

45-Year Follow-up of Hepatitis C Virus Infection in Healthy Young Adults

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Background: The sequelae during the first two decades after acute hepatitis C virus (HCV) infection have been well studied, but the outcome thereafter is unknown.

Objective: To conduct an extended study of the natural history of HCV infection by using archived serum specimens originally collected between 1948 and 1954.

Design: Retrospective cohort study.

Setting: A university, a Veterans Affairs medical center, and a medical follow-up agency that had access to the serum specimens and accompanying demographic and medical records.

Participants: 8568 military recruits who were evaluated for group A streptococcal infection and acute rheumatic fever between 1948 and 1954. Blood samples were taken from the recruits and, after testing, were stored frozen for almost 45 years.

Measurements: The presence of antibodies to HCV was determined by enzyme-linked immunoassay, supplementary recombinant immunoblot assay, and polymerase chain reaction for HCV RNA. Morbidity and mortality were also assessed.

Results: Of 8568 persons, 17 (0.2%) had positive results on enzyme-linked immunosorbent assay and recombinant immunoblot assay. The rate was 1.8% among the African-American persons and 0.1% among the white persons in the total sample (relative risk, 25.9 [95% CI, 8.4 to 80.0]). During the 45-year follow-up, liver disease occurred in 2 of the 17 HCV-positive persons (11.8%) and 205 of the 8551 HCV-negative persons (2.4%) (ethnicity-adjusted relative risk, 3.56 [CI, 0.94 to 13.52]). Seven of the 17 HCV-positive persons (41%) and 2226 of the 8551 HCV-negative persons (26%) had died by December 1996 (ethnicity-adjusted relative risk, 1.48 [CI, 0.8 to 2.6]). Of persons who were HCV-positive, 1 (5.9%) died of liver disease 42 years after the original phlebotomy, 5 (29%) died of non-liver-related disease a median of 37 years after the original phlebotomy, and 1 (5.9%) died of unknown causes. One hundred nineteen HCV-negative persons (1.4%) died of liver disease.

Conclusions: The rate of HCV infection from 1948 to 1954 among a sample of military recruits parallels that among present-day military recruits and volunteer blood donors. During 45 years of follow-up, HCV-positive persons had low liver-related morbidity and mortality rates. This suggests that healthy HCV-positive persons may be at less risk for progressive liver disease than is currently thought.

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The long-term outcome of patients with hepatitis C virus (HCV) infection is difficult to determine for several reasons. First, the initial bout of acute HCV infection is rarely recognized because of the paucity or complete absence of symptoms (1). Second, even persons who have established chronic hepatitis are rarely symptomatic. Third, end-stage liver disease, when it occurs, can often take more than three decades to develop (2, 3). To address these issues, investigators have studied HCV infection in settings that allow accurate determination of onset, such as transfusion-transmitted HCV (4–8) or outbreaks of HCV infection that can be attributed to receipt of contaminated immunoglobulin (9, 10).

Several studies (9–12) have shown that major sequelae of chronic HCV infection, decompensated cirrhosis and hepatocellular carcinoma, are relatively uncommon. In contrast, clinical observations in tertiary care and liver transplantation centers suggest that chronic HCV infection is highly likely to have serious or fatal outcomes (2, 3, 13, 14). Some researchers believe that prospective studies have been too short to accurately establish outcomes or, in the case of transfusion-related studies, have been limited by premature deaths due to the underlying illness. These concerns could be addressed by studying young, healthy persons with laboratory-confirmed HCV infection for more extended periods. Through access to frozen serum specimens obtained during the late 1940s and early 1950s from more than 9000 healthy young persons, we were able to implement a retrospective cohort study spanning 45 years to determine liver-related morbidity and mortality in HCV-positive and HCV-negative persons.

Methods

The Repository

Between 1948 and 1955, epidemiologic and clinical studies were conducted at Fort Francis E. Warren Air Force Base, Wyoming, to investigate the problem of group A streptococcal infection and acute rheumatic fever among military recruits (15, 16). At the conclusion of the study, remaining serum specimens that had been collected from recruits were frozen in rubber-capped glass vials at

–20 °C. The specimens and the study records were archived by the original investigators. After the subsequent discovery of HCV (17) and the development of sensitive serologic assays, it became apparent that this unique collection of serum samples could be a valuable tool for determining the long-term outcome of HCV-positive persons.

