

Increased Hepatitis C Virus Load among Injection Drug Users Infected with Human Immunodeficiency Virus and Human T Lymphotropic Virus Type II

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Coinfection of hepatitis C virus (HCV) and human immunodeficiency virus (HIV) and/or human T-lymphotropic virus type II (HTLV-II) is common among drug users. We compared HCV RNA detection and load in a cohort of 6570 injection drug users from 9 US cities during 1987-1991. Of 385 subjects selected from 16 strata by sex, race (black or nonblack), and HIV/HTLV-II group (HIV positive [HIV⁺]/HTLV-II⁺, HIV⁺/HTLV-II negative [HTLV-II⁻], HIV⁻/HTLV-II⁺, and HIV⁻/HTLV-II⁻), 376 had HCV antibodies, of whom 305 had detectable HCV load. HCV RNA detection was unrelated to sex, race, and virus groups, but differed by study site. The mean HCV load was 5.4 log₁₀ IU/mL and was 0.24 log₁₀ higher in men than in women. Virus load increment with HIV or HTLV-II infection was higher among white subjects than among other subjects. Compared with HIV⁻/HTLV-II⁻ subjects, virus load was 0.50, 0.22, and 0.56 log₁₀ higher in HIV⁺/HTLV-II⁻, HIV⁻/HTLV-II⁺, and HIV⁺/HTLV-II⁺ subjects, respectively. HTLV-II infection significantly increased HCV load in white subjects but not in other racial groups.

Persons with long-term hepatitis C virus (HCV) infection are at increased risk of liver cirrhosis and hepatocellular carcinoma [1]. Although the overall prevalence is low (<2%) in the general population, the infection is common (>80%) among persons with high risk of parenteral exposure, including those with hemophilia or those with a history of injection drug use (IDU). Many of these individuals also are coinfecting with human immunodeficiency virus (HIV) [1-3]. Because HIV impairs the host's immune response, the natural course of HCV infection may be affected by the presence of HIV infection. A number of studies to date have shown that HCV load is elevated among persons with HIV infection [3-5]. Furthermore, risk of end-

stage liver disease is elevated among HCV carriers who are coinfecting with HIV [6-11], which raises concerns that HCV infection presents a significant burden of disease among HIV-infected persons. To complicate the natural history of HCV infection still more, many IDUs also are infected with human T lymphotropic virus type II (HTLV-II) [2, 12], a retroviral infection of uncertain consequences. HCV can infect and replicate in an HTLV-I-infected cell line [13], raising the possibility that, if coinfecting, HCV may also interact with HTLV-II in vivo. In the present study, we analyzed the independent and joint associations of HIV and HTLV-II infections on HCV load in a well-characterized population of US IDUs.

SUBJECTS, MATERIALS, AND METHODS

Study population. The subjects of the present investigation were participants of the National Institute on Drug Abuse (NIDA) Study, which investigates risk factors and trends in HIV seroprevalence among IDUs from methadone maintenance and detoxification clinics in 9 US cities [12, 14, 15]. Participating treatment

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centers were located in Baltimore, Chicago, Los Angeles, San Antonio, New York City, Asbury Park (New Jersey), Trenton (New Jersey), Philadelphia, and Miami, enrolling ~9000 participants aged ≥ 18 years who were admitted for treatment between March 1987 and December 1991. Sixty-six percent of enrolled participants were men. Thirty-eight percent were non-Hispanic black (hereafter "black"), 30% were non-Hispanic white (hereafter "white"), 30% were Hispanic, and $< 2\%$ were Asian/Pacific Islanders or Native Americans. Three percent were coinfecting with HIV and HTLV-II, 10% were infected with HIV alone, 15% were infected with HTLV-II alone, and 72% were negative for both infections [12]. Blood samples were collected at the time of study enrollment. Specimens were stored at -70°C in a central repository until used. Informed consent was obtained from all study participants. Study protocol followed the human experimentation guidelines of the US Department of Health and Human Services and institutional review boards of the National Cancer Institute and the National Institute on Drug Abuse.

