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**FINAL REPORT:
NATURAL HISTORY STUDY of NON-A, NON-B POST-
TRANSFUSION HEPATITIS (1988 - 2001)**

A MULTI-CENTER APPROACH TO DETERMINING THE NATURAL HISTORY OF POST-
TRANSFUSION NON-A, NON-B HEPATITIS

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Non-A, Non-B Hepatitis Study CONTRACT and Sub-CONTRACT NEGOTIATIONS

Inter-agency Agreement: Veterans Affairs Medical Center
Washington, DC

Sub-Contractor: Medical Follow-up Agency - NAS
Washington, DC

Sub-Contractor: Armed Forces Institute of Pathology
Washington, DC

Intra-agency Agreement: Department of Transfusion Medicine
National Institutes of Health
Bethesda, MD

Primary Contractor: Georgetown University
Washington, DC

Sub-Contractor: George Washington University
Washington, DC &
New England Research Institutes
Watertown, MA

Sub-Contractor: Baylor College of Medicine
Houston, TX

Sub-Contractor: Westat, Inc.
Rockville, MD

Sub-Contractor: St. Joseph's Hospital
for Walter Reed
Towson, MD

New York Blood Center
New York, NY

UCLA School of Medicine
Los Angeles, CA

Washington University
St. Louis, MO

Baylor College of Medicine
Houston, TX

KEY CONTRACT PERSONNEL

INTER-AGENCY AGREEMENT--

PRINCIPAL INVESTIGATOR:

Leonard B. Seeff, MD
Veterans Affairs Medical Center
Washington, DC

STUDY COORDINATOR &
CO-INVESTIGATOR:

Zelma J. Buskell, BS
Veterans Affairs Medical Center
Washington, DC

Sub-Contractors &
PRINCIPAL CO-INVESTIGATORS:

James Norman/Dennis Robinette, PhD's
Medical Follow-up Agency - NAS
Washington, DC

Kamal Ishak/Zachary Goodman, MD's
Armed Forces Institute of Pathology
Washington, DC

INTRA-AGENCY AGREEMENT--

PRINCIPAL CO-INVESTIGATOR:

Harvey J. Alter, MD
NIH Blood Bank
Bethesda, MD

PRIMARY CONTRACTOR--

PRINCIPAL INVESTIGATOR:

GEORGETOWN UNIVERSITY

Leonard B. Seeff, MD

Contracting Officer:

Earnest Porta, JD
Washington, DC

Sub-Contractors &
PRINCIPAL CO-INVESTIGATORS:

Elizabeth C. Wright, PhD
George Washington University
Washington, DC &
New England Research Institutes
Watertown, MA

Stephen J. Durako, BA
Westat, Inc.
Rockville, MD

Blaine Hollinger, MD
Baylor College of Medicine
Houston, TX

NHLBI Contracts NO1-HB-87047 and NO1-HB-37093

CLINICAL CENTER'S CO-PRINCIPAL INVESTIGATORS: (Alphabetical)

Harvey Alter, MD	NIH Blood Bank Bethesda, MD
Evelio Bravo/Doris Toro, MD's	VA Medical Center San Juan, PR
Gary Gitnick/Ron Koretz, MD's	UCLA Los Angeles, CA
Blaine Hollinger, MD	Baylor College of Medicine Houston, TX
Frank Iber, MD	VA Medical Center Hines, IL
Robert Knodell, MD	Walter Reed thru St. Joseph's Towson, MD
Stephen Lasky, MD	VA Medical Center Pittsburgh, PA
Timothy Morgan, MD	VA Medical Center Long Beach, CA
Robert Perrillo, MD	Washington University St. Louis, MO
Arun Samanta, MD	VA Medical Center East Orange, NJ
Eugene Schiff, MD	VA Medical Center Miami, FL
Leonard Seeff, MD	VA Medical Center Washington, DC
Stuart Spechler, MD	VA Medical Center Boston, MA
Cladd Stevens, MD	New York Blood Center New York, NY
Reno Vlahevic, MD	VA Medical Center Richmond, VA
Elizabeth Weinshel, MD	VA Medical Center Manhattan, NY

**COORDINATING CENTER
Veterans Affairs Medical Center
Washington, DC**

STAFF

Principal Investigator: Leonard B. Seeff, MD
Coordinator &
Co-Investigator: Zelma J. Buskell, BS

Research Assistants: David Kalloo*, BS (1988-95)
Justine Montgomery, BS (1993-94)
Barbara Harris, BS (1995-01)

*Deceased

**DATA MANAGEMENT CENTER
WESTAT, INC.
Rockville, MD**

STAFF

Project Director &
Co-Investigator: Stephen J. Durako (Vice-President)

Study Managers: Janet Archer, MSc (1988-89)
Casey Cain, MDiv (1989-99)
Janet Bykoski, BS (1999-01)

Epidemiologists: James Korelitz, PhD
Parivash Nourjah, PhD

Biostatisticians: Robert Harris, PhD
Rene Gonin, PhD

Systems Analysts: Judith Walsh, BA
Robert McConnell, BS
Steve Williamson, BS
Peter Ohan, BS

Coding Supervisor: Janet Bykoski, BS

Forms Management
Supervisor: Judy Reilly

STATISTICAL CENTER
George Washington University Biostatistics Center (1988-1992)
Rockville, MD
New England Research Institutes (1992-2001)
Watertown, MA

STAFF

Biostatistician &
Co-Investigator:

Elizabeth C. Wright, PhD

Statisticians:

Lisa Mele, MS (1988-92)
George Washington Bio. Center

Maria Liu, MS (1994-96)
New England Research Inst.

Jennie Jiang, MS (1997-98)
New England Research Inst.