The serum samples had been stored frozen and thawed for at least two decades. When we examined the collection in preparation for this study, we found that vials had been broken and specimens lost for only 0.5% of the 9451 identified persons. The previously frozen samples were separated into 1-mL aliquots and were refrozen at –20 °C. One aliquot was used for all subsequent tests, and a computerized database was generated.

Serologic Assays

Initial anti-HCV assays were performed in the Division of Pediatric Infectious Disease at the University of Minnesota, Minneapolis, Minnesota. Samples from 9427 persons were assayed in duplicate for antibodies to HCV by third-generation enzyme-linked immunoassay (EIA 3.0) (Ortho Diagnostics, Raritan, New Jersey) (18). Repeatedly reactive samples were analyzed by using supplemental recombinant immunoblot assay (RIBA 3.0, Ortho Diagnostics); these tests were performed by the manufacturer. Samples were then tested for HCV RNA by polymerase chain reaction (19) and by genotyping in the hepatitis research laboratory of the Veterans Affairs Medical Center in Washington, D.C. (20). Persons were classified as having HCV infection if their serum samples demonstrated antibodies to HCV on both EIA 3.0 and RIBA 3.0. If an EIA-positive result yielded an indeterminate or negative result on RIBA 3.0, the EIA 3.0 result was considered false-positive (21). Serum aminotransferase levels were not measured because activity was probably lost through prolonged storage and because freeze-thaw cycles may have occurred after the original phlebotomy (22).

Construction of the Study Cohort

The criteria for selection of recruits, the periods of observation, and the objectives of the original studies were defined in the original published reports (15, 16). During these studies, only names and military service numbers were recorded. We used this information to obtain additional data, such as Social Security numbers and demographic variables (including age, ethnicity, and sex), from records of the Department of Defense, the National Archives and Records Administration, and the Department of Veterans Affairs. Our analysis was restricted to persons with identifiable Social Security numbers because this information was essential for docu-

menting data on morbidity and mortality. Death certificate information was obtained from the Department of Veterans Affairs and through the National Death Index.

Outcomes

Morbidity

Morbidity diagnoses were obtained from the Department of Veterans Affairs and the Health Care Financing Administration (HCFA). Veterans Affairs data came from Patient Treatment Files, which code patients' diagnoses at discharge from Veterans Affairs medical centers, and from documentation of disabilities in Compensation and Pension files. The HCFA data came from three files: the Medicare Provider Analysis and Review file, which includes information on diagnoses and procedures for each hospital discharge in the United States; the Health Information Skeletonized Eligibility Write-off file, which records dates of birth and death that are abstracted from Social Security records; and the Standard Analytical File, which provides data on outpatients.

Mortality

We collected data on all-cause and cause-specific mortality from death certificates in Veterans Affairs claims files. The Department of Veterans Affairs maintains a computer file of beneficiaries, including recipients of veterans' death benefits, and also records deaths that occur during hospitalizations in Veterans Affairs medical centers. Investigation of the extent of reporting has shown that the file is almost complete (23). In some instances, we obtained evidence of death from HCFA files, which are derived from Social Security records. The National Death Index Plus service of the National Center for Health Statistics provided additional coded, cause-specific death certificate information from national mortality data. A qualified nosologist coded the underlying causes of death according to the rules that existed at the time of death and recoded them by using the International Classification of Diseases, Ninth Revision, Clinical Modification. Some death certificates and Veterans Affairs claims may be incomplete or inaccurate, but the frequency of these events should not differ between the HCV-positive and HCV-negative groups.

Statistical Analysis

Relative risks and CIs (Cornfield and exact) (24) and results of chi-square tests and the Fisher exact test were calculated by using Epi-Info, version 6.04 (Centers for Disease Control and Prevention, Atlanta, Georgia) (25). A Kaplan–Meier survival analysis curve (26) was calculated by using SAS, version 6.12 (SAS Institute, Inc., Cary, North Carolina)

(27). A *P* value less than 0.05 was considered statistically significant.

