of hepatitis B virus, hepatitis D virus and hepatitis C virus among patients with human immunodeficiency virus or acquired immunodeficiency syndrome in the Brazilian Amazon basin. Rev Soc Bras Med Trop 39: 519-522, 2006.
43. García-Samaniego J, Soriano V, Miró JM, *et al*: Management of chronic viral hepatitis in HIV-infected patients: Spanish consensus conference. October 2000. HIV Clin Trials 3: 99-114, 2002.
44. Sullivan PS, Hanson DL, Teshale EH, Wotring LL and Brooks JT: Effect of hepatitis C infection on progression of HIV disease and early response to initial antiretroviral therapy. AIDS 20: 1171-1179, 2006.
45. Tedaldi EM, Baker RK, Moorman AC, *et al*: Influence of coinfection with hepatitis C virus on morbidity and mortality due to human immunodeficiency virus infection in the era of highly active antiretroviral therapy. Clin Infect Dis 36: 363-367, 2003.
46. Mascheretti S, Hintichsen H, Ross S, Hampe J, Folesch UR and Schreiber S: Genetic variants in the CCR gene cluster and spontaneous viral elimination in hepatitis C-infected patients. Clin Exp Immunol 136: 328-333.
47. Reiche EM, Bonametti AM, Voltarelli JC, Morimoto HK and Watanabe MAE: Genetic polymorphisms in the chemokine and chemokine receptors: impact on clinical course and therapy of the human immunodeficiency virus type 1 infection (HIV-1). Curr Med Chem 14: 1325-1334, 2007.
48. Haynes BF, Pantaleo G and Fauci AS: Toward an understanding of the correlates of protective immunity to HIV infection. Science 271: 324-328, 1996.
49. Lifson AR, Buchbinder SP, Sheppard HW, *et al*: Long term human immunodeficiency virus infection in asymptomatic homosexual and bisexual men with normal $CD4^+$ lymphocyte counts: immunologic and virologic characteristics. J Infect Dis 163: 959-965, 1991.
50. Cocchi F, DeVico AL, Garzino-Demo A, Arya SK, Gallo RC and Lusso P: Identification of RANTES, MIP-1 alpha, and MIP-1 beta as the major HIV-suppressive factors produced by $CD8^+$ T cells. Science 270: 1811-1815, 1995.
51. Mikawa AY, Tagliavini SA and Costa PI: CCR5 genotype and plasma ß-chemokine concentration of Brazilian HIV-infected individuals. Braz J Med Biol Res 35: 1333-1337, 2002.
52. Cheng DM, Nunes D, Libman H, *et al*: Impact of hepatitis C on HIV progression in adults with alcohol problems. Alcohol Clin Exp Res 31: 829-836, 2007.
53. Bartlett JG and Gallant JE: Medical Management of HIV Infection. Johns Hopkins School of Medicine, Baltimore, MD, pp25-27, 2003.
54. Tanuri A, Caride E, Dantas MC, *et al*: Prevalence of mutations related to HIV-1 anti-retroviral resistance in Brazilian patients failing HAART. J Clin Virol 25: 39-46, 2002.
55. Cerqueira DM, Amorim RMS, Silva RR, Camara GNL, Brigido MM and Martins CRF: Anti-retroviral resistance and genetic diversity of human immunodeficiency virus type 1 isolates from the Federal District, Central Brazil. Mem Inst Oswaldo Cruz 99: 877-882, 2004.
56. Eyer-Silva WA and Morgado MG: A genotyping study of human immunodeficiency virus type-1 drug resistance in a small Brazilian municipality. Mem Inst Oswaldo Cruz 100: 869-873, 2005.