Jeff Allen, BA (Summer Intern – 1999)
New England Research Inst.

Latha Padmanabhan, MS (2000-01)
New England Research Inst.

MEDICAL FOLLOW-UP AGENCY
National Academy of Sciences
Washington, DC

STAFF

Co-Principal Investigators:

James E. Norman, PhD (1988-89)
Dennis Robinette, PhD* (1990-92)

Manager – Data Operations:

Harriet Crawford, BS

Sr. Programmer:

Chiquita Benson, BS

*Deceased

ARMED FORCES INSTITUTE OF PATHOLOGY
Walter Reed Army Medical Center
Washington, DC

STAFF

Co-Investigators: Kamal Ishak, PhD
Zachary D. Goodman, MD, PhD

BAYLOR COLLEGE OF MEDICINE
Department of Virology
Houston, TX

STAFF

Co-Principal Investigator: Blaine F. Hollinger, MD
Investigators: Hsiang J. Lin, DSc
Tawesak Tanwandee, MD
Molecular Biologist: Ron G. Nachtman, PhD

OCHSNER CLINIC
New Orleans, LA

STAFF

Co-Principal Investigator: Robert Perrillo, MD
Investigator: Andy Mason, MD

FINAL REPORT

Transfusion-Associated Non-A, Non-B Hepatitis

Introduction

A series of studies of transfusion-associated hepatitis were undertaken between 1969 and 1980 to define diagnostic criteria for hepatitis following transfusion, to determine the hepatitis incidences, and to establish the specific virologic cause for the illness. These studies described the existence of non-A, non-B hepatitis, later defined as the result predominantly of hepatitis C virus infection. Recognizing that most infected persons failed to recover from the infection and therefore advanced to chronic hepatitis, some of whom progressed further to cirrhosis and even hepatocellular carcinoma, the original transfusion studies were believed to represent ideal sources for future long-term follow-up evaluation.

What follows is a description of the original transfusion studies, the justification for conducting long-term follow-up studies, a description of the methods used to perform the long-term follow-up studies, and the results and implications of the findings.

Background to Natural History Follow-Up Studies

1. Initial studies to identify cases of transfusion-associated hepatitis

Beginning in the late 1960's, the first of what was to be two Veterans Administration (VA) Cooperative Studies, aimed at preventing transfusion-associated hepatitis (TAH), was begun. The first trial (VA-1), that involved 2,204 patients, started in January 1969 and terminated in February 1973 (1) (Figure 1). The aim of this study was to determine whether immune serum globulin (IG) was capable of preventing or ameliorating TAH. TAH was sought by monitoring transfusion recipients for alanine aminotransferase (ALT) levels at 2-weekly interval for a period of 26 weeks. At its inception, viral hepatitis was still considered to consist of two forms – “infectious” or type A hepatitis and “serum” or type B hepatitis. Furthermore, the view at the time was that TAH and hepatitis B were synonymous entities. The “Australia” antigen, later to be recognized as a component of the hepatitis B virus (HBV), was only just emerging as the marker of this virus (2,3). The development of specific tests for HBV permitted the recognition during the course of this first study that, contrary to prevailing opinion, type B hepatitis was responsible for only a minority of cases of hepatitis. It also made it possible to develop a specific form of immune globulin, termed hepatitis B immune globulin (HBIG). Because it became clear in this first VA study that the IG did not prevent, although it did modify TAH, it was elected to conduct a second VA Cooperative Study, this time utilizing HBIG. Shortly after initiating the second study, the virus responsible for hepatitis A was identified (4), leading to the realization that the non-B TAH cases were also non-A, thus introducing the concept of non-A, non-B hepatitis. In this first study, 241 (10.9%) of the patients developed hepatitis, 52 (2.4%) of the cases being attributed to type B hepatitis and 189 (8.6%) to type non-A, non-B hepatitis (1).

The second VA study (VA-2) began in March 1973 and was concluded in September 1975 (5). A total of 969 patients entered this treatment trial. Because the first study suggested limited efficacy for IG, no placebo was used, so that the trial compared the efficacy of HBIG with IG. Concomitant with initiation of the study, routine screening of donor blood for hepatitis B surface antigen (HBsAg) by a third generation test was introduced. The result is that hepatitis B accounted for only 8 (0.8%) of the 127 cases of hepatitis, the remaining 119 (12.3%) being designated non-A, non-B hepatitis. There was a significantly higher frequency of hepatitis among the HBIG recipients (14.0%) than among those who had received IG (10.6%).

Three other large-scale studies were also performed around this period, two of them designed as epidemiologic studies, attempting to determine the incidence of TAH and the risk factors associated with its occurrence (6,7), and one other designed to test the efficacy, in a randomized placebo-controlled trial, of both IG and HBIG (8).

The first epidemiologic survey study, part of a continuing series, came from the National Institutes of Health (NIH) Clinical Center Blood Bank (6). Because this is an ongoing survey, the follow-up natural history, to be described later, was restricted to the period 1968 to 1980. Like the two VA studies, the NIH study monitored blood recipients with biweekly tests for ALT as a marker for hepatitis development. Among the 1142 patients who entered the study after undergoing open-heart surgery, 103 (9.0%) developed non-A, non-B hepatitis.

The second epidemiologic study, termed the Transfusion Transmitted Virus (TTV) study, was a multicenter prospective study also designed to determine the incidence of TAH (7). The study took place in New York City, Saint Louis, Houston, and Los Angeles. Patients scheduled for transfusion were evaluated prior to the transfusion. Blood samples were collected from all donors and recipients were monitored, as described above, at biweekly interval. A total of 1942 persons entered the survey between 1974 and 1979, 161 (8.3%) of whom developed non-A, non-B hepatitis.

