

Effect of Human Immunodeficiency Virus Infection on Hepatitis C Virus Infection in Hemophiliacs

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Chronic liver disease due to hepatitis C virus (HCV) infection is a major problem in hemophiliacs. Recent reports suggested that hemophiliacs coinfecting with hepatitis C virus and human immunodeficiency virus (HIV) have an increased incidence of liver failure but the mechanism of accelerated liver injury is not clear. We tested plasma from 100 hemophiliacs for anti-HCV by second generation ELISA, anti-HIV by EIA, and HCV RNA and HIV RNA by branched DNA and polymerase chain reaction assays to determine if hemophiliacs coinfecting with HCV and HIV have higher HCV RNA levels and more active liver disease. Seventy-nine (79%) patients were anti-HCV positive, of whom 85% were HCV RNA positive. None of the anti-HCV-negative patients had detectable HCV RNA in plasma. Forty-two (42%) patients were anti-HIV positive, of whom 47% had detectable HIV RNA. All the anti-HIV-positive patients were also anti-HCV positive. The prevalence of both anti-HCV and anti-HIV increased significantly with age. There was no difference in HCV RNA levels between anti-HIV-positive and anti-HIV-negative patients (mean: 21 ± 4 vs 18 ± 5 Meq/ml), although HCV RNA levels were significantly higher in anti-HIV-positive patients with CD4 counts $<200/\text{mm}^3$ ($P = 0.008$). There was an inverse correlation between HCV RNA levels and CD4 counts but no correlation was found between HCV RNA and serum aminotransferase levels. We found a high prevalence of HCV and HIV coinfection in our hemophiliacs. Hepatitis C virus replication appears to be increased in patients with severe immunodeficiency secondary to progressive HIV infection. However, there was no correlation between HCV RNA and serum ALT level, suggesting that HCV is not directly cytopathic.

KEY WORDS: hepatitis C virus; hepatitis C virus RNA; human immunodeficiency virus; chronic liver disease; hemophilia.

More than 50% of multitransfused hemophiliacs who received untreated clotting factors have chronic or intermittent abnormalities in liver biochemistries (1–

3), and up to 15% have established cirrhosis on liver biopsies (3–6). The predominant cause of chronic liver disease in hemophiliacs has now been shown to be due to hepatitis C virus (HCV) (7–11). It has also been shown that multitransfused hemophiliacs who received clotting factors prior to 1985 have a high prevalence of human immunodeficiency virus (HIV) infection (12). Thus, many hemophiliacs are dually infected with HCV and HIV.

Three studies suggested that patients with concur-

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rent HCV and HIV infection have an increased incidence of liver failure compared to patients infected with HCV alone (13–15). Martin et al reported that all three patients with concurrent HIV infection and non-A, non-B (NANB) hepatitis developed hepatic decompensation within three years of overt hepatitis in contrast to 8 (8.5%) of 94 patients who had NANB hepatitis alone (13). Eyster et al found that 8 (9%) of 91 hemophiliacs with concurrent HCV and HIV infection developed liver failure compared to none of 58 hemophiliacs with HCV alone (age adjusted $P = 0.034$, relative risk = 3.2) (14). Telfer et al reported that the relative risk of developing liver failure was 21 in hemophiliacs coinfecting with HCV and HIV compared to hemophiliacs with HCV infection alone (15). However, other factors such as age, alcohol, medications, and concurrent opportunistic infections secondary to HIV infection may have contributed to the liver failure in some of these patients. Thus, the mechanism(s) by which HIV infection accelerates the progression of HCV related liver disease is unclear.

Several studies have now shown that patients with concurrent HCV and HIV infection have higher serum HCV RNA levels, suggesting that concurrent HIV infection increases HCV replication (16, 17). Eyster et al found a significant increase in HCV RNA levels in anti-HCV-positive hemophiliacs who developed superinfection with HIV and an eightfold faster rate of increase in HCV RNA levels during follow-up compared to those who remained HIV negative (17). They also found a negative correlation between HCV RNA levels and CD4 counts and a positive correlation between serum HCV RNA and aspartate aminotransferase (AST) levels, suggesting that acceleration of liver disease in hemophiliacs with concurrent HCV and HIV infection may be due to enhanced HCV replication secondary to progressive immunodeficiency. However, the correlation between serum HCV RNA levels and activity of liver disease remains controversial (16–21). This is in part related to the difficulty in accurate quantitation of serum HCV RNA levels using polymerase chain reaction (PCR) assays, and the lack of longitudinal studies with adequate time points.

