HHS Public Access

Author manuscript

Transfusion. Author manuscript; available in PMC 2017 July 06.

Published in final edited form as:

Transfusion. 2014 November ; 54(11): 2833–2841. doi:10.1111/trf.12682.

Transfusion-associated hepatitis before the screening of blood for hepatitis risk factors

Ronald E. Engle[1], **Jens Bukh**[2], **Harvey J. Alter**[3], **Suzanne U. Emerson**[1], **Joni L. Trenbeath**[3], **Hanh T. Nguyen**[1], **Alicia Brockington**[1], **Tanaji Mitra**[1], and **Robert H. Purcell**[1]

[1]Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland [2]Copenhagen Hepatitis C Program (CO-HEP), Department of Infectious Diseases and Clinical Research Centre, Hvidovre Hospital, Hvidovre, and the Department of International Health, Immunology and Microbiology, Faculty of Health and Medical Sciences, Copenhagen N, University of Copenhagen, Copenhagen, Denmark [3]Department of Transfusion Medicine, Grant Magnuson Clinical Center, National Institutes of Health, Bethesda, Maryland

Abstract

BACKGROUND—The true incidence of transfusion-associated hepatitis (TAH) before blood screening is unknown. Our aims were to reevaluate blood recipients receiving unscreened blood and analyze hepatitis viruses circulating more than 45 years ago.

STUDY DESIGN AND METHODS—Cryopreserved serum samples from 66 patients undergoing open heart surgery in the 1960s were reevaluated with modern diagnostic tests to determine the incidence of TAH and its virologic causes.

RESULTS—In this heavily transfused population receiving a mean of 20 units per patient of predominantly paid-donor blood, 30 of 66 (45%) developed biochemical evidence of hepatitis; of these, 20 (67%) were infected with hepatitis C virus (HCV) alone, four (13%) with hepatitis B virus (HBV) alone, and six (20%) with both viruses. Among the 36 patients who did not develop hepatitis, four (11%) were newly infected with HCV alone, nine (25%) with HBV alone, and one (3%) with both viruses. Overall, 100% of patients with hepatitis and 39% of those without hepatitis were infected with HBV and/or HCV; one patient was also infected with hepatitis E virus. The donor carrier rate for HBV and/or HCV was estimated to be more than 6%; contemporaneously prepared pooled normal human plasma was also contaminated with multiple hepatitis viruses.

CONCLUSION—TAH virus infections were a larger problem than perceived 50 years ago and HCV was the predominant agent transmitted. All hepatitis cases could be attributed to HCV and/or

Address correspondence to: Ronald E. Engle, Laboratory of Infectious Diseases, 50 South Drive, Room 6345, Bethesda, MD 20892; rengle@niaid.nih.gov.

A preliminary report of this study was presented by RHP at the 2012 International Society of Blood Transfusion meeting, July 10, 2012, Cancun, Mexico.

CONFLICT OF INTEREST

The authors have disclosed no conflicts of interest.

HBV and hence there was no evidence to suggest that an additional hepatitis agent existed undetected in the blood supply.

Today, blood is extremely safe, but it was not always so. Advances in transfusion medicine in the first half of the 20th century led to increasing use of blood and blood components and a parallel increase in transfusion-associated hepatitis (TAH). World War II accelerated the use of blood and led to the recognition, in the 1940s, that there were probably two types of transmissible hepatitis, homologous serum jaundice and infectious catarrhal jaundice. Studies in Europe and the United States confirmed the existence of two hepatitis viruses, leading to their designation as hepatitis B and hepatitis A,[1] but neither virus could be isolated with the emerging technology of tissue culture nor could they be transmitted to laboratory animals.