Results

Completeness of Identifier and Demographic Data

Of 9427 persons, 8568 (91%) were included in our analysis because they had a Social Security number with which we could obtain information on morbidity and mortality. Most participants were white men who were younger than 25 years of age at the original phlebotomy. Of 6805 persons whose sex was known, 6742 (99%) were men. Among 6611 persons whose ethnicity was known, 89.3% were white, 10.3% were African American, and 0.4% were Asian. The mean age of surviving cohort members as of 31 December 1996 was 64.8 years, and 95% were between 60 and 69 years of age.

Serologic Data

Among the 8568 persons tested, 34 (0.4%) were repeatedly positive for anti-HCV. Seventeen of these 34 participants (50%) had positive results on RIBA 3.0, 9 had indeterminate results, and 8 had negative results. Therefore, the prevalence of RIBA-confirmed anti-HCV reactivity was 0.2% (17 of 8568). At original phlebotomy, the mean age of the HCV-positive group was 21.5 years (range, 19 to 28 years; median, 20.5 years) and the mean age of the HCV-negative group was 20.7 years (range, 18 to 46 years; median, 20.0 years). A significant difference was seen between the number of African-American persons with confirmed HCV infection (12 of 684 [1.8%]) and the number of white persons

Table 1. Liver-Related Hospitalizations, Outpatient Visits, and Compensation Awards among Persons with (*n* = 17) and Those without (*n* = 8551) Hepatitis C Virus Infection*

ICD-9-CM Code	Diagnosis	HCV-Positive Persons	HCV-Negative Persons
571.0–571.9†	Chronic liver disease and cirrhosis	1	92
572.0–572.8‡	Liver abscess and sequelae of chronic liver disease	0	20
573.0–573.9§	Other liver disorders	0	24
155.0	Liver cancer, primary	0	1
155.2	Liver cancer, not specified as primary or secondary	0	1
Total		1	115¶

* Ethnicity-adjusted relative risk, 2.7 (95% CI, 0.4–20.0). HCV = hepatitis C virus; ICD-9-CM = International Classification of Diseases, Ninth Revision, Clinical Modification.

† Includes C&P (Compensations and Pensions) code 7312 (liver, cirrhosis of).

‡ Includes C&P code 7313 (liver, abscess of, residuals).

§ Includes C&P code 7311 (liver, injury of).

|| Coded as 155.0 on one occasion and 155.2 on another occasion.

¶ Numbers do not sum because multiple episodes were coded as 571.0–571.9 and 572.0–572.8 in 12 persons, 571.0–571.9 and 573.0–573.9 in 9 persons, 572.0–572.8 and 573.0–573.9 in 1 person, and 155.0 and 155.1–155.2 in 1 person.

Table 2. Mortality Rate as a Function of Hepatitis C Virus Status and Ethnicity*

Ethnicity	HCV-Positive Group	HCV-Negative Group	Relative Risk (95% CI)†
	<i>n/n</i> (%)		
African American	6/12 (50)	209/672 (31)	1.6 (0.9–2.9)
White	1/4 (25)	1470/5898 (25)	1.00 (0.2–5.5)
Unknown or other	0/1 (0)	547/1981 (28)	–
Total	7/17 (41)	2226/8551 (26)	1.48 (0.8–2.6)

* HCV = hepatitis C virus.

† Total relative risk adjusted for ethnicity by using the Mantel-Haenszel method.

with confirmed HCV infection (4 of 5902 [0.07%]) (relative risk, 25.9 [CI, 8.4 to 80.0]).

Polymerase chain reaction detected HCV RNA in 11 (65%) of the 17 persons who had positive results on EIA 3.0 and RIBA 3.0 but in no persons who had positive results on EIA 3.0 and indeterminate or negative results on RIBA 3.0. Ten of the 11 persons who were positive for HCV RNA were classified as having genotype 1b. One person could not be classified.

Morbidity

Eight HCV-positive participants (47%) and 3566 HCV-negative participants (42%) had had at least one hospitalization, outpatient visit, or disease-related compensation award (relative risk, 1.24 [CI, 0.5 to 3.2]). One HCV-positive person had a liver-related diagnosis and was recorded as having “chronic liver disease and cirrhosis” (Table 1). Most of the 115 persons who were originally negative for HCV and had liver-related diagnoses were recorded as having “chronic liver disease and cirrhosis.” No HCV-positive persons had received a diagnosis of liver cancer or had been treated for liver cancer. One HCV-negative person had received outpatient treatment for liver cancer that was described on one occasion as “primary” and on another as “not specified as primary or secondary.”