Study subjects. After excluding Asian/Pacific Islanders and Native Americans, the 6570 participants [12, 14, 15] previously evaluated for HIV/HTLV-II infection status were classified into 16 groups, according to HIV/HTLV-II infection status (HIV positive [HIV⁺]/HTLV-II positive [HTLV-II⁺], HIV⁺/HTLV-II negative [HTLV-II⁻], HIV⁻/HIV⁺, and HIV⁻/HTLV-II⁻), sex (male vs. female), and race (black vs. white and Hispanic combined). Thirty-five individuals who were HTLV-I positive and 16 others who were HTLV-I and HTLV-II dually positive were excluded. We sought 25 subjects in each of these 16 sex-, race-, and virus-specific strata. Selection was random whenever > 25 persons in a category were available. Of the 400 sought, we found and tested stored serum samples from 385 subjects (96.3%) for HCV antibody detection and HCV RNA level (virus "load"). For non-black, female, HIV⁺/HTLV-II⁺ subjects, there were only 15 subjects available.

Laboratory methods. Serum samples were screened for antibodies to HIV by use of a whole-virus EIA (Du Pont NEN), with a confirmatory Western blot (Du Pont NEN). Detection of bands for gp120/160 and to p24 or gp41 defined the HIV positivity. Serum samples also were screened for HTLV-I/II antibodies by recombinant p21e EIA (Cambridge Biotech) or whole-virus EIA (DuPont NEN or Genetic Systems). Reactive sera in either EIA were confirmed by Western blot (Biotech Research Laboratories). Reactivity to both p24 and rp21e were defined as HTLV-I/II⁺. Infection with HTLV-II was confirmed by synthetic peptide EIA (United Biomedical/Olympus), recombinant protein-enhanced Western blot (Diagnostic Biotechnology), or an algorithm comparing Western blot p24 and p19 band strength [12, 16]. HCV antibody was detected by RIBA3.0 (Chiron). The presence of at least 2 of the 4 bands (C22, C33, C100, or NS5) with the intensity of > 1 on the 0–

10 gray scale was defined as positive [17]. Plasma HCV load was measured by a branch DNA assay (HCV bDNA version 3.0; Bayer), which has a quantitation limit of $2500\text{--}40 \times 10^6$ copies/mL. Virus loads were converted to international units (IU)/mL, using the lot-specific conversion factor, according to the manufacturer's specification.

Statistical analysis. After excluding 7 HCV antibody negative and 2 indeterminate subjects, a total of 376 HCV-seropositive subjects were included in the main analyses. Age and HCV load were treated as continuous variables. HCV load was \log_{10} transformed. Proportions of subjects with detectable HCV load were compared across 3 stratifying variables (sex, race, and HIV/HTLV-II virus groups), as well as study site. Mean ages of subjects also were compared across these strata separately for those with and without detectable HCV RNA. To obtain population-based estimates of the means and proportions from the stratified random sample of subjects, we weighted subjects by the inverse of the sampling fraction of their sex, race, and virus-specific strata [18]. For example, in the stratum of black male subjects who were HIV⁻/HTLV-II⁻, we selected 25 of a total of 1088 subjects (sampling fraction, 0.023). In contrast, in the stratum of white female subjects who were HIV⁺/HTLV-II⁺, all 17 subjects who were available were selected (sampling fraction, 1.0). In the weighted analysis approach, each subject in the former stratum was weighted by $1/0.023 = 44$, and each subject in the latter stratum was weighted by $1/1 = 1.0$. Thus, a stratum with a low sampling fraction was overweighed, so that its contribution was proportional to its fraction in the population.