The final study was a randomized, placebo-controlled trial designed to evaluate prophylaxis for hepatitis among patients undergoing cardiac surgery (8). It took place at Walter Reed Army Hospital and Letterman Army Medical Center, patients being randomized to receive IG, HBIG, or an albumin placebo. Although 342 persons entered the study, 63 were excluded because they either died following surgery, or received no transfusions, or because of poor follow-up. Among the 279 remaining patients, 42 (15.1%) developed non-A, non-B hepatitis.

In all but the study conducted at the NIH Blood Bank, follow-up of the patients in these studies terminated 12 to 18 months after their initiation. Investigators at the NIH Blood Bank continued to study the problem of TAH and therefore maintained surveillance of transfusion recipients for an additional 20 years.

2. Reason for planning a natural history follow-up study

In the decade following the conduct of these studies, it became clear that non-A, non-B hepatitis, although a seemingly benign condition, commonly asymptomatic and, more frequently than not, unassociated with jaundice, was nevertheless an illness of concern because of its apparent failure to resolve in 50% or more of instances (9,10). Even though people with the condition generally continued to have no symptoms, in

about one-half of them, serum enzymes remained elevated far in excess of 6 months, the period designated at the time to be representative of chronic hepatitis. Liver biopsies performed as a result showed persistent inflammation and the presence of fibrosis, ranging in extent from minimal, to bridging fibrosis, to fully established cirrhosis (11). Concern about the condition heightened considerably when reports began to emerge of cases of hepatocellular carcinoma (HCC) in individuals whose only preceding illness was the occurrence of non-A, non-B hepatitis (12). That the condition appeared to be precipitated by an infectious agent became clearer as a result of chimpanzee inoculation studies undertaken by two separate sets of investigators who had conducted two of the above studies, using material derived from patients with TAH (13,14). Efforts to identify a specific infectious agent, however, proved fruitless for a decade-and-a-half, despite intense efforts, until 1989, when a unique approach by investigators in California led to the discovery of the hepatitis C virus (15,16).

The idea for a natural history study of non-A, non-B hepatitis began to germinate in the mid-1980's. Evidence was mounting that the rate of chronicity was extremely high, that evolution to cirrhosis was not uncommon, and that a proportion of these latter individuals advanced to end-stage liver disease, and in some instance, to the development of HCC. However, because of the paucity of associated signs and symptoms, and because there was at the time no specific marker to identify the entity, it was apparent that it would be exceedingly difficult to design a study that would permit accurate assessment of the long-term consequences of this form of viral hepatitis. In seeking a circumstance in which disease onset could be identified, it was recalled that the earlier transfusion studies described above had all identified disease onset by careful serum enzyme monitoring. Thus, because all five studies had monitored blood recipients with ALT values at biweekly intervals for about 6 months following the transfusion, and because the criteria used to diagnose hepatitis was similar in all instances, it was felt that important information on natural history could be gleaned by tracing the cases identified in all of them in order to determine their subsequent status. Accordingly, the principal investigators of the five studies met to devise a plan and develop the logistics to conduct a long-term follow-up study of non-A, non-B TAH. The basic concept was to combine the cases from all five studies that were designated non-A, non-B hepatitis, and to compare their long-term outcome with a group of transfused individuals, matched carefully with the index cohort, who had not developed hepatitis. A complex protocol was developed (to be described) and, in 1988, the Blood Resources Branch of the National Heart, Lung, and Blood Institute funded a multi-center follow-up study of the individuals who had participated in the several studies of TAH conducted between 1967 and 1980.

3. Hypotheses developed for the follow-up study

Based on the availability of a large cohort of individuals who had developed TAH under careful scrutiny, and in which the time of onset of hepatitis was well delineated through close-spaced serum enzyme monitoring; and given the fact that it was possible to carefully match the "infected" cohort with transfused individuals who had not developed hepatitis, it was possible to construct the following hypotheses:

Primary Hypotheses

1. Death from liver disease is more common among persons who develop TAH than among those transfused patients who do not develop hepatitis;

2. Development of serious liver disease that usually causes morbidity (whether fatal or non-fatal) is more common among persons who develop TAH than among those who do not develop hepatitis.

Secondary Hypotheses

1. Mortality from all causes is higher among persons who develop TAH than among those who do not develop hepatitis;
2. The presence of chronic liver-related abnormalities not associated with serious morbidity is more common among surviving persons who develop TAH than among surviving persons who do not develop hepatitis. Chronic liver-related abnormalities are defined as:
 - a. At least two ALT values above the upper limit of normal, with the two abnormal values separated by at least 6 months, on blood samples drawn during this study;
 - b. Colloid shift in liver-spleen scintogram (salt-and-pepper distribution of colloid in liver with uptake demonstrated in spleen and spinal vertebrae) together with hypoalbuminemia (albumin < 3.0 mg/dL) or hyperprothrombinemia (prothrombin time > 2 seconds prolonged).

Other Objectives

1. Does the use of prophylactic gamma globulin at the time of transfusion affect the risk of developing chronic liver disease among persons who were infected at transfusion?
2. Does the time to development of chronic liver disease or death from liver disease vary by age, sex, race, or other factors?
3. How does the disease progress over time? For example, does chronic active hepatitis progress to cirrhosis? This will be evaluated by conducting a long-term follow-up study of living persons with chronically raised serum enzymes.

Methods

This section describes the methods for the first and second follow-up studies. The timeline is shown in Figure 1. The original transfusion-associated hepatitis (TAH) studies were conducted between 1967 and 1980. The first follow-up study began in 1988 and was scheduled to end in 1993. In 1993 it was extended for an additional 5 years (Follow-up Study 2).