Mortality

Through December 1996, the mortality rate for the entire cohort was 26.0% (2233 of 8568). Mortality rates, stratified by ethnicity and serologic status, are shown in Table 2. Death from all causes was somewhat more frequent among HCV-positive persons (41%) than among HCV-negative persons (26%) (ethnicity-adjusted relative risk, 1.5 [CI, 0.8 to 2.6]). The mean age at death was 56.5 years for HCV-positive persons and 54.2 years for HCV-negative persons, not a clinically significant difference. Survival curves for HCV-positive persons and HCV-negative persons during the 45-year follow-up are shown in the Figure.

Data on cause-specific death were available for

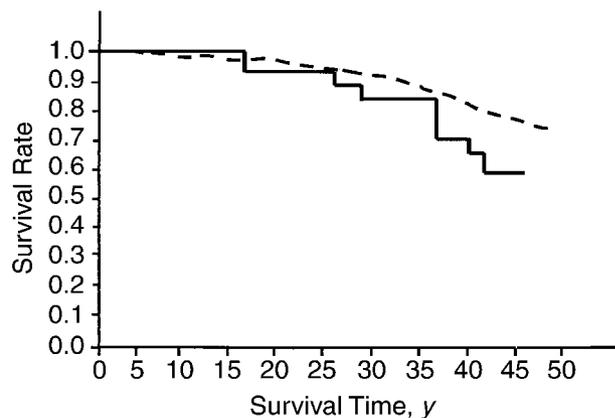


Figure. Forty-five-year survival curve comparing 17 hepatitis C virus (HCV)-positive persons (solid line) with 8551 HCV-negative persons (dashed line).

1896 of the 2233 persons who died (85%). Liver-related causes of death for HCV-positive and HCV-negative persons are presented in **Table 3**. Seven of the 17 HCV-positive persons died. One of these 7 persons is omitted from **Table 3** because his death in 1984 was attributed to unknown causes (no cause was listed on his death certificate). Only 1 of the remaining 6 persons (16.7%) died of liver disease, 42 years after the original phlebotomy. The other 5 persons died of causes unrelated to liver disease (**Table 4**). Of the 1890 persons who were negative for HCV, 119 died of liver disease (6.3%) (**Table 3**). No deaths from liver cancer were recorded for any of the HCV-positive persons; however, 9 HCV-negative persons died of liver cancer (0.5%). Three of these 9 deaths were coded as “carcinoma of the liver, primary,” and 6 were coded as “carcinoma of the liver, not specified as primary or secondary.”

Data were also examined as a composite of morbidity and mortality according to the original serologic and virologic status. Among the 17 persons who were positive for HCV, liver-related events (1 death, 1 case of chronic liver disease) occurred only in those in whom HCV RNA was detected (2 of 11 [18.2%]). None of the 6 HCV-positive persons who were negative for HCV RNA and none of the 17 persons who had indeterminate or negative results on RIBA 3.0 had a record of liver disease. None of the 17 HCV-positive persons reported alcohol or drug dependence, although 1 death in this group was attributed to alcohol abuse. Records of the 8551 HCV-negative persons showed 316 hospitalizations for alcohol dependence but no deaths from or hospitalizations for alcohol or drug abuse.

Discussion

Discordant views about the risk for serious long-term sequelae of HCV infection are related to the

different strategies that have been used to study the natural history of this infection. Prospective studies that begin with onset of acute illness have identified such outcomes as hepatocellular failure, decompensated cirrhosis, and hepatocellular carcinoma in a relatively small proportion of infected persons (9–12). No study, however, has exceeded 25 years of follow-up, and therefore critical information has not been available beyond that period. In contrast, retrospective studies of persons who have chronic, clinically obvious HCV infection may overemphasize more serious outcomes because they risk omitting persons with subclinical infection as well as those in whom infection spontaneously resolves (2, 3, 13, 14).