Standard multiple regression analysis was used to associate \log_{10} HCV load to age, sex, race, HIV/HTLV-II infection status, study site, and time since first drug use. Exploratory analysis, using kernel smoothing regression [19], suggested a more rapid increase in HCV load with years of drug use among subjects whose first drug use was < 10 years before study entry in 1987–1991. This shift in trend was modeled by use of change point linear regression analysis [20], with a node at 10 years. Because the exploratory analysis suggested significant interactions between race and some of the covariates, race-specific estimates from the regression analysis are presented, with white subjects separated a posteriori from Hispanic subjects. We checked the adequacy of the fitted regression models by residual-based regression diagnostics methods. These explorations suggested that variances of \log_{10} (HCV RNA) were unequal across virus status. Because "heteroscedastic" variance may influence the SE estimates (confidence intervals) obtained from standard software, we also obtained bootstrap standard errors [21]. We found these robust variance estimates to be very similar to the standard variance estimates and chose to report the latter. Statistical significance was based on 2-sided tests at $\alpha = 0.05$.

RESULTS

Characteristics of the 376 HCV-positive study subjects are presented in table 1. The mean age of the 376 subjects was 37.6 years (range, 20–74 years), which was similar to the mean age of the whole sample (36.9 years). The mean age was higher in men than in women, in Hispanics and blacks than in whites, and in HTLV-II⁺ subjects than in HTLV-II⁻ subjects. Three hundred five (81.1%) of the 376 HCV-seropositive subjects had detectable HCV RNA. The sampling-adjusted proportion with detectable HCV RNA for the underlying population was 80.9%. Probability of detection did not differ significantly by race, sex, or virus group (table 1), but did differ by study site ($P = .03$), being lowest in Philadelphia (46%) and Trenton/Asbury Park (51%), and highest in New York (94%). Mean age was similar for those with and without detectable HCV RNA (37.7 vs. 36.7 years; $P = .48$).

Among subjects with detectable HCV loads, the unadjusted and sampling-adjusted mean HCV load was 5.5 and 5.4 (range, 2.7–6.9) \log_{10} IU/mL, respectively. The adjusted mean HCV load was 5.5 \log_{10} IU/mL among men and 5.4 \log_{10} IU/mL among women. The adjusted mean HCV loads for white, black, and Hispanic subjects were 5.8, 5.5, and 5.4 \log_{10} IU/mL, respectively. The adjusted mean HCV load was 5.4 \log_{10} IU/mL among subjects with neither infection, 5.6 \log_{10} IU/mL among subjects with HIV infection alone, 5.5 \log_{10} IU/mL among subjects with HTLV-II infection alone, and 5.8 \log_{10} IU/mL among subjects with both infections.

The kernel smoothing regression plot of the nonparametric relationship between the time since first injection drug use and the mean HCV load is shown in figure 1. The mean HCV RNA increased with time since the initial injection drug use, but only during the first 10 years. There was little association with time since first drug use thereafter. Accounting for this change in trend, we estimated the differences in the mean HCV load across groups by a change point multiple linear regression analysis with a node at 10 years (table 2).

With adjustment for age, sex, race, and study site, HCV load was significantly elevated in the HIV⁺/HTLV-II⁺, HIV⁺/HTLV-II⁻, and HIV⁻/HTLV-II⁺ subjects, compared with that in the HIV⁻/HTLV-II⁻ subjects. HCV loads in men were 0.24 \log_{10} higher, on average, than those in women. HCV loads in Hispanic subjects were significantly lower, compared with those in white subjects ($P = .05$); HCV loads also were lower in blacks than in whites, but this was not statistically significant ($P = .50$). HCV load was 0.56, 0.50, and 0.22 \log_{10} IU/mL higher among the HIV⁺/HTLV-II⁺, HIV⁺/HTLV-II⁻, and HIV⁻/HTLV-II⁺ subjects, respectively, compared with that in the HIV⁻/HTLV-II⁻ subjects. Although the joint association of HIV and HTLV-II was smaller than the sum of the association by each virus alone, there was no statistically significant interaction between HIV and HTLV-II on HCV load ($P = .45$).