This study was conducted to determine: (1) the prevalence of HCV and HIV infection in a cohort of hemophiliacs, (2) if HCV RNA levels are higher in hemophiliacs with concurrent HIV infection and if there is an inverse correlation with CD4 counts, (3) if liver disease is more active in hemophiliacs with concurrent HIV infection, and (4) if there is a positive

correlation between serum ALT and HCV RNA levels.

MATERIALS AND METHODS

Patients. A total of 99 hemophiliacs and one patient with von Willebrand's disease who were followed at the Louisiana Comprehensive Hemophilia Care Center at Tulane University School of Medicine were studied. There were 99 males and one female, with a mean age of 23.2 ± 1.4 years, (range 2–65 years). None of the patients were known to abuse drugs or alcohol.

Methods. Citrated plasma samples were prospectively collected between 1993 and 1994 and stored at -70°C prior to testing. All patients were tested for HCV antibody (anti-HCV), HIV antibody (anti-HIV), hepatitis B surface antigen (HBsAg), hepatitis B core antibody (anti-HBc), HCV RNA, and liver chemistries. HIV RNA and CD4 counts were obtained in all anti-HIV-positive patients. Anti-HCV was tested by ELISA-2 (Chiron Corporation, Emeryville, California), and quantitative HCV RNA level by the branched DNA (bDNA) assay (Chiron). All patients who had undetectable HCV RNA levels in the bDNA assay were retested for HCV RNA by qualitative reverse transcription-PCR assay as previously described (22). Anti-HIV was detected by EIA (Abbott Laboratories, North Chicago, Illinois), and all positive samples were confirmed by western blot. HIV RNA levels were quantitated by the bDNA assay (Chiron). The cutoff values for detection of HCV RNA and HIV RNA were 0.35×10^6 eq/ml and 1.0×10^4 eq/ml, respectively. HBsAg and anti-HBc were tested by ELISA (Abbott). Liver chemistries and CD4 counts were determined by standard methods. Activity of liver disease was assessed by serum alanine aminotransferase (ALT) levels, as we think that it was not justifiable to perform liver biopsies on these patients because of the risks of bleeding and the extra costs of factor concentrates and prolonged hospital stay.

Statistics. Group comparisons were made with Student's t test and chi-square test as indicated. Associations were made with Spearman's correlation; patients who were HCV RNA negative in the bDNA assay were excluded from the analysis. Arbitrary values were not assigned to these patients since their true HCV RNA levels may range from 0 to 0.34×10^6 eq/ml.

RESULTS

Anti-HCV was detected in 79 (79%) patients. Of these, 65 (82%) were positive for HCV RNA by the bDNA assay. Of the 14 anti-HCV-positive patients who had HCV RNA levels below the level of detection in the bDNA assay, only 2 (14%) tested positive for HCV RNA by RT-PCR. None of the anti-HCV-negative patients was found to be HCV RNA positive by the bDNA as well as the RT-PCR assays. There was a significant difference in age and mean ALT levels between anti-HCV-positive and anti-HCV-

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TABLE 1. COMPARISON BETWEEN HCV-POSITIVE AND HCV-NEGATIVE PATIENTS

	Anti-HCV+ (N = 79)	ANTI-HCV- (N = 21)
HBV serology		
HBsAg positive	6 (7.6)*	0 (0)
Anti-HBc positive	46 (58.2)	1 (4.8)
HIV serology		
Anti-HIV positive	42 (53.2)	0 (0)
Elevated ALT (N)	49 (62)	1 (4.8)
ALT (units/liter)		
(mean ± SEM)	66 ± 6.2†	20 ± 2.5‡
Age (yr)		
(mean ± SEM)	27 ± 2‡	9 ± 1‡

* Numbers in parentheses are percentages.

† $P = 0.0002$.

‡ $P < 0.0001$.

negative patients ($P < 0.0001$ and $P = 0.0002$, respectively) (Table 1).

Anti-HIV was detected in 42 (42%) patients. Plasma samples from 38 anti-HIV-positive patients were available for HIV RNA testing. Of these, HIV RNA was detected in 18 (47%). HBsAg was detected in 6 (6%) and anti-HBc in 47 (47%) patients. All the anti-HIV-positive and all the HBsAg-positive patients were also anti-HCV positive.

