CLINICAL TRIALS AND OBSERVATIONS

Correlates of spontaneous clearance of hepatitis C virus among people with hemophilia

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People with hemophilia were formerly at very high risk of infection with hepatitis C virus (HCV). Approximately 20% of HCV-infected patients spontaneously clear the virus. To identify correlates of spontaneous clearance of HCV, we studied a cohort of HCV-infected hemophilic subjects without human immunodeficiency virus infection who had never been treated with interferon. Plasma HCV RNA was persistently undetectable in 192 (27.0%) of 712 HCV-seropositive subjects. In multivariate analyses, HCV clearance was more **likely in subjects infected with HCV at younger age, especially with infection before age 2 years (40.1%) compared with after age 15 years (14.9%, P_{trend} < .0001), and with relatively recent infection, especially after 1983 (42.8%) compared with before 1969 (18.2%, P_{trend} < .0001). HCV clearance was marginally reduced with African ancestry (19%) and greatly increased with chronic hepatitis B virus (HBV) infection (59.1%, P = .001). Resolved HBV infection, coagulopathy types and severity, types of clotting factor treat-** **ment, and sex were not associated with HCV clearance. In conclusion, hemophilic subjects coinfected with chronic HBV and those infected with HCV before age 2 years or after 1983 were significantly more likely to spontaneously clear HCV viremia. These data highlight and clarify the importance of nongenetic determinants in spontaneous recovery from HCV infection. (Blood. 2006;107:892-897)**

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Introduction

The majority of patients infected with hepatitis C virus (HCV) fail to mount an effective immune response and develop persistent infection that may lead to chronic liver disease. Epidemiologic data from populations at risk of HCV exposure suggest that 14% to 40% of people infected with HCV spontaneously clear the virus and have no detectable HCV RNA in plasma.[1-7] Single exposure to HCV, younger age at infection, female sex, and certain major histocompatibility complex genes are likely to be associated with a higher spontaneous clearance rate.[2,5-8] African Americans and perhaps Asian Americans appear to be less likely than Hispanic or white Americans to clear the virus spontaneously.[7,9-11] Coinfection with human immunodeficiency virus 1 (HIV-1) is associated with significantly lower spontaneous HCV clearance, while chronic infection with hepatitis B virus (HBV) is associated with significantly higher spontaneous HCV clearance.[10,12]

People with hemophilia receiving clotting factor replacement therapy were at very high risk of infection with HCV before the introduction of donor screening and virus inactivation in the mid-1980s.[13-15] Of HCV antibody–positive patients with hemophilia, spontaneous clearance of HCV RNA has been reported in about 25% of those without HIV-1 coinfection compared with 10% or less of those with HIV-1 coinfection.[6,16-17] The clinical and epidemiologic determinants of HCV clearance in hemophilic patients are not well defined, although clearance may be more frequent among those infected at a younger age.[6] Clearance, when it occurs, is almost always within 1 to 2 years of primary infection.[18] The purpose of the current study was to identify clinical correlates of spontaneous HCV clearance in a well-characterized cohort of people with hemophilia who were infected with HCV without HIV-1.

Materials and methods

Study subjects

The second Multicenter Hemophilia Cohort Study (MHCS-II) was established to better identify, quantify, and develop markers for complications of HCV, as well as later complications of HIV-1 and its treatment.[19] All patients at the collaborating comprehensive hemophilia treatment centers were invited to participate in the MHCS-II if they had reached age 13 years, had a congenital coagulation disorder (hemophilia A or B [congenital factor VIII or IX deficiency], von Willebrand disease, or other, hereafter referred to as hemophilia), and if, since January 1, 1993, they had had a positive