Table 1. Hepatitis C virus (HCV) RNA detection among 376 HCV antibody-positive US injection drug users in the National Institute on Drug Abuse Study, stratified by sex, race, human immunodeficiency virus (HIV)/human T lymphotropic virus type II (HTLV-II) status, and study site.

Characteristic	Total	Detectable (n = 305)	Undetectable (n = 71)	P^a
Sex				
Male	195	162 (82)	33 (18)	.52
Female	181	143 (78)	38 (22)	Referent
Race/ethnicity				
Black	197	157 (80)	40 (20)	.63
Hispanic	106	88 (83)	18 (17)	.92
White	73	60 (82)	13 (18)	Referent
HIV/HTLV-II status				
HIV ⁺ /HTLV-II ⁺	85	70 (84)	15 (16)	.49
HIV ⁺ /HTLV-II ⁻	98	84 (87)	14 (13)	.30
HIV ⁻ /HTLV-II ⁺	99	77 (77)	22 (23)	.80
HIV ⁻ /HTLV-II ⁻	94	74 (80)	20 (20)	Referent
Study site				
Los Angeles	78	60 (78)	18 (22)	.03
Trenton/Asbury Park	22	16 (51)	6 (49)	
Baltimore	58	50 (84)	8 (16)	
New York City	67	61 (94)	6 (6)	
San Antonio	34	26 (76)	8 (24)	
Chicago	104	83 (85)	21 (15)	
Philadelphia	13	9 (46)	4 (54)	

NOTE. Data are no. (%) of subjects with detectable HCV RNA but excludes 7 negative for HCV antibody and 2 with indeterminate infection status. Proportions are adjusted for the sampling fraction per sex, race/ethnicity, and virus category. +, Positive; -, negative

^a P values compare sampling-adjusted proportion of subjects with and without detectable HCV RNA. The referent groups are female, white, HIV⁻/HTLV-II⁻. P value for study site is derived by test for heterogeneity.

The associations of HIV and HTLV-II coinfection with HCV load varied by racial group. Among whites, the \log_{10} IU/mL increase in HCV RNA among the HIV⁺/HTLV-II⁺, HIV⁺/HTLV-II⁻, and /HTLV-II⁺ subjects, compared with that in the HIV⁻/HTLV-II⁻ subjects, was 1.08, 0.87, and 1.05, respectively (table 2). The associations were much smaller among black subjects, with only HIV⁺ subjects having significantly elevated HCV loads. There was no significant elevation of HCV loads in any of the virus groups among Hispanics. In addition, the association of male sex with increased HCV loads was much larger among whites (0.72 \log_{10} IU/mL) than among Hispanics or blacks (0.17 and 0.15 \log_{10} IU/mL, respectively).

DISCUSSION

To compare the detection of HCV RNA between subjects with HIV or HTLV-II infection and to estimate the independent and joint associations of these infections on HCV load in serum,