Another strategy involves conducting a study with a cohort from the remote past that can be stratified as “exposed” or “unexposed” on the variable of interest. The two strata are then compared through direct determination of risk for each developing outcome. This method permits acquisition of data over a considerably extended observation period.

At least two such studies have been reported, one involving long-term follow-up of transfusion-associated hepatitis (28) and the other involving follow-up of persons whose hepatitis is attributed to receipt of HCV-contaminated immunoglobulin (9). Both studies found that few infected persons had histologically advanced liver disease; however, follow-up has not yet exceeded 23 years in either study. In addition, it has been argued that adverse outcomes in the study involving transfusion recipients might have been modified by the relatively advanced ages of the patients and by their often serious underlying diseases, which could have compromised health before end-stage liver disease could

Table 3. Liver-Related Causes of Death in 6 Persons with and 1890 Persons without Hepatitis C Virus Infection*

ICD-9-CM Code	Diagnosis	HCV-Positive Persons	HCV-Negative Persons
571.0–571.9	Chronic liver disease and cirrhosis	0	82
572.0–572.8	Liver abscess and sequelae of chronic liver disease	1†	52
573.0–573.9	Other liver disorders	0	6
155.0	Liver cancer, primary	0	3
155.2	Liver cancer, not specified as primary or secondary	0	6
Total		1	119‡

* Seven persons with hepatitis C virus infection died, but the cause of one death is unknown. Causes of death were obtained from death certificate data. HCV = hepatitis C virus; ICD-9-CM = International Classification of Diseases, Ninth Revision, Clinical Modification.

† Positive results on enzyme-linked immunoassay, recombinant immunoblot assay, and polymerase chain reaction; ICD-9 code, 572.8.

‡ Numbers do not sum because multiple diagnoses were coded as 571.0–571.9 and 572.0–572.8 in 26 persons, 572.0–572.8 and 573.0–573.9 in 1 person, 571.0–571.9 and 155.0 in 1 person, 572.0–572.8 and 155.0 in 1 person, and 571.0–571.9 and 155.0–155.2 in 1 person.

develop. Our study eliminates these deficiencies because we evaluated young adults who were presumably healthy at the time of serum collection.

We found that 0.2% of the 8568 participants were confirmed by RIBA 3.0 as being positive for HCV. This rate does not differ substantially from that found in a recent study conducted among recruits at five U.S. Navy and Marine Corps training centers (29) and is similar to the 0.3% rate found among first-time blood donors (30, 31). It is, however, lower than the 1.8% rate reported in the Third National Health and Nutrition Examination Survey (NHANES III) (32). Of note, HCV RNA was detected in 65% of the HCV-positive persons in our study even though the blood samples were not originally handled in the manner that is currently thought to be necessary for preserving nucleic acids (33). Also of note, all but 1 of the 11 persons in whom HCV RNA was detected were characterized as having genotype 1b; the remaining person could not be genotyped. This frequency of genotype 1b is higher than that currently found in the United States and other western countries (34). Although the reason for this prevalence is unknown, presumably genotype 1b was the predominant genotype when the original studies were conducted.

To our knowledge, we describe the earliest confirmed detection of HCV infection in the United States. Because the recruits were obviously not interviewed for potential risk factors, the sources of infection cannot be determined. Our results suggest that a low rate of HCV infection has been present in the United States for the past five decades, especially in military populations. Presumably, the rate in the general population began to increase in the 1960s along with parenteral drug abuse; this may account for the higher prevalence of HCV infection found in NHANES III (32). It is unknown why more African-American Air Force trainees than white Air Force trainees in our study were positive for HCV; however, this trend has also been seen in the recent NHANES III study (32).