result on a locally performed, licensed assay for HCV antibodies (anti-HCV), HIV-1 antibodies, or HIV-1 RNA. Earlier HCV and HIV-1 results were not considered to avoid false-positive results with first-generation assays. All 1449 HIV-uninfected subjects who enrolled in the MHCS-II from 2001 to 2004 were eligible for the current analysis. As described,[18] some small fraction of subjects had lost anti-HCV prior to MHCS-II enrollment, but this was not considered in the current analysis. Data on consumption of alcohol during the year before knowledge of being infected with HCV were collected by questionnaire. One drink was defined as 45 mL (1.5 oz) liquor, 120 mL (4 oz) wine, or 360 mL (12 oz) beer and was estimated to contain 12 g alcohol.[19] Additional information is available at https://mhcs-ii.rti.org. Excluding those who lacked HCV RNA data sufficient to define HCV clearance (n = 473, see "Definition of HCV clearance") and those with a history of interferon treatment (n = 264), a final total of 712 subjects were included. Approval was obtained from the Special Studies Institutional Review Board of the National Cancer Institute, NIH, as well as from the institutional review board of each of the participating centers. Informed consent was provided according to the Declaration of Helsinki.

Types of plasma exposures

Before the early to mid-1980s, when heat inactivation of enveloped viruses in clotting factor concentrate became standard practice, people with hemophilia were treated with non–heat-treated clotting factor concentrate (NHT), which was derived from large pools of plasma (20 000 to 50 000 donors), and with cryoprecipitate, which pooled a few to a maximum of 20 single-donor units or fresh-frozen plasma (subsequently referred to as cryo/plasma). Based on the types and the order of the treatment received, each subject in the current study was classified as having 1 of the 3 types of exposure: (1) cryo/plasma only; (2) cryo/plasma before NHT factor concentrate; (3) NHT concentrate before or without cryo/plasma.

Imputation of age and year at HCV infection

We assumed that concentrate used after December 31, 1986, was noninfectious, but concentrate used prior to December 31, 1986, resulted in infection with any single exposure. This date was well after use of NHT concentrate had ceased in the mid-1980s. However, sporadic cases of HCV transmission attributed to heat-treated clotting factor concentrates occurred over the next few years, until development and implementation of more stringent virucidal treatments and exclusion of anti-HCV–positive donors.[15] Therefore, for subjects in group 3 (see "Types of plasma exposures"), we assumed that HCV infection occurred as a consequence of the first exposure. For subjects in groups 1 and 2 above, the precise dates of infection were unknown but occurred in the interval between the first and last potentially infectious exposures. We assumed that for "similar" groups of subjects, defined below, the age-at-infection curve was proportional to the age-at-exposure curve. On the basis of this assumption, we estimated the age-at-exposure distribution using statistical methods, and imputed each subject's most likely age-at-infection as the expected value of this distribution, conditional on exposure within the individual's window. The imputed dates of infection were calculated by adding the imputed ages to the corresponding dates of birth. Exposure probabilities by age were estimated from exposure window data using an expectation maximization EM algorithm and nonparametric locally weighted scatter plot smoother (EMS algorithm).[20,21] While defining groups of subjects who could be pooled for purposes of imputation, we noted that naive estimates of age-at-exposure defined using the midpoints of the exposure windows were strongly dependent on the severity of hemophilia and the year of birth (data not shown). Therefore, for each severity group and date of birth, we applied the EMS algorithm using all subjects with the same severity and with dates of birth occurring within 2.5 years.

For analysis, age at infection was divided into approximate quartiles. These were age younger than 2, 2 to 5, 6 to 15, and 16 to 60 years. In contrast, year of infection was divided into approximate quintiles to reflect eras of therapeutic plasma products. Specifically, these were 1947 to 1968 (pre-NHT era), 1969 to 1974 (early NHT era), 1975 to 1979 (pre-HIV NHT era), 1980 to 1983 (peak HIV NHT era), and 1984 to 1990 (heat-treatment era).

Laboratory methods

Two assays were used to determine the presence or absence of HCV RNA in plasma separated from venous blood collected in acid-citrate-dextrose anticoagulant at each MHCS-II visit. First, each specimen was screened with a real-time reverse transcription–polymerase chain reaction (RT-PCR) assay developed in our laboratory. The reagents and protocol were optimized by modifying an existing assay to detect and quantify simian immunodeficiency virus RNA.[22] Detailed methods for the HCV RT-PCR assay are available upon request. Analytic sensitivity for HCV RNA in plasma was 9.1 international units (43 copies) per mL with the World Health Organization international standard. Mean HCV load by RT-PCR was 5 779 050 copies/mL (standard error: 474 585 copies/mL; interquartile range: 814 286-7 285 714 copies/mL).