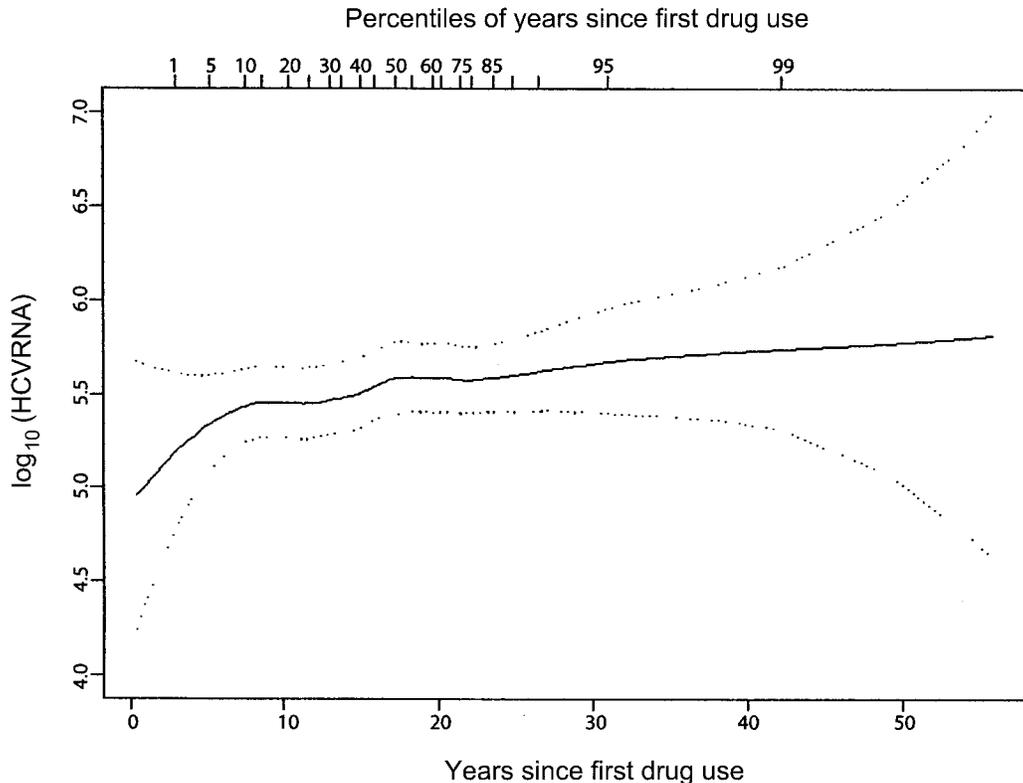


Figure 1. Association of hepatitis C virus (HCV) loads (\log_{10} IU/mL) with years since first injection drug use in 305 subjects in the National Institute on Drug Abuse Study. Percentage of distribution of subjects by years since first injection drug use is shown on top axis. Those who first used injection drugs within 10 years of enrollment (1987–1991) were likely to have acquired HCV infection during the human immunodeficiency virus epidemic in the US. Slope of the association between HCV load and time since infection is flat ($=0$) for subjects who were likely to have acquired HCV infection before the AIDS era. Figure is generated by the loess function of S-plus 6.1 software [19], with the default parameter options that include the tri-cube weight (Kernel) function and a span parameter of $\alpha = 0.75$.

we evaluated stratified random samples of IDUs who were enrolled during 1987–1991. Coinfection with HIV, with HTLV-II, or with both of these retroviruses was associated with significant elevation of HCV load but not with mere detection of HCV RNA. Being white, male, and an IDU for <10 years also were associated with a higher HCV load.

The association of HIV coinfection with higher HCV load has been described elsewhere [22] and is not surprising, given the devastating effect of HIV infection on cellular immunity. In contrast, a potential relationship between HTLV-II and HCV load has not been evaluated previously and may be surprising, given the paucity of clinical disease associated with HTLV-II [15, 23]. No previous studies of interactions between HTLV-II and HCV have been published, but the plausibility of a relationship is supported by 3 studies of the closely related virus, HTLV-I. First, the HTLV-I-infected T cell line, MT-2, appears to be capable of supporting low-level replication of HCV, which implies that intracellular interactions could occur [13]. Second, in a prospective cohort study of HCV-seropositive persons in Japan, HTLV-I coinfection was associated with a 6-fold increased risk of liver disease and a 2.6-fold increased risk of liver

cancer mortality [24], which suggests that HTLV-I-induced immune perturbation and inflammatory cytokine dysregulation could affect HCV persistence and progression to disease [25]. Third, also in Japan, HTLV-I coinfection was associated with a higher prevalence of HCV RNA detection and with a lower rate of clearance of HCV RNA with human lymphoblastoid interferon- α therapy, as well as with an elevated HCV load [26].