The most obvious limitation of our study is the relatively small number of HCV-positive persons. However, we nevertheless present important, provocative data. We found that all-cause mortality was somewhat higher among HCV-positive persons than among HCV-negative persons more than 40 years after detection of anti-HCV antibodies. Similarly, liver-related deaths occurred only slightly more frequently in the HCV-positive group; only one liver-related death in this group has so far been recorded. The similarity of the two groups with respect to ethnicity-specific mortality cannot be accounted for by earlier death from any cause in the HCV-positive group. Mean age at death did not differ between the two groups, which indicates that

Table 4. Death Information for Six Persons with Hepatitis C Virus Infection for Whom Data on Cause of Death Were Available*

Age at Death	Interval from Time of Original Phlebotomy to Death	Cause of Death
y		
37	16	Alcohol abuse
46	26	Trauma
61	37	Congestive heart failure, diabetes mellitus
62	37	Chronic ischemic heart disease
61	41	Multiple myeloma
64	42	Viral hepatitis and chronic liver disease

* Seven persons with hepatitis C virus infection died, but the cause of one death is unknown.

persons in both groups lived long enough for liver-related sequelae to have developed. In addition, all-cause morbidity did not differ substantially between the two groups, although liver-related morbidity was somewhat higher in the HCV-positive group than in the HCV-negative group. Efforts are under way to contact and clinically evaluate all of the surviving HCV-positive persons.

It is more difficult to assess the data regarding hepatocellular carcinoma because of the small number of events. No deaths from hepatocellular carcinoma were recorded in the HCV-positive group; however, of the 1890 death certificates that were available for review in the HCV-negative group, 9 (0.5%) listed liver cancer as an underlying or associated cause of death. The Surveillance, Epidemiology, and End Results (SEER) database estimates that the lifetime risk for liver cancer is 0.5% (35). The data on liver cancer in our study must be viewed in the context of recent evidence, which shows that the incidence of hepatocellular carcinoma is increasing in the United States, possibly a result of previous infection with HCV and hepatitis B virus (36).

Unmistakable clinical evidence shows that chronic HCV infection is associated with end-stage liver disease; that HCV infection is the most common cause of liver transplantation; and that hepatocellular carcinoma is a terminal event for some persons with chronic HCV, especially in Japan, Italy, and Spain. How can our data be reconciled with this evidence? Several explanations can be considered.

First, the relatively small number of HCV-positive persons in our study might have hindered precise determination of an association. Second, more persons may have been HCV-positive than were actually identified; false-negative results could have been caused by prolonged storage of the serum specimens. This explanation cannot be entirely excluded but seems unlikely in view of the generally strong reactivity that was seen among persons who

tested positive for HCV. Furthermore, as already noted, the frequency of anti-HCV reactivity detected 45 years ago is nearly identical to that seen in current military recruits (29). Third, death certificate data regarding cause of death are sometimes inaccurate (41). Fourth, HCV infection may progress more slowly when contracted by young, healthy persons than by older persons whose health has already been compromised. Data from a large French survey support this possibility (42); however, the survey was restricted to HCV-infected women.

If our data represent an accurate estimate of the frequency and rate of progression of chronic HCV infection, only a small fraction of HCV-infected persons progress to end-stage liver disease. The current concern that such a progression is common or inevitable may be a result of the fact that most evaluations focus on only a subset of infected persons, usually those who are most seriously affected.

Despite the relatively small number of HCV-positive persons identified by our prospective evaluation, our results indicate that progressive liver disease in persons with HCV infection is not inevitable. When the combined morbidity and mortality are considered, less than 15% of HCV-positive persons (2 of 17) progressed to overt chronic liver disease, leaving more than 85% without obvious end-stage liver disease. All but 1 of the deaths that were not related to liver disease occurred at least 26 years after the original phlebotomy, an interval sufficient for liver disease to have developed.

Many researchers believe that HCV-infected persons in Japan, Italy, and Spain have a high rate of serious liver disease because HCV was introduced and disseminated much earlier in those countries than it was in the United States. Indeed, the rate of liver cancer in the United States is well below that reported in Japan (37, 38). Our study shows that HCV has been present in the United States for a sufficient time to have detected a greater incidence of hepatocellular carcinoma than current data indicate; this suggests that additional undefined factors may play a role in the carcinogenic potential of chronic HCV infection.

Future studies should focus on efforts to determine the additional contributing factors that are associated with progressive liver disease. Some possible influences have been mentioned, such as age at the time of HCV infection, sex, concomitant alcoholism, viral genotype, viral concentration, and the extent of development of quasi-species (43). However, further study is required. Future research must also include efforts to determine and define markers that will help to predict outcome in the asymptomatic person who receives an unexpected diagnosis of chronic HCV infection.

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