Second, the qualitative COBAS Amplicor Hepatitis C Virus Test, version 2.0 (Roche Diagnostic Systems, Branchburg, NJ) was performed in an independent laboratory (Pennsylvania State University, Hershey, PA) on a masked, stratified random sample of 440 MHCS-II specimens. Compared with the Amplicor results, sensitivity of the screening RT-PCR was 100%. Specificity of the RT-PCR, compared with the COBAS Amplicor, was only 88%. Investigation revealed low-level false-positives by RT-PCR (median: < 429 copies/mL; maximum: 16 286 copies/mL) due to well-to-well contamination during processing. To rectify this, the independent laboratory retested all specimens that were RT-PCR positive up to 100 000 copies/mL (5% of all screening-positive samples and 6-fold higher than the maximum false-positive found in the screening assay) by COBAS Amplicor using a previously unthawed aliquot of plasma. The Amplicor result was considered definitive.

Definition of HCV clearance

Subjects with a negative HCV RNA, by RT-PCR or COBAS Amplicor, at both enrollment and first annual follow-up were defined to have cleared HCV infection.[18] Subjects with a positive HCV RNA (by COBAS Amplicor or > 100 000 copies/mL by RT-PCR) at enrollment were considered to have chronic HCV infection.

HBV infection status

HBV status for each subject in the current study was defined using the results of licensed serologic assays as used for clinical care and as reported by each clinical center. Those with no detectable HBV serologic markers or with isolated antibody against HBV surface antigen (anti-HBs) following HBV vaccination were classified as HBV uninfected. Those with unresolved HBV surface antigen (HBsAg) in serum for more than 6 months were classified as having chronic HBV infection. Those with other serologic markers, including anti-HBs with no history of HBV vaccination, were classified as having resolved HBV infection. Testing for antibody against HBV core antigen was not routinely performed by the clinical centers.

Statistical analyses

Chi-square analysis was used to compare characteristics of subjects with cleared and chronic HCV infection. Exact test was used if appropriate. Median and interquartile range (IQR) of age at infection were calculated. Differences in mean age were compared with a t test. Odds ratios measuring potential associations of variables and HCV clearance were estimated by logistic regression analysis, after adjusting for other covariates. Variables with P values of .15 or below in univariate analysis were included in the multivariable model. Variables with adjusted P values of .10 or below were retained in the final model. All statistical analyses were done with the Statistical Analysis System version 8.0 (Cary, NC).

Results

Spontaneous HCV clearance by ancestry and sex

Among the 712 HIV-negative, interferon-untreated subjects who currently or previously were anti-HCV–positive, 192 (27.0%) had cleared HCV infection as established by the inability to detect HCV

RNA in plasma (Table 1). Subjects of African ancestry had a marginally lower HCV clearance rate (19.1%) than did those of non-African ancestry (28.0%, $P = .08$). As is typical for inherited coagulation deficiencies, only 41 (5.8%) of 712 subjects in the study were females. HCV clearance had occurred in 22.0% of the female subjects compared with 27.3% of the male subjects ($P = .46$).

Spontaneous HCV clearance by type and severity of the coagulation deficiency

The HCV clearance rate did not differ significantly between subjects with hemophilia A (28.5%), hemophilia B (26.0%), or other coagulation deficiencies (17.2%, $P = .15$). By severity, the HCV clearance rate was 30.0% with severe bleeding diathesis, 25.2% with moderate bleeding diathesis, and 20.5% with mild bleeding diathesis ($P = .06$).

Spontaneous HCV clearance by type of factor treatment

There was no significant difference in HCV clearance rate by type of clotting factor replacement therapy. The HCV clearance rate was 24.6% among subjects who received only cryoprecipitate or plasma. It was 27.5% among subjects who received cryoprecipitate or plasma prior to later receipt of NHT factor concentrates. And it was 30.2% among subjects who received only NHT factor concentrates or who received the concentrates before cryoprecipitate or plasma ($P = .52$).