In our data, detection of HCV RNA was not associated with race/ethnicity, which is consistent with a study of patients receiving dialysis in Los Angeles [27], but is seemingly inconsistent with a higher likelihood of chronic HCV viremia among blacks in a study of HIV-infected patients in New Orleans and a cohort of IDUs in Baltimore [28, 29]. Differences in laboratory techniques, specimen integrity, and definitions probably account for some of the inconsistencies. The Baltimore cohort was evaluated for viral clearance, using a sensitive polymerase chain reaction (PCR) technique and a stringent definition—that is, inability to detect HCV RNA on 2 specimens collected >5 months apart [29]. In contrast, we measured HCV RNA with a less-sensitive technique, bDNA, on a single specimen per subject that had been stored frozen for ~10 years. All these

Table 2. Estimates of differences in hepatitis C virus (HCV) load by time since first injection drug use, age, sex, race, and virus group in 305 injection drug users in the United States.

Variable	All racial groups	White	Hispanic	Black
Intercept	4.69 (3.87 to 5.51)	5.50 (3.89 to 7.10)	3.83 (2.27 to 5.40)	4.33 (2.95 to 5.72)
Years since first drug use				
≤10	0.02 (−0.05 to 0.09)	−0.14 (−0.29 to 0.005)	0.02 (−0.09 to 0.14)	0.06 (−0.04 to 0.16)
>10	−0.02 (−0.10 to 0.05)	0.14 (−0.04 to 0.33)	−0.003 (−0.14 to 0.13)	−0.07 (−0.18 to 0.05)
Age, per 10 years	0.04 (−0.18 to 0.27)	0.08 (−0.43 to 0.59)	−0.17 (−0.65 to 0.32)	0.11 (−0.19 to 0.42)
Male sex	0.24 (0.03 to 0.46)	0.72 (0.22 to 1.21)	0.17 (−0.30 to 0.65)	0.15 (−0.13 to 0.43)
Race/ethnicity				
Hispanic	−0.28 (−0.63 to −0.06)
Black	−0.10 (−0.40 to 0.20)
HIV/HTLV-II status				
HIV ⁺ /HTLV-II ⁺	0.56 (0.26 to 0.87)	1.08 (0.35 to 1.81)	0.43 (−0.33 to 1.21)	0.38 (−0.02 to 0.79)
HIV ⁺ /HTLV-II [−]	0.50 (0.20 to 0.81)	0.87 (0.17 to 1.58)	0.21 (−0.60 to 1.03)	0.60 (0.21 to 0.99)
HIV [−] /HTLV-II ⁺	0.22 (−0.09 to 0.52)	1.05 (0.31 to 1.78)	−0.15 (−0.84 to 0.53)	0.20 (−0.21 to 0.61)

NOTE. Data are log₁₀ international units (IU)/mL difference (95% confidence interval). Reference groups for the overall model are 20 years old for age, female for sex, white for race, and human immunodeficiency virus (HIV)[−]/human T lymphotropic virus type II (HTLV-II)[−] for virus group. Estimates for age are per 10-year increase in level. Models were adjusted for all variables shown, as well as for study site. Change-point multiple linear regression models with the node at 10 years since first drug use were used. Positive value for each variable indicates increment in HCV load associated with the variable. The negative estimate for “>10 years since first drug use” indicates the change in slope from “10 years since first drug use.” Thus, the estimate of trend after the 10-year node is given by 0.02 − 0.02 = 0. +, Positive; −, negative.

differences could have contributed to the higher proportion of specimens (19%) that were negative for HCV RNA in our study, compared with the 10% HCV clearance reported for the Baltimore cohort [29]. The composition of the study population also could determine the magnitude of the association of race/ethnicity, because differential distribution of the human leukocyte antigen (HLA) and other polymorphic genes across ethnic groups may affect the likelihood of HCV clearance [30–32]. The varying HCV detection by study site in the present study may have, in part, resulted from differential genetic characteristics and/or risk behaviors of individuals in these areas. Further investigation is needed to confirm or refute an association between race/ethnicity and likelihood of recovery from HCV infection.