Fifty (50%) patients had elevated serum ALT levels, of whom only one was anti-HCV negative. This patient was an 8-year-old boy with severe factor 8 deficiency and persistently elevated ALT levels. He was HBsAg, anti-HBc, anti-HCV, and anti-HIV negative.

The prevalence of both anti-HCV and anti-HIV increased significantly with age ($P < 0.0001$ and $P = 0.0004$ respectively, Table 2). The prevalence of anti-HCV was significantly higher than that of anti-HIV for every age group. Among the children aged ≤ 10 years, 33% were anti-HCV positive but none was anti-HIV positive. The prevalence of anti-HCV in-

creased rapidly to almost 100% in patients older than 20 years, while the corresponding prevalence of anti-HIV increased to 60% only.

A higher proportion of anti-HCV- and anti-HIV-positive patients were HCV RNA positive by the bDNA assay compared to the anti-HCV-positive, anti-HIV-negative patients, but the difference did not reach statistical significance. There was no significant difference in HCV RNA levels between the anti-HIV-positive and the anti-HIV-negative patients. However, as can be seen in Table 3 and Figure 1, HCV RNA levels were significantly higher in the subgroup of patients with severe immunodeficiency (CD4 counts less than 200/mm³). In addition, there was a significant inverse correlation between HCV RNA levels and CD4 counts (Figure 2). There was no correlation between age and HCV RNA levels (Figure 3). This was true in both anti-HIV-positive and in anti-HIV-negative patients. Of the six HBsAg-positive patients, only three were HCV RNA positive by the bDNA assay; exclusion of these three patients did not alter the results of the above analyses. Likewise, inclusion of the two anti-HCV-positive patients who were HCV RNA positive in the RT-PCR assay but negative in the bDNA assay had no impact on the above analyses.

There was no difference in the percentage of patients with elevated ALT levels as well as the mean ALT levels between the HCV-positive patients with and without concomitant HIV infection irrespective of the CD4 counts (Table 3).

There was no correlation between HCV RNA and ALT levels. This was true for anti-HCV- and anti-HIV-positive patients as well as anti-HCV-positive, anti-HIV-negative patients (Figure 4).

Of the 38 anti-HIV-positive patients who were tested for HIV RNA, 18 (47%) were HIV RNA positive by the bDNA assay, 17 of whom were also HCV RNA positive. There was no statistical difference in the percentage of patients who were HCV

TABLE 2. AGE-SPECIFIC PREVALENCE OF HCV, HIV, AND HBV INFECTIONS

Age (yr)	Patients (N)	Anti-HCV+ (N)	Anti-HIV+ (N)	HBsAg+ (N)	Anti-HBc+ (N)
0-10	18	6 (33)*	0 (0)	0 (0)	1 (6)
11-20	30	22 (73)	11 (37)	3 (10)	11 (37)
21-30	26	25 (96)	15 (58)	1 (4)	20 (80)
31-40	15	15 (100)	10 (67)	2 (13)	8 (53)
>41	11	11 (100)	6 (55)	0 (0)	7 (64)
Total	100	79	42	6	47
		$P < 0.0001$	$P = 0.0004$	$P = NS$	$P < 0.0001$

*Numbers in parentheses are percentages.

TABLE 3. COMPARISON BETWEEN ANTI-HCV+/ANTI-HIV+ AND ANTI-HCV+/ANTI-HIV-PATIENTS

	Anti-HCV+ (N = 79)			
	Anti-HIV+		Total	Anti-HIV-
	CD ₄ <200	CD ₄ >200		
Anti-HCV+ (N)	16	26	42	37
HCV RNA+ [N(%)]	14 (88)*	24 (92)	37 (88)*	29 (78)
HCV RNA level Meq/ml [mean (range)]	35.6 ± 7.7a†	13.5 ± 4.0b	21.3 ± 4.1c	17.5 ± 4.6d
Elevated ALT [N (%)]	8 (50)	19 (73)	27 (64)	22 (59)
ALT (U/L), (mean ± SEM)	64 ± 13e	71 ± 9f	68 ± 7g	63 ± 10h

* Includes one patient HCV RNA positive in RT-PCR assay.

† a vs b, $P = 0.008$; a vs d, $P = 0.04$; b vs d, $P = NS$; c vs d, $P = ns$; e vs f, $P = NS$; e vs h, $P = NS$; f vs h, $P = ns$; g vs h, $P = ns$.