Figure 1: Study Timetable, Original Studies and Follow-up Studies 1 and 2

Date	Activity	NIH	VA1	WR	VA2	TTV	
1967	Cases and controls transfused in original studies	[Shaded]					
1968							
1969				[Shaded]			
1970							
1971							
1972					[Shaded]		
1973						[Shaded]	
1974							[Shaded]
1975							
1976							
1977							
1978							
1979							
1980							
1981							
1982							
1983							
1984							
1985							
1986	Planning for follow-up Study.						
1987							
1988	Matching and tracing.	[Shaded]				Follow-up Study 1	
1989	Initial visits.						
1990	Six-month follow-up.						
1991	Continued follow-up for patients with CH						
1992	Annual NDI.						
1993		[Shaded]				Follow-up Study 2	
1994	Annual follow-up for normal patients. Six month						
1995	follow-up patients with CH.						
1996	Annual NDI						
1997							
1998	Six-month follow-up for pts. with CH, HCV RNA, ALT Elev.						
1999	Annual NDI. Analysis						
2000							
2001	Annual NDI. Analysis						

Summary of Follow-up Study 1

Follow-up Study 1 was conducted in three phases. During **Phase I** (months 1-12), the protocol was developed and agreements were made by the study Investigators regarding the accepted diagnostic criteria for acute NANB hepatitis in the original studies and of chronic hepatitis during follow-up evaluation. Matching criteria were formulated so that control subjects could be chosen and the protocol was refined and data forms and a Procedures Manual were developed.

Cases and controls were participants in one of the five studies of TAH. Cases were defined as participants in the original studies who developed transfusion-associated non-A, non-B hepatitis according to the diagnostic criteria employed in the individual study (see Section III, Part C). Controls were selected from participants in the original study whose ALT levels after day 17 remained below the upper limit of normal. Cases and controls were excluded from the follow-up study if they were followed for fewer than 18 weeks after transfusion or if they had a history of cirrhosis. Controls were also selected for cases who developed transfusion-associated hepatitis B. Results for these cases and controls are reported separately.

The matching criteria included study and center, treatment with immune globulin, history of alcoholism, race (black or non-black), sex, age at transfusion, date of transfusion, and number of units transfused. Data required for matching were sent from the coordinating centers of the five studies to the DMC. Details of the matching procedure are provided in sections II and III.

Tracing of cases and controls was performed by the Data Management Center. Once cases and controls had been selected, requests for tracing information were sent to the investigators at each center. The information requested included name, Social Security number, date-of-birth, date of last known address, last known address, telephone number, name of spouse if married, name and address of next-of-kin, and vital status (presumed alive or known dead). This information had been stored separately from other patient data in order to maintain confidentiality.

Subjects were assumed to be alive until evidence of death was found. The National Death Index (NDI)(17,18), Social Security Death Tapes (SSDT), and records of the Health Care Financing Administration (HCFA) were consulted in order to find records of patient deaths. If a date-of-death was found, then copies of the death certificate were requested from the appropriate state. Next-of-kin were contacted and asked to complete a questionnaire and give approval for access to medical records.

The Medical Follow-up Agency (MFUA) of the National Research Council (NRC) was asked to search for addresses for patients from the VA and WR studies using the Beneficiary Identification and Record Locators System (BIRLS)(18). They also obtained copies of death certificates and abstracted VA medical records.

Living subjects were traced through information supplied by individual investigators, by HCFA and BIRLS, and from directory assistance, departments of motor vehicles, voter registration lists, and public libraries.

In **Phase II** of Follow-up Study 1 (months 13-24), tracing of cases and controls and proxies continued. Proxies of deceased persons were contacted by telephone and questioned about their knowledge of the medical history and other relevant information relating to the deceased person. Living subjects were invited to come to one of the participating study centers for an initial interview, physical examination, and phlebotomy

for measurement of specific biochemical tests (the ALT in particular) and for assays for serologic markers of viral hepatitis. This same process was repeated on at least two other occasions over the course of a six-month period (Table 1).

Table 1: Schedule of tests and procedures

	1989-1993					1993-2000	1994-1997	1997-2000
	All			CH		CH	Others	Single ALT or HCV RNA +
	Start of Study	Month 3	Month 6	Every 6 months	Last follow-up	Every 6 months	Every 12 months	Every 6 months
Telephone Interview	X						X	
Medical History	X	X	X	X	X	X		X
Physical Exam	X			X	X	X		X
Hematology*	X			X	X	X		X
Hepatitis Serology**	X		X	X	X	X		X
Alpha-fetoprotein	X				X	X		X
Liver Chemistries***	X	X	X	X	X	X		X
Liver Biopsy ****			X		X			

* Hematology: CBC with differential, platelets, prothrombin time, partial thromboplastin

** Hepatitis Serology: HBsAg, anti-HBs, Anti-HBc, anti-HAV, anti-HCV

*** Liver Chemistries: Total protein, albumin, globulin, direct bilirubin, alkaline phosphatase, AST, ALT

**** Only for patients with CH (at least two abnormal ALT values). A second liver biopsy should have been performed for CH patients once during 1994 – 1995 if not performed earlier.

Patients were considered to have chronic hepatitis if an elevated ALT was found in at least two of three blood samples obtained during the six-month or longer initial evaluation period. Hepatitis C was held responsible if there was accompanying and persisting RIBA-confirmed anti-HCV reactivity.