Age at infection and spontaneous HCV clearance

Based on available data on age at first treatment and the types of treatment, age at HCV infection could be imputed for 620 of 712 subjects. The median age at HCV infection for subjects who spontaneously cleared HCV viremia was 3.0 years (IQR: 8.0 years), which was significantly younger than that for subjects with chronic HCV infection (median: 8.0 years, IQR: 16 years, $P < .001$). By quartile, HCV clearance rate was 40.1% for those infected before age 2 years, 29.7% for those infected at ages 2 to 5 years, 26.1% for those infected at ages 6 to 15 years, and 14.9% for those infected after age 15 years (Table 2). Compared with subjects who were infected with HCV before 2 years of age, subjects infected at

Table 1. Characteristics of hemophilic subjects with cleared and chronic HCV infection

	HCV cleared, no. (%)	HCV chronic, no. (%)	P
Ancestry			
African	16 (19.1)	68 (80.9)	
Non-African	176 (28.0)	452 (72.0)	.08
Sex			
Male	183 (27.3)	488 (72.7)	
Female	9 (22.0)	32 (78.0)	.46
Type of coagulation deficiency			
Hemophilia A	141 (28.5)	353 (71.5)	
Hemophilia B	40 (26.0)	114 (74.0)	
Other	11 (17.2)	53 (82.8)	.15
Severity of coagulation deficiency			
Mild	34 (20.5)	132 (79.5)	
Moderate	31 (25.2)	92 (74.8)	
Severe	127 (30.0)	296 (70.0)	.06
Type of factor treatment*			
NHT only or first	65 (30.2)	150 (69.8)	
Both, cryo/plasma first	78 (27.5)	206 (72.5)	
Cryo/plasma only	32 (24.6)	98 (75.4)	.52

*Non-heat-treated (NHT) factor concentrate; cryoprecipitate or fresh-frozen plasma (cryo/plasma) used first (before the other) or exclusively.

Table 2. Univariate analysis of correlates of HCV clearance

	Cleared HCV (%)	Chronic HCV	OR (95% CI)	P
Age at infection (quartile)				
Younger than 2 y	67 (40.1)	100	1.00	
2 to 5 y	41 (29.7)	97	0.63 (0.39-1.02)	.059
6 to 15 y	42 (26.1)	119	0.53 (0.33-0.84)	.007
16 to 60 y	23 (14.9)	131	0.26 (0.15-0.45)	< .001
Year of infection (quintile)				
1947 to 1968	25 (18.2)	112	1.00	
1969 to 1974	25 (21.6)	91	1.23 (0.66-2.29)	.51
1975 to 1979	30 (27.0)	81	1.66 (0.91-3.03)	.099
1980 to 1983	34 (28.8)	84	1.81 (1.01-3.27)	.048
1984 to 1990	59 (42.8)	79	3.35 (1.93-5.80)	< .001
HBV coinfection				
HBV uninfected	94 (28.7)	234	1.00	
Resolved HBV	44 (24.3)	137	0.80 (0.52-1.24)	.29
Chronic HBV	13 (59.1)	9	3.60 (1.38-9.49)	.003
Alcohol use*				
None	106 (30.3)	243	1.00	
Less than 36 g/wk	24 (27.3)	64	0.86 (0.51-1.45)	.57
36 to 95 g/wk	14 (16.3)	72	0.45 (0.24-0.83)	.01
96 or more g/wk	15 (18.8)	65	0.53 (0.29-0.97)	.04
Ancestry				
Non-African	176 (28.0)	452	1.00	
African	16 (19.0)	68	0.60 (0.33-1.10)	.08

*Alcohol use before knowledge of being infected with HCV. One drink was defined as 45 mL (1.5 oz) liquor, 120 mL (4 oz) wine, or 360 mL (12 oz beer) and was estimated to contain 12 g alcohol.

ages 2 to 5 years tended to be less likely to clear HCV (OR: 0.63; 95% CI: 0.39-1.02). The likelihood of HCV clearance was even less for subjects infected with the virus at ages 6 to 15 years (OR: 0.53; 95% CI: 0.33-0.84) and for subjects infected at age 16 years or older (OR: 0.26; 95% CI: 0.15-0.45). Spontaneous HCV clearance decreased with age at infection ($\chi^2_{trend} = 24.9$, $P < .001$).