RNA positive or in the mean HCV RNA levels between the patients in whom HIV RNA was detected and those in whom HIV RNA was not detectable, although the numbers were too small to draw meaningful conclusions. Furthermore, there was no correlation between HCV RNA and HIV RNA levels. Surprisingly, there was no correlation between HIV RNA levels and CD4 counts, but this may again be related to the small sample size.

DISCUSSION

The prevalence of anti-HCV in our cohort of multitransfused hemophiliacs was 79%; this is in keeping with other published data (7–11, 14, 18). The obser-

vation that all anti-HIV- and all HBsAg-positive patients were also anti-HCV positive indicates that all of these infections were most likely acquired through the same route: transfusion of clotting factor concentrates. The significant increase in prevalence of anti-HCV, anti-HIV, and anti-HBc with age reflects not only an increase in the number of units of clotting factor concentrates received in older patients but also changes in donor screening and implementation of virus-inactivating procedures in the last decade. HBsAg screening was introduced in the early 1970s, while anti-HIV screening began in 1985, and anti-HCV screening did not take place until 1990 (23–27). Various virus-inactivation procedures have been em-

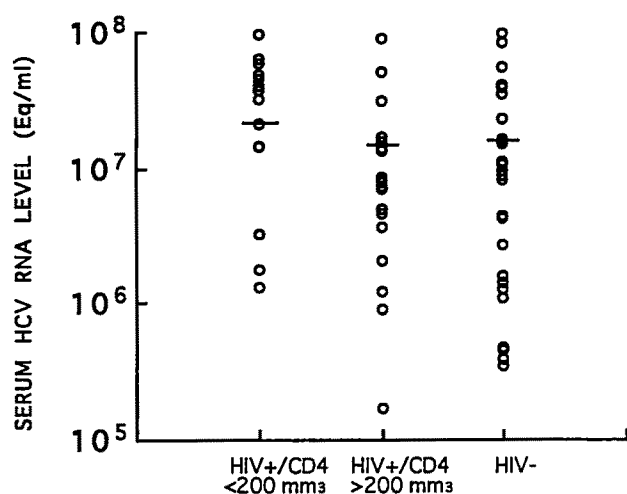


Fig 1. Serum HCV RNA levels in anti-HIV-positive and anti-HIV-negative patients. Bars denote mean HCV RNA levels.

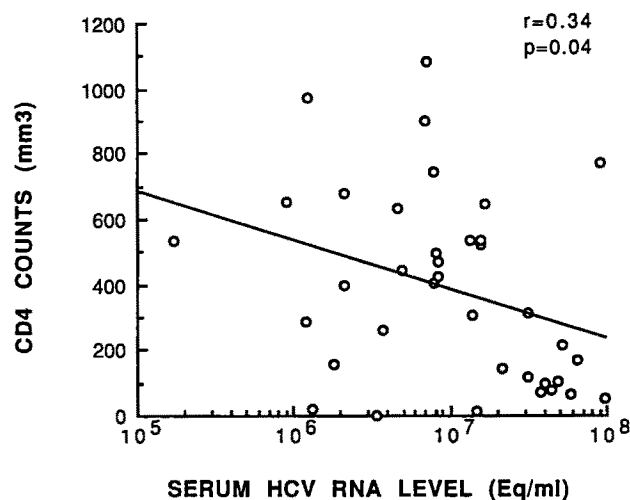


Fig 2. Correlation between serum HCV RNA levels and CD4 counts in anti-HIV-positive patients.

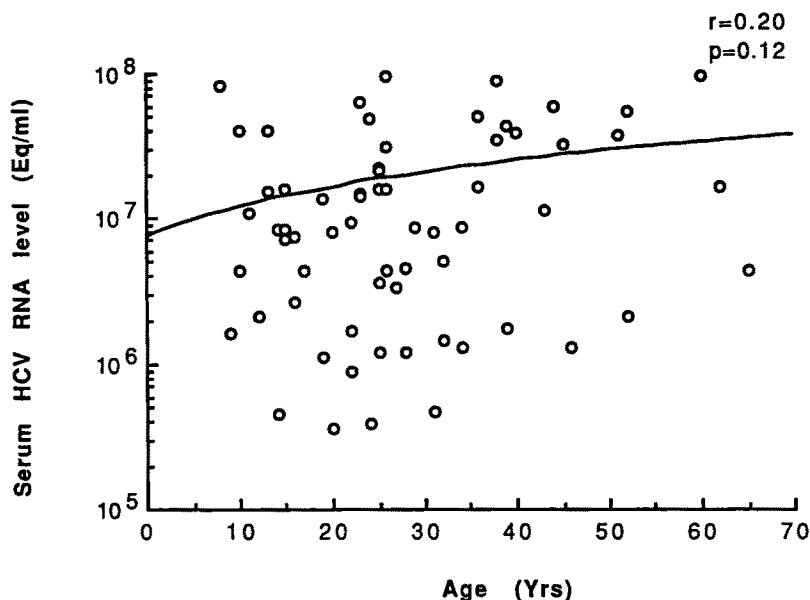


Fig 3. Correlation between age and serum HCV RNA levels in all patients.