Phase III was directed toward determining, with more accuracy, the extent of the chronic disease. For this purpose, subjects with chronic hepatitis continued to be followed at six-month intervals with a medical history survey, physical examination, and phlebotomy for biochemical, hematologic, and serologic evaluations. A liver biopsy was requested of patients with established chronic hepatitis. For those who refused or in whom biopsy was not possible, other measures were taken to seek evidence of portal hypertension, namely a liver-spleen scintiscan or an upper endoscopy looking for the presence of varices. Sections of the liver biopsies were submitted under code to Drs. Kamal Ishak and Zachary Goodman at the Armed Forces Institute of Pathology (AFIP), Washington, D.C.

The underlying cause of death as recorded on the death certificates was coded according to the *International Classification of Diseases, 9th Revision* (19). Any liver-related conditions mentioned on the death certificate were also coded. In addition, medical abstract data were reviewed to identify subjects with chronic liver disease, cirrhosis, or hepatocellular carcinoma at the time of their death.

Testing for HBsAg and for antibodies to the hepatitis B surface and core antigens and the hepatitis A virus was accomplished using routine radioimmunoassays (Abbott Laboratories, North Chicago, IL). Testing for antibody to hepatitis C of both current and stored original samples was performed by enzyme-linked immunoassay (ELISA 2.0; Abbott Laboratories, North Chicago, IL). The following additional tests for anti-HCV and HCV RNA were conducted on stored samples:

1. Matrix test for confirmation of anti-HCV (Abbott)
2. RIBA confirmation of anti-HCV (Chiron)
3. Branch bDNA
4. HCV RNA by PCR
5. HCV genotype

The primary outcomes of this study were all cause mortality, death from liver disease, and the development of chronic liver disease. Kaplan-Meier survival curves, stratified by study, were calculated and cases and controls were compared by means of the log-rank test. Further details are provided in Section III.

Summary of follow-up study 2

In 1993 the study was continued for an additional five years of follow-up. During this phase, subjects with evidence of liver disease continued to be seen at six-month intervals, living subjects without evidence of liver disease were contacted by telephone annually, and data were requested from the National Death Index annually (Table 1).

In 1998 the study was extended for an additional 3 years. Patients with CH continued to be followed at 6-month intervals. In addition, patients who had had a single ALT abnormality that was not classified as CH and patients who had normal ALT values but were HCV RNA positive were brought in for follow-up. A final NDI search was conducted in 2001 (included deaths through 1999).

Results

The first report of the results of the study appeared in December 1992 after a follow-up period that averaged 18 years after the original diagnosis of non-A, non-B hepatitis (20). Tracing of subjects for mortality from all five original studies proved highly successful; the vital status was established for over 94% of the 568 patients who were designated as having originally developed non-A, non-B hepatitis and their 984 controls (526 first controls and 458 second controls).

Through examination of the National Death Index and Social Security Death Tapes, it was found at the outset of the follow-up study that death from all causes occurred among 287 (51%) of the 568 subjects with non-A, non-B hepatitis, among 273 (52%) of the 526 first controls, and among 228 (50%) of the 458 second control subjects. Obviously, there was no statistical difference between these mortality figures. Survival curves plotted for all three cohorts were virtually identical. Death from liver disease was determined by examining the cause-of-death recorded in death certificates. Liver disease accounted for 19 deaths among the subjects with non-A, non-B hepatitis (3.3%), for 6 deaths among the first controls (1.1%), and for 9 deaths among the second controls (2.0%). The differences were significant when comparison was made with the first control ($P = 0.022$) and with the two controls combined ($P = 0.033$) but not with the second control ($P = 0.26$). Most of the liver-related deaths occurred in the two VA Cooperative Studies. The explanation offered for this finding is that the VA studies were the only ones that permitted enrollment of persons with a history of alcoholism, suggesting that previous heavy alcohol intake might have acted in concert with HCV infection to promote advancement to end-stage liver disease. Interestingly, death from cirrhosis and hepatocellular carcinoma occurred with nearly equal frequency among both the controls and the hepatitis cases. Examination of the survival curves indicated that the all-cause mortality rate was almost identical for the cases and controls participating in these studies, and that these mortality rates were both significantly higher than that of the general population, indicating that the bulk of deaths were a consequence of conditions not specifically associated with intrinsic liver disease.

It must be noted that at the time of the publication, the hepatitis C virus had not yet been identified so that it was not yet possible to determine whether all cases of non-A, non-B hepatitis were in fact a consequence of HCV infection. At this juncture, an average of 18 years after the transfusion episodes, the conclusion reached in the study was that "there was no increase in mortality from all causes after transfusion-associated non-A, non-B hepatitis, although there was a small but statistically significant increase in the number of deaths related to liver disease." (20)

Subsequently, several presentations of study data were made at national meetings, leading to a number of published abstracts (21-24). The second major publication, however, appeared in late 2001 (25). This report updated the mortality data after extending follow-up evaluation from approximately 18 years to approximately 25 years beyond the initial transfusion(s). In addition, the report summarized morbidity outcome among the living study subjects. Unlike the first report, follow-up was now restricted to study subjects in three of the original five studies. By this time, HCV had been identified and sophisticated virologic and molecular biologic assays had been developed to test for the presence of the hepatitis C virus. In two of the original studies – the first VA study and the Walter Reed Army Hospital study, no original archived sera were available to establish the precise viral etiology. In the three remaining studies,

original samples were available in repository permitting specific identification of the viral hepatitis source. Because outcome was to be determined according to the defined viral type of infection, the first VA and the Walter Reed Army studies were excluded for this analysis.