Calendar year of infection and spontaneous HCV clearance

To investigate whether the likelihood of spontaneous HCV clearance varied chronologically with the year in which a subject was infected with the virus, we calculated the calendar year of infection for each subject with imputed age at infection. Spontaneous HCV clearance increased from 18.2% to 42.8% with calendar year of infection ($\chi^2_{trend} = 21.4$, $P < .001$, Table 2). Compared with subjects who were infected with HCV between 1947 and 1968, subjects infected with the virus in later years were more likely to clear HCV. Subjects infected with the virus between 1980 and 1983 were significantly more likely to clear HCV with an OR of 1.81 (95% CI: 1.01-3.27). Of those infected with HCV most recently, between 1984 and 1990, 42.8% had cleared HCV (OR: 3.35; 95% CI: 1.93-5.80). Twenty-eight subjects were infected with HCV from 1987 through 1990, all of whom received only cryo/plasma, and 9 (32.1%) of whom cleared the virus.

Coinfection with HBV and spontaneous HCV clearance

Sufficient information was available to define the HBV infection status for 531 (74.6%) of the 712 subjects. HCV clearance rates were 28.7% among the 328 HBV-uninfected subjects, 24.3% among the 181 subjects with resolved HBV infection, and 59.1% among the 22 subjects with chronic HBV infection (Table 2). Compared with HBV-uninfected subjects, those with chronic HBV infection were significantly more likely to clear HCV (OR: 3.60; 95% CI: 1.38-9.49; Table 2). Resolved HBV infection was not associated with spontaneous HCV clearance (OR: 0.80; 95% CI: 0.52-1.24).

Alcohol use and spontaneous HCV clearance

Information was available for 603 (84.7%) of the 712 subjects on alcohol use during the year before the subject learned that he or she had HCV antibodies. As shown in Table 2, HCV clearance rates by tertile of alcohol consumption were 27.3%, 16.3%, and 18.8%, compared with 30.4% among those who denied alcohol consumption ($P_{trend} = .004$).

Multivariate analysis

Because many subjects lacked information on alcohol use and HBV infection status, the primary multivariate analysis was restricted to the 620 subjects with complete data on the other variables (Table 3). With adjustment for all variables in the model, sex and bleeding severity were unrelated to HCV clearance, but African ancestry was associated with a significantly lower rate of HCV clearance with an OR of 0.46 (95% CI: 0.23-0.91). Subjects infected with HCV at age 16 years or older were significantly less likely to clear the virus when compared with subjects infected before 2 years of age (OR: 0.34; 95% CI: 0.18-0.65). Subjects who were infected with HCV most recently (between 1984 and 1990) were significantly more likely to clear the virus than subjects infected between 1947 and 1968 (OR: 2.37; 95% CI: 1.25-4.49). A variable to test for interaction between age at and year of infection was not significant ($P = .52$).

In a secondary multivariate analysis of the 388 subjects who had complete data on alcohol use and HBV status, HCV clearance continued to be independently associated with chronic HBV infection (OR: 9.08; 95% CI: 2.54-32.45) but not with consuming at least 96 g alcohol per week (OR: 0.87; 95% CI: 0.39-2.00). In this full model, subjects infected with HCV at age 16 years or older remained significantly less likely to clear the virus compared with subjects infected before 2 years of age (OR: 0.19; 95% CI: 0.07-0.53); and those infected with HCV most recently (between 1984 and 1990) remained significantly more likely to clear the virus than those infected between 1947 and 1968 (OR: 3.93; 95% CI: 1.52-10.19). In this secondary multivariate analysis, African ancestry was no longer significantly associated with HCV clearance (OR: 0.68; 95% CI: 0.29-1.62).