ployed in the preparation of clotting factor concentrates in the past 10 years, but many of the earlier methods were ineffective, particularly in the elimination of non-A, non-B hepatitis (hepatitis C) (28, 29). Thus, effective elimination of HBV and HIV preceded that of HCV. This accounts for the higher overall as well as age-specific prevalence of anti-HCV. The significantly lower prevalence of HBsAg compared to anti-HBc is related to the lower risk of chronicity of hepatitis B infection when acquired during childhood (10–20%) or adult life (<5%). In contrast, almost 100% of HIV and approximately 70% of HCV infections progress to chronic infection (30).

We found that most (82%) of our anti-HCV-positive hemophiliacs were HCV RNA positive by the bDNA assay. The bDNA assay is less sensitive than PCR assays, but it has a high degree of internal consistency and is a more reliable quantitative assay (16). Only two (14%) of our anti-HCV-positive patients who had undetectable HCV RNA levels in the bDNA assay were HCV RNA positive by RT-PCR, supporting the validity of using HCV RNA levels by the bDNA assay in our correlation analyses. Some studies have shown that HCV RNA may be present in anti-HCV-negative, multitransfused patients who are immunosuppressed (31–33). Hepatitis C virus RNA was not detected in any of our anti-HCV-negative hemophiliacs, even when tested by the RT-PCR assay. This may be related to the fact that none of our anti-HCV-negative patients was immunosuppressed by concurrent HIV infection.

As a group, the anti-HIV-positive hemophiliacs had similar HCV RNA levels as the anti-HIV-negative ones, suggesting that HIV infection alone does not enhance HCV replication. This is supported by a lack of correlation between HCV RNA and HIV RNA levels, although the number of patients studied was small. In contrast, the anti-HIV-positive hemophiliacs with severe immunodeficiency as defined by CD4 counts below 200/mm³ had significantly higher HCV RNA levels compared to the anti-HIV-negative hemophiliacs as well as the anti-HIV positive ones who had CD4 counts above 200/mm³. We also found a negative correlation between HCV RNA levels and CD4 counts. These findings suggest that HCV replication is enhanced by immunodeficiency secondary to progressive HIV infection. This is in agreement with the finding of Eyster et al (17). Although the anti-HIV-positive patients with CD4 counts <200/mm³ were significantly older, the higher serum HCV RNA levels in these patients is probably unrelated to the longer duration of HCV infection, since we found no correlation between age and serum HCV RNA levels for the entire patient population or when the patients with and without HIV coinfection and those with CD4 counts above or below 200/mm³ were analyzed separately.

Fifty percent of our hemophiliacs had elevated ALT levels. Although we did not perform liver biopsies on our patients, we suspect that most of them have chronic liver disease based on histological studies of other investigators (3–6). Ninety-eight percent