The three studies yielded 314 persons with a diagnosis of non-A, non-B hepatitis. Two hundred and twenty-two (70.7%) of them could be attributed to HCV infection, the cause for the remaining 92 cases being undetermined. The 222 anti-HCV positive cases were matched with 377 controls, and the 92 anti-HCV negative cases were matched with 168 controls. By this time, all-cause mortality had risen among the HCV positive cases from 44.6% at initial tracing to 67.1% about 25 years later, whereas liver-related mortality rose from 2.3% initially to 4.1% ($P = 0.05$). Comparison of these numbers with those derived from the controls revealed no significant difference with respect to all-cause mortality, but a significant difference was found among persons who had suffered a liver-related death (4.1% vs. 1.3%; $P = 0.05$). The 9 liver-related deaths among the original cases consisted of hepatocellular carcinoma in three, cirrhosis in three, chronic hepatitis in one, hepatitis unspecified in one, and "hepatic failure" in one. Four of the five liver-related deaths among the controls were a result of cirrhosis, and one from "other sequelae of liver disease". Twenty-year survival curves again were virtually overlapping.

Among the anti-HCV negative cases, all-cause mortality increased from 39.1% initially to 51.1% at follow-up, while liver-related mortality rose insignificantly from 2.2% to 3.3%. None of these numbers differed significantly from their control counterparts. The liver-related deaths were a result of HCC in one, cirrhosis in one, and "unspecified liver disorder" in one. The single liver-related death in the control group was a patient with HCC.

Follow-up of living subjects was possible for 129 cases (90 anti-HCV positive, 39 anti-HCV negative) and 209 controls. Cases were significantly more likely to experience tiredness and anorexia, and to have hepatomegaly, a tender liver, thrombocytopenia, and elevated levels of ALT, AST, and bilirubin.

Serologic and clinical outcome of the 90 anti-HCV positive cases approximately 25 years after the initial infection revealed that 69 persons (77%) were still anti-HCV positive and were viremic (HCV RNA-positive), 15 persons (17%) had antibody but were not viremic, and surprisingly, 6 persons (7%) showed no virologic markers whatsoever of their original HCV infection; they were not viremic, did not have detectable anti-HCV, and had normal serum enzymes. Half of the viremic group had raised ALT values, consistent with chronic hepatitis, and among this group of 34 persons, 12 (35%) had histologic evidence of cirrhosis. The other half had normal ALT values and hence, based on our original protocol requirement, could not be biopsied. We conjectured that had a liver biopsy been performed, fewer than 5% (perhaps 2 person) might have had cirrhosis. Among the two nonviremic groups, (one anti-HCV positive and the other anti-HCV negative), consisting of 21 persons, only two had raised ALT values consistent with a diagnosis of chronic hepatitis. Again, most were not biopsied but we assumed that had they been biopsied, perhaps one of them might have had cirrhosis. Thus, based on observation and projection, we concluded that about 15% of those infected in this study would have developed cirrhosis 20-25 years later.

Finally, among the 20 persons who actually underwent biopsy, 13 (65%) had histologic evidence of mild to moderate chronic hepatitis without bridging or cirrhosis.

Ten of them (77%) had no clinical manifestations of chronic liver disease whatsoever, the remaining three (23%) showing only minor evidence of chronic liver disease (any one of the following – splenomegaly, hypoalbuminemia, thrombocytopenia). In contrast, among the seven persons with cirrhosis, four (57%) had severe clinical manifestations and two had clinical features of mild chronic live disease.

Thus it was concluded that, all-cause mortality approximately 25 years after acute TAH was high but was no different between cases and controls. Liver-related mortality attributable to chronic hepatitis C, though low (<3%), was significantly higher among the cases. Among living patients originally HCV-infected, 23% had spontaneously lost HCV RNA. Also, of great interest, is the evidence that 30% of the non-A, non-B hepatitis cases were unrelated to the hepatitis viruses A, B, C, and G, suggesting the existence of another unidentified agent.

In the effort to examine factors associated with progression to cirrhosis, the data were analyzed further in order to quantify the relationship of HCV-related TAH to a history of heavy alcohol abuse as one determinant of disease progression. All 1030 persons from the original studies whose sera had been stored in the repository were initially included for this analysis (25). However, 194 were later excluded for several reasons, 108 because their original sera samples were exhausted, 79 because their ALT levels or HCV assays did not permit reliable classification of the hepatitis status, one because of cirrhosis that predated the index transfusion, and six with data inconsistencies that could not be resolved. For analysis purposes, the remaining group was divided into three categories – those with established HCV-related TAH, persons with TAH unrelated to HCV and designated non-A, non-B, non-C hepatitis, and controls.

To determine whether cirrhosis had developed following the transfusion, medical records, death certificates, and biopsy and autopsy reports were reviewed by trained abstractors for the diagnosis of cirrhosis, based on the occurrence of features of portal hypertension and overt clinical manifestations of cirrhosis. A sample of these records was evaluated by the principal investigator to check agreement with the abstractors.

Potential risk factors for disease progression were sought, with a focus on heavy alcohol intake. A composite variable aimed at identifying a history of heavy alcohol abuse was designed. It was based on the following criteria: loss of friends, family, or a job because of drinking; evidence of heavy drinking abstracted from medical records; or quantification of usual intake of more than 80g of alcohol per day during the years when the patient drank.

Logistic regression was used to estimate the risk for cirrhosis associated with TAH-HCV infection and a history of heavy alcohol abuse.

The absolute risk for developing cirrhosis among these individuals was 17% for those with HCV-related TAH, 3.2% for the non-A, non-B, non-C hepatitis group, and 2.8% for the controls. A history of heavy alcohol abuse was associated with a fourfold increased risk for cirrhosis. In comparison with the control group, HCV infection plus a history of heavy alcohol abuse led to a substantial increase for the risk of cirrhosis (odds ratio, 31.1 [CI, 11.4-84.5]). A note of interest in this study was the finding that African-American patients who had developed HCV-related TAH were significantly ($P=0.022$) less likely to have developed cirrhosis (2.2%) than did non-African-American study subjects (7.2%).