Table 3. Multivariate logistic regression model of correlates of HCV clearance

Variable	OR (95% CI)	P
Sex		
Male	1.00	
Female	0.95 (0.41-2.20)	.90
Ancestry		
Non-African	1.00	
African	0.46 (0.23-0.91)	.03
Severity of coagulation deficiency		
Severe	1.00	
Moderate	1.05 (0.63-1.74)	.86
Mild	1.01 (0.61-1.68)	.97
Age at infection (quartile)		
Younger than 2 y	1.00	
2 to 5 y	0.65 (0.40-1.08)	.10
6 to 15 y	0.72 (0.40-1.28)	.26
16 to 60 y	0.34 (0.18-0.65)	.001
Year of infection (quintile)		
1947 to 1968	1.00	
1969 to 1974	1.14 (0.60-2.14)	.69
1975 to 1979	1.41 (0.75-2.68)	.29
1980 to 1983	1.26 (0.66-2.43)	.49
1984 to 1990	2.37 (1.25-4.49)	.008

Discussion

The outcome of HCV infection is determined by the balance of the dynamic interaction between the virus and the host immune defense, with many environmental and other host factors modifying this interaction.[10,12] In this study of HIV-uninfected hemophilic subjects enrolled in the MHCS-II cohort, we examined the association of spontaneous HCV clearance with several variables postulated to be important. Like others, we found that spontaneous HCV clearance was significantly more frequent among subjects of non-African ancestry and those with chronic HBV infection. Our major new findings were that HCV clearance also was significantly more frequent among those infected at a very young age and those infected after 1983.

Most of our subjects had been infected with HCV during childhood, and there was a striking and highly significant gradient between the age at infection and the proportion who had cleared HCV. Overall, 27% of our subjects had cleared HCV. However, we observed that a much higher proportion, 40.1%, of those infected before age 2 years had cleared HCV compared with only 14.9% of those infected after age 15 years. This difference was not confounded by the other variables. The median age at infection was 3 years for subjects who cleared HCV infection compared with 8 years among those in whom viremia persisted.

Our findings corroborate those of Messick et al,[6] who showed that the rate of spontaneous HCV clearance among 49 HIV-negative, anti-HCV–positive hemophilic patients was significantly higher with younger age at infection. Similarly, Vogt et al[23] found HCV clearance in 45% of 67 children infected by blood transfusion at a mean age of 2.8 years. Despite these data, clearance is not a simple function of age. Among volunteer blood donors, all of whom were adults, Busch et al[11] recently found no association between HCV clearance and age. At the other end of the spectrum, only 2 (11%) of 18 children infected through mini-transfusions at birth spontaneously cleared HCV.[24] Perhaps the likelihood of HCV clearance is related to age-specific differences in immune response that modify the interaction between virus and host immune defense, leaving the neonate vulnerable to chronic infection while an older child may be primed for clearance. The size of the inoculum may also influence the clearance rate. Because clotting factor therapy is calibrated to body size, younger hemophilic subjects may have received a smaller inoculum of HCV that might have increased the success of an immune response and the likelihood of viral clearance. The complex relationship between age and clearance of HCV contrasts with the higher likelihood of clearance of HBV with older age at infection.[25]

We also observed that the likelihood of spontaneous HCV clearance increased with the calendar year in which the patient was infected. Only 18.2% of those infected before 1969 had cleared HCV, compared with 42.8% of those infected after 1983, a highly significant difference that was not confounded by age at infection or the other variables. Change in the pathogenesis of HCV itself is unlikely. Rather, the association probably reflects a lower likelihood of HCV clearance following HCV reinfection.[26,27] We speculate that the higher clearance in subjects first infected after 1983 might be due to less frequent reexposure and repeated infection because of improvements in heat treatment and other procedures to inactivate viruses in factor concentrates that occurred during the 1980s. Alternatively, bias could account for the calendar association. Specifically, persons infected decades before the discovery of HCV in 1989 may have cleared not only HCV RNA but also HCV antibodies,[18,28,29] in which case they would not have

enrolled in MHCS-II, falsely reducing the denominator of those infected before 1969. Conversely, nonenrollment in MHCS-II because of reduced survival with chronic HCV infection would present an opposing bias.