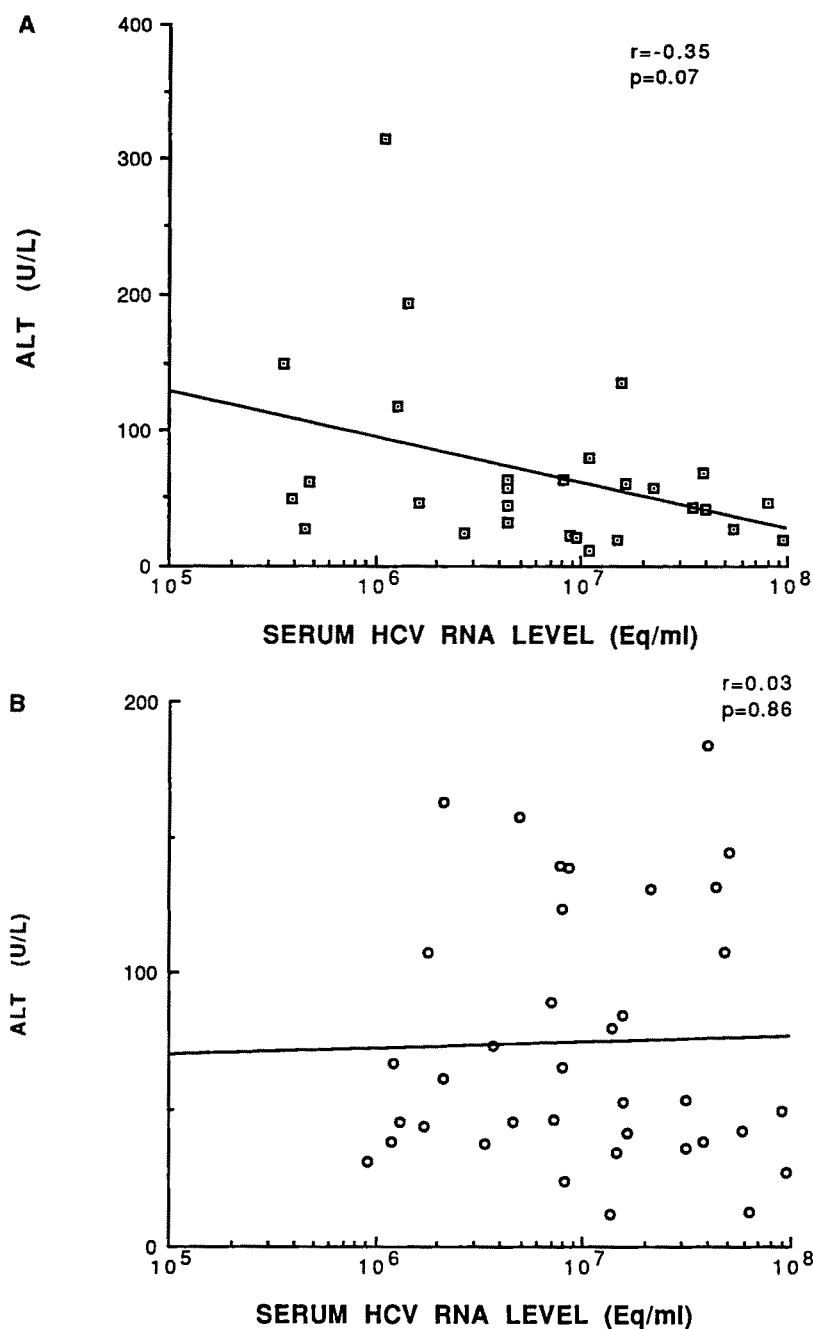


Fig 4. Correlation between serum HCV RNA and ALT levels in anti-HIV-positive (A) and anti-HIV-negative (B) patients.

of our hemophiliacs with elevated ALT levels were anti-HCV positive [including five patients (10%) who were also HBsAg positive], confirming that HCV is the predominant cause of liver disease in hemophiliacs. Thirty-eight percent of our anti-HCV-positive hemophiliacs had normal ALT levels despite the detection of HCV RNA in 73% of them. Some of these patients may have chronic liver disease since liver

biopsies of anti-HCV-positive nonhemophiliacs with normal ALT levels have shown a wide range of liver pathology from minimal changes to chronic hepatitis and cirrhosis (19, 20, 34–36). Contrary to the findings of Eyster et al (17), we found no correlation between HCV RNA and ALT levels and no difference in ALT levels between anti-HCV-positive hemophiliacs with or without concurrent HIV infection irrespective of

their CD4 counts. The lack of correlation between HCV RNA and ALT level or liver histology has also been reported by other investigators, suggesting that HCV-induced liver injury may not be due to a direct cytopathic effect of the virus (16, 19, 20, 33, 37, 38).

Surprisingly, there was no correlation between the HIV RNA levels and CD4 counts, although there was a trend toward higher HIV RNA levels with lower CD4 counts. This may be related to the small number of patients.

In summary, we found a high prevalence (79%) of anti-HCV in our cohort of hemophiliacs. More than half of these patients were also infected with HIV. There was a significant increase in the prevalence of HCV and HIV infection with age. HIV infection on its own did not seem to enhance HCV replication, but HCV replication may be increased as a result of severe immunodeficiency secondary to progressive HIV infection. We found no correlation between HCV RNA and ALT levels and no evidence of increased activity of liver disease as assessed by ALT levels in hemophiliacs with concurrent HIV infection, even in those with low CD4 counts and high HCV RNA levels.

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