Thus the conclusion of this report is that heavy alcohol abuse greatly exacerbates the risk for cirrhosis among patients with HCV infection, emphasizing the need to counsel such patients about their drinking habits.

In order to study characteristics that link the blood donor to the recipient with respect to HCV transmission, careful analysis was undertaken of 30 transfusion recipients and 120 blood donors who had participated in the original Transfusion-Transmitted Viruses Study (TTVS) and who had been recalled for evaluation in the current follow-up study (26). Phylogenetic analysis of hypervariable region 1 (HVR1) and HCV genotyping were carried out on the genomic region encoding amino acids 329-410. Using a variety of relevant techniques to accrue the data, linear regression analysis demonstrated no differences between donor and recipient HVR1 sequences in the initial 4.4 weeks following transfusion, but they diverged over the subsequent 20 weeks. The lag phase in HVR1 evolution in the infected recipient was thought to be the consequence of the time required to mount host immunologic defenses against the virus.

To determine the rate and extent of divergence over time in the recipients themselves, the same evaluations were directed at three specimens of each individual under investigation – one collected within 2 weeks of the ALT peak in the original study, one at the end of that study, and one collected during the long-term follow-up. HVR1 remained invariant over a period of 5.5 to 9.9 days during the acute infection but diverged over a period of 11 to 15 years, reaching the degree of divergence observed between unlinked subjects. In cases where transfusion involved more than one subtype, only one of them established infection in the recipient. The results support the hypothesis that the dominant HCV in the donor is established in the recipient during the post-transfusion period, and that the transmitted HCV remains invariant during an initial period of 4-6 weeks, evolving rapidly after the development of acute hepatitis.

Implications of the Natural History Studies

Soon after the recognition of non-A, non-B hepatitis, evidence began to emerge that about 50% of infected individuals failed to recover as evidenced by persistence of serum enzymes abnormalities, ALT in particular. Because enzymes remained abnormal for over six months, these individuals were now considered to have chronic hepatitis. When liver biopsies showed the presence of marked bridging fibrosis and even cirrhosis in as many as 20% of instances, and when anecdotal evidence began to appear apparently linking chronic non-A, non-B hepatitis and hepatocellular carcinoma, virtually always in persons who had developed cirrhosis, the illness began to be viewed as being extremely serious. The discovery of the hepatitis C virus as the agent responsible for most cases of non-A, non-B hepatitis escalated the concern because of the recognition that a larger proportion of infected persons, between 75% and 85%, actually persisted with infection, and also because numerous reports appeared describing the presence of hepatitis C virus infection in the majority of persons with hepatitis B virus-negative hepatocellular carcinoma.

The advent of the test for hepatitis C also made it possible to identify persons with chronic hepatitis C and attempt to track them back to the presumed time of disease onset, often 20 to 40 years earlier (29). These retrospective studies yielded highly disconcerting information because of the clear linkage of end-stage liver disease and primary hepatocellular carcinoma to long-term infection with the hepatitis C virus. In retrospect, these studies, while clearly of great importance in describing the potential

consequences of long-term HCV infection, suffered from the fact that they tended to emphasize the very severe outcomes that could occur, but obviously were unable to detect the frequency of less severe disease or of recovery from the infection without any serious effect. In summary, they tended to suggest that evolution to end-stage liver disease was extremely common.

Later a series of prospective studies were reported, mostly a result of follow-up of persons infected through blood transfusion, thus permitting prospective studies beginning with identified disease onset. A lower rate of development of end-stage cirrhosis and hepatocellular carcinoma was noted. While these studies were more comforting, they suffered from the fact that they were of relatively short duration, ranging in length from 8 to 16 years, far too short to permit firm conclusions of the true rate of progressive disease.

More information has derived from several newer studies, that include those described above, that were conducted as combined retrospective/prospective (or non-concurrent prospective) studies. Each of these were able to track back to a confirmed time of disease onset, to have original samples stored in repository permitting accurate diagnosis, and then to have an opportunity to conduct a further prospective study (29,30).

A series of these recent studies, involving follow-up of infected children (30), young women exposed to contaminated Rh immune globulin (31-34), and injection drug users (35,36) have all reported lower rates of advancement to cirrhosis approximately two decades after infection than had previously been anticipated as well as a lower mortality rate. The studies described above involving transfusion recipients and young men infected almost a half-century ago tend to support these data. The problem regarding the studies of young children and young women is that they have conducted surveillance over a period of two decades only. Although they show clearly that the rate of progression is far lower than would have been expected, they provide little evidence of what might be expected over the two to three decades that follow. It would appear that evolution to cirrhosis is more likely, and occurs more rapidly, when older people are infected. The question that must then be raised is whether there will be an increase rate of progression as the younger infected person ages or whether the long-term outcome is defined for life if infection occurs initially at an early age. This speaks to the continuing controversy regarding the question of whether fibrosis progression follows a linear pathway, progressing forward inexorably, or whether the course is defined early in the infection, perhaps modified by extraneous factors, such as heavy alcohol abuse, perhaps other environmental contaminants or even dietary factors.

The studies conducted as part of the present contract also produced interesting and informative data that were not anticipated at its outset. The finding of all-cause mortality that has been identical over the past 25 years for both the non-A, non-B hepatitis cases and their transfused non-hepatitis controls, and for the subgroup within this category identified as having hepatitis C and their controls, was somewhat surprising. When compared with the expected mortality rate of the general public (mostly non-transfused and non-hepatitis controls), there is clearly a highly significant difference between the identical survival curves of the cases and controls and that of the general public, indicating that the equally high all-cause mortality of the cases and controls is a consequence of the underlying disease that led to the transfusion in the first place. On

the other hand, death from liver disease, although surprisingly low, was significantly higher among the patients with hepatitis than among the controls.