Coinfection with other viruses, especially HIV-1 and HBV, has been shown to modify the natural history of HCV infection.[10,12] To better understand other correlates of HCV clearance without the potential confounding effect of HIV-1 infection, we restricted our current study to HIV-uninfected hemophilic subjects. While resolved HBV infection was not associated with spontaneous HCV clearance, the relatively few subjects with chronic HBV infection were 3.6-fold more likely to clear HCV. Similar findings were reported previously among people with hemophilia and other populations.[9,30-34] This could be due to reciprocal inhibition in viral replication between HBV and HCV in patients coinfected with these 2 viruses.[35] Additionally, lower survival of patients chronically infected with both HBV and HCV could have enriched the MHCS-II cohort with those chronically infected with HCV infection alone. However, this seems unlikely, as the 10.8% prevalence of chronic HBV infection in our cohort is nearly identical to that found 15 years earlier by Troisi et al.[36]

African ancestry was associated with lower spontaneous HCV clearance in other populations.[7,10,11] The current study, with only 84 subjects of African ancestry, was not ideal for assessing the impact of this characteristic. Nonetheless, we observed HCV clearance in only 19.0% of our subjects of African ancestry compared with 28.0% among other subjects, a difference that was statistically significant after adjustment for age at and calendar year of infection. We did not have sufficient statistical power to disentangle the role of ancestry from that of chronic HBV infection, nor did we have enough females to assess the role of sex.

Alcohol use was reported to be associated with lower spontaneous HCV clearance.[9] As HCV clearance usually occurs within 1 to 2 years after infection[6] and as changes in health or medication use may have prompted MHCS-II participants to change their drinking habits, we analyzed historical use of alcohol (drinking during the year before knowledge of the participant's HCV positivity) rather than recent alcohol use. In our univariate analysis, alcohol use had a highly statistically significant association with a lower rate of HCV clearance. However, alcohol consumption was no longer associated with HCV in a multivariate analysis. This suggests that the association of alcohol consumption and HCV clearance was confounded by other variables, especially age at infection.

There are several potential limitations of our study. First, because age at HCV infection with hemophilia is seldom known precisely, we used information on types of and age at first exposure to infectious HCV through clotting factor replacement therapy to impute age at infection. Although we used statistical methods to maximize the probability of an accurate imputation, these historical data are inherently imprecise. Similarly, our data on use of alcohol were self-reported, allowing not only for inaccuracy but also the possibility for bias. To focus on spontaneous HCV clearance, we excluded subjects who had received interferon. The included and excluded subjects were very similar in all variables that we analyzed except for alcohol use and African ancestry (data not presented). Finally, the hemophilic population differs from others, particularly with respect to very young age at infection, multiple and possibly simultaneous infections, and inclusion of only survivors in the cohort. Thus, the results from this study may not generalize to nonhemophilic populations.

In conclusion, among people with hemophilia, the likelihood of spontaneous HCV clearance was increased more than 3-fold with each of 3 variables—chronic HBV coinfection, HCV infection at a very young age, and HCV infection since 1983. These findings illustrate the importance of nongenetic determinants in spontaneous recovery from HCV infection.