To determine the incidence and natural history of TAH, retrospective and prospective studies of transfused patients were undertaken.[2–5] Two studies, one in Tokyo[6] and the other in Philadelphia,[7] were performed shortly after the development, by Karmen[8] in 1955, of sensitive tests for serum levels of transaminase enzymes indicative of liver damage and these tests were incorporated into the prospective studies, thereby permitting the determination of the incidence of anicteric as well as icteric hepatitis. The incidence of hepatitis was markedly different in the two studies: 113 of 175 transfused patients (65%) in Tokyo and 10 of 56 patients (18%) in Philadelphia. This was not surprising, since the Japanese patients received a mean of 11 units of blood, whereas the US patients received a mean of 2.1 units. However, when a binomial expansion of risk factors was applied to the data from the two studies, the calculated proportion of blood donors in Japan and in the United States who presumably were infected with a hepatitis virus was virtually identical: 9%.[9]

This finding was alarming to surgeons and blood bankers and precipitated a number of additional prospective studies of TAH.[10–15] One of the first was the study of heart surgery patients at the National Institutes of Health in the United States. The National Heart Institute had an active open heart surgery program, which, at the time, required large quantities of blood to prime the extracorporeal heart–lung pump. Separate studies by the NIH Clinical Center Blood Bank and the Laboratory of Infectious Diseases, NIAID, were subsequently combined to yield a multi-institute project that continued for decades and documented the progressive decline in TAH as new control measures were instituted, until the disease was no longer detected among blood recipients after 1994.[15–22] However, when the studies began, there were no tests for the diagnosis or screening of hepatitis A virus (HAV) or hepatitis B virus (HBV) infections; hepatitis types C, D, and E and their etiologic agents (hepatitis C virus [HCV], hepatitis D virus [HDV], hepatitis E virus [HEV]) were yet to be discovered; and serum enzyme tests for diagnosing hepatitis, although available, were not used for screening blood donors. Thus, large amounts of blood were being transfused without any screening related to viral hepatitis. The initial purpose of this study was to determine the true incidence of TAH with serum samples collected before and after open heart surgery at the NIH between December 1964 and June 1968. We have tested them for infection with hepatitis viruses A through E, utilizing serological and molecular tests currently available.

Blood was not the only clinical material at risk of transmitting hepatitis. Normal human plasma (NHP), used as a plasma volume expander, was often prepared from outdated blood, pooled into lots of various sizes, and stored frozen or lyophilized. It soon became apparent that hepatitis was a significant risk associated with infusion of pooled plasma. Attempts to inactivate the causative agent of the hepatitis by a variety of chemical and physical treatments failed to find a fully satisfactory method, but ultraviolet radiation or storage at room temperature for at least 6 months was believed to diminish the risk.[23,24] There was considerable debate about this approach until Redeker and colleagues[25] performed a controlled prospective study of plasma that had been irradiated and stored at room temperature and demonstrated a 10% incidence of hepatitis in recipients of the plasma but no hepatitis in recipients of human albumin that had been heated at 60°C for 10 hours. Based on this study, the Committee on Plasma and Plasma Substitutes of the Division of Medical Sciences, National Research Council, recommended that “the use of whole, pooled human plasma be discouraged and even discontinued unless a clear-cut case can be made for its unique requirements.”[26] We examine herein the markers of hepatitis in commercial pooled NHP from the 1960s.

MATERIALS AND METHODS

Patients

Patients scheduled for open heart surgery were bled the day before the operation and again at weekly to monthly intervals for the first 6 months after surgery and once or twice a year thereafter.[15] Consecutive patients between January 1965 and June 1968 who met the following criteria were followed: at least 21 years of age, undergoing cardiac surgery associated with transfusion of large quantities of blood, agreement to periodic blood collection at home after surgery, residence within reasonable driving distance from NIH. Sixty-six patients met the criteria and, in addition, provided sufficient serum samples (a mean of 10 samples per patient) for analysis; only two patients were rejected because of early cardiac death and/or insufficient serum samples. Informed consent was obtained from each patient and the study met all of the ethical criteria in place at the time. The demographic and clinical characteristics of the patients are listed in