To summarize, the natural history of transfusion-associated hepatitis among middle-aged adults, as defined by this study, indicates that there is high mortality in the first two decades following transfusion, but this is predominantly a consequence of disease unrelated to hepatitis C; in many instances, mortality derives from the reason(s) for the initial transfusion. Although chronic liver disease represents only a small fraction of the causes of death, this outcome is significantly more common among those initially infected with HCV than among transfused individuals not so infected. Also, the frequency of progression to cirrhosis, about 15% in this study, is in keeping with the generally accepted figure of about 20% approximately 20 years after infection. It is possible that this relatively high rate of development of cirrhosis is a consequence of factors associated with older age, since transfusion-associated hepatitis C occurring in young children appears to lead to a significantly lower rate of cirrhosis (30). Similarly, a low rate of evolution of cirrhosis has been found in young women infected with HCV-contaminated Rh immune globulin (31,32).

Of particular interest in this study were the serologic, virologic and biochemical outcomes among HCV-infected persons still living 25 years later. Although 77% of persons originally infected with HCV remained viremic, of whom only a half also had raised serum enzymes, 17% were anti-HCV positive but HCV RNA negative, almost all with normal enzymes, and 7% had negative tests for both anti-HCV and HCV RNA and hence showed no evidence whatsoever to indicate that they had previously been infected with hepatitis C. These data, indicating that complete recovery that includes loss of all viral markers can occur is not without precedence. A similar outcome has been identified in follow-up of young German women who had been infected with HCV-contaminated Rh immune globulin (34,37).

In keeping with the evidence that there seems to be differential rates of progression and frequencies of evolution to cirrhosis are the data of follow-up involving the young, healthy military recruits who had been phlebotomized almost 50 years ago and had their blood samples stored frozen since that time. Among the 17 persons whose original samples were found to be anti-HCV positive, only one had died of liver disease in the interval between the original phlebotomy and the follow-up evaluation. Among the 10 survivors, eight could be traced and seven could be further evaluated, access being denied to the eighth person by his family because he had earlier suffered a stroke. When the seven living persons were initially contacted, none complained of liver-related symptoms and most were not aware of the fact that they had been exposed to HCV. All were anti-HCV positive, six of the seven were also viremic, all had raised serum enzymes, and all but one had normal albumin levels and normal platelet counts. The single person whose indices were abnormal had a long history of chronic alcoholism. Over the course of the subsequent 9 months, his health began to deteriorate, and he died carrying a diagnosis of "hepatocellular/cholangiolar carcinoma" based on scrutiny of a CT scan. No liver biopsy or autopsy was performed to permit confirmation of the diagnosis. A subsequent re-appraisal of the CT scan raised the possibility that the patient might have had primary lung cancer with metastases to the liver. Regardless, it is noteworthy that after at least 50 years of HCV infection, only 2(11.8%) developed end-stage liver disease, one of whom may not have died of primary liver disease.

What accounts for the apparent differences in natural history of these various cohorts, the rate of evolution of the chronic liver disease being low in some and high in others? Differences are probably a consequence of both viral and host factors. Several host factors seem apparent in these studies. First, as noted, age at the time of infection may be of importance. The average age of patients entering the transfusion studies was close to 50, and about 15% were found to have cirrhosis approximately 20 years after initial infection. In contrast, the cirrhosis rate in the study of military recruits was lower, approximating the frequencies reported in infected children and young woman.

Another frequently cited co-factor for disease progression is concomitant chronic alcohol abuse (38,39). As noted above, examination of this issue among individuals participating in the transfusion studies identified a strong association between heavy alcohol intake and progression to cirrhosis. It is also of note that the one person in the study of military recruits who progressed to severe end-stage liver disease had a history of heavy alcohol intake. Clearly, persons with chronic HCV infection should be strongly advised to strongly moderate or, preferably, to discontinue alcohol intake altogether.

Finally, of intriguing interest is the possibility that evolution to cirrhosis may be slower among African Americans than among Caucasians despite the higher rate of infection in African Americans and their poorer response rated to therapy. (40). Data from the transfusion study clearly show a significantly lower rate of progression to cirrhosis among African American than among Caucasians. Also tantalizing is the evidence that half of the long-term survivors in the military recruit study were African American although this racial group represented a tiny fraction of the persons stationed at that base.

Many questions remained unanswered regarding outcome following acute HVC infection, some of which include the following. Why do so many infected persons fail to eradicate HCV? Does fibrosis progression following established viral persistence follow a linear pattern so that culmination in cirrhosis and end-stage liver disease is an inevitable consequence of the infection unless the infected person succumbs first to another disease process, or is progression limited to only a proportion of infected individuals, the process being affected by not yet fully characterized viral, host, or extraneous factors? Is spontaneous viral loss higher than has heretofore been described and, if so, when after infection might this occur? Is it possible to identify serum markers that will regularly predict disease progression? Is there a geographic, racial, or ethnic difference in the rate and frequency of disease progression that might explain apparent differences in outcome according to these characteristics? The studies just concluded have helped to establish the fact that the rate of evolution of chronic HCV infection is generally exceedingly slow and that the outcome seems to vary based on a number of characteristics of the affected population. Prior to conduct of these studies, the outcome of the disease was seen as grim, with the view that a high proportion of affected persons were likely to advance to end-stage liver disease. The results of the studies described herein provide a far more optimistic view of the natural history of hepatitis C.

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