Acknowledgments

Appendix 1: collaborators and institutions in MHCS-II

North American Hemophilia Centers: Cardeza Foundation Hemophilia Center, Thomas Jefferson University Hospital, Philadelphia, PA (Dr Jamie Siegel, Ms Kay Miller); Case Western Reserve University, UHC Center for AIDS Research, Cleveland, OH (Dr Michael M. Lederman); Chicago Children's Memorial Hospital, Comprehensive Hemophilia Program, Chicago, IL (Dr Alexis Thompson, Ms Brittan Browning and Ms Susan Gamerman); Children's Center for Cancer and Blood Disorders, Palmetto Richland Memorial Hospital, Columbia, SC (Dr Kevin McRedmond, Ms Janice Withycombe); Children's Hospital Medical Center, Cincinnati, OH (Dr Ralph Gruppo, Ms Gina Stack); Children's Hospital of Michigan, Hemophilia Treatment Center, Detroit, MI (Dr Jeanne Lusher, Ms Linda Percy); Children's Hospital of Orange County, Department of Hematology/Oncology, Orange, CA (Dr Diane Nugent, Ms Marianne McDaniel); Children's Hospital of Philadelphia, Division of Hematology, Philadelphia, PA (Dr Catherine Manno, Ms Regina Butler, and Ms Amanda Wade); Children's National Medical Center, Washington, DC (Drs Anne Angiolillo and Naomi L. C. Luban, Ms Christine Guelcher); Comprehensive Bleeding Disorders Center, Peoria, IL (Dr Michael Tarantino, Ms Mary Brooks); Comprehensive Center for Bleeding Disorders, Wauwatosa, WI (Dr Joan Gill, Ms Jodie Nelson); Comprehensive Hemophilia Diagnostic and Treatment Center, University of North Carolina at Chapel Hill, Chapel Hill, NC (Drs Gilbert White, Alice Ma, and Michael Fried, Ms Aime L. Grimsley); Cornell Medical Center, Hemophilia Treatment Center, New York, NY (Dr Donna DiMichele, Ms Ilene Goldberg); Dayton Children's Medical Center, Dayton, OH (Dr James French, Ms Sandra Hibner); Emory University School of Medicine, Atlanta, GA (Dr James Steinberg, Mr Steven Faust, Ms Francie Lassiter); Fairview University Medical Center, Minneapolis, MN (Dr Nigel Key, Ms Vicky Hannemann); Georgetown University Medical Center, Lombardi Cancer Center, Washington, DC (Dr Craig M. Kessler, Ms Anastasia E. Lee); Hemophilia Center of Central Pennsylvania, Penn State College of Medicine at Hershey, Hershey, PA (Dr M. Elaine Eyster, Ms Kathryn Galli, and Ms Gillian Jenkins); Hemophilia Center of Western New York, Buffalo, NY (Dr Zale P. Bernstein, Ms Linda Belling); Indiana Hemophilia and Thrombosis Center, Indianapolis, IN (Dr Amy Shapiro, Ms Patti Noblet); London Health Sciences Center, South Western Ontario Regional Hemophilia Program, London, ON (Dr Lawrence Jardine, Ms Lori Laudenbach); Long Island Jewish Medical Center, Hemophilia Treatment Center, New Hyde Park, NY (Dr Richard Lipton, Ms Christine Pece); Louisiana Comprehensive Hemophilia Care Center, Tulane University Medical School, New Orleans, LA (Dr Cindy A. Leissinger, Ms Cecilia Schmidt); Medical College of Virginia, Richmond Hemophilia Treatment Center, Richmond, VA (Dr Marcus Carr, Ms Melinda Nolte); Mountain States Regional Hemophilia and Thrombosis Center, Aurora, CO (Dr Marilyn Manco-Johnson, Ms Ruth Ann Kirschman); South-East Health Care, Moncton Hemophilia Clinic, Moncton, NB (Dr Sheldon H. Rubin, Ms Dorine Belliveau); Mount Sinai School of Medicine, New York, NY (Dr Louis Aledort, Ms Johanna McCarthy); Mountain States Regional Hemophilia Center-Utah, Primary Children's Hospital, Salt Lake City, UT (Dr Richard Lemons, Ms Shirley Bleak); Ohio State University Hemophilia Center, Columbus, OH (Dr Eric Kraut, Ms Leslie Witkoff); Oklahoma Center for Bleeding Disorders, Children's Hospital, Oklahoma City, OK (Dr Charles Sexauer, Ms Felicia Kiplinger); Puget Sound Blood Center, Seattle, WA (Dr Arthur Thompson, Mr Charles Cooper); South Texas Comprehensive Hemophilia Center, Santa Rosa Children's Hospital, San Antonio, TX (Dr Howard Britton, Ms Karen Aufdemorte); St Louis University Cancer Center, Missouri/Illinois Regional Hemophilia Center-Adult, St Louis, MO (Dr Hans Joachim Reimers, Ms Judy A. Bagato); UCD Northern Central California

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