Among our IDUs who had detectable HCV viremia, the associations of HIV and HTLV-II infections with increased HCV load were profound among whites, intermediate among blacks, and least evident among Hispanics. Adjusted for the prevalence of HIV and HTLV-II in each group, as well as for age, sex, study site, and time since first injection drug use, HCV load was similar among blacks and whites ($P = .50$) but was significantly lower among Hispanics versus whites. Why Hispanics should have a lower HCV load is not readily apparent.

In some oncogenic virus infections, virus load differs by sex. In a cohort of HTLV-I carriers in Japan, HTLV-I provirus detection and load were significantly higher in men than in women [33]. In a cohort of HIV-infected patients in Italy, the mean HIV RNA level was 0.29 log₁₀ higher in men, compared with that in women, after adjustment for duration of infection

[34]. Detection and persistence of hepatitis B virus also have been shown to be higher in men than in women [35]. A higher HCV load in men in our study is consistent with these observations, but it does not agree with other studies on HCV that found no difference based on sex [27, 28].

The trend between HCV load and years since first injection drug use is noteworthy. Virus load was directly related to time among subjects who began to use injection drugs and probably acquired HCV infection within 10 years of the study entry through 1991, but was not related to time among those who had used injection drugs before the early 1980s (figure 1). The former group represents IDUs who acquired HCV infection during the AIDS epidemic. Therefore, one may speculate that concurrent HIV infection and resulting immune suppression might contribute to a significant increase in HCV load. An alternative explanation for our finding is a rapid increase of HCV load during the early years of HCV infection, regardless of HIV infection. Because of the limited number of subjects in our study, the results of kernel smoothing regression analysis stratified by HIV status could not conclusively distinguish between these possibilities (data not shown).

Older age was associated with a higher HCV load in patients with hemophilia [7]. Among our IDUs, after adjustment for HIV, HTLV-II, race, study site, and time since first injection drug use, HCV load was strongly associated with age, albeit not significantly. The association of age with HCV load was more evident among blacks than among other racial groups. The lack of significant association between age and HCV load in our study may in part be attributable to highly variable

duration of HCV infection among older study subjects, as well as oversampling of some subgroups, particularly white and Hispanic subjects with HTLV-II infection.

There were other limitations in our study. We relied on self-report to indicate when injection drug use began and assumed that HCV infection occurred shortly after initiation of injection drug use. Although this assumption is supported by a prospective study of IDUs [36], enrollment of participants before acquiring HCV infection would accurately estimate the date of infection and enable direct evaluation of the association between HCV load and duration of infection. Such a prospective study design also would have allowed for evaluation of longitudinal samples drawn from the same individual over time. As noted previously, use of a more sensitive PCR-based assay for the detection of HCV RNA, other than bDNA, would have reduced the misclassification of subjects with virus clearance. Lack of information on therapies for HCV infection is a concern. However, because all subjects had been enrolled by December 1991, which was before or immediately after the discovery of HCV, very few, if any, would have received any treatment that would have affected our results. Finally, although a priori sampling combined white and Hispanic subjects into a single group, unanticipated differences between Hispanic and white subjects during the preliminary analysis forced us to analyze a posteriori these 2 racial groups separately. Although our point estimates are valid, the confidence intervals were wide because of further stratification.

In summary, white male subjects with HIV and HTLV-II infections had the highest HCV loads. Because infection with retroviruses is relatively common among HCV-infected IDUs, HIV and HTLV-II may contribute substantially to the propagation of HCV transmission and to what is already a large public health problem. Ultimately, understanding the interactions among these viruses may be critical in developing policies and programs for the control of HCV in the population and for the assessment and care of those who have been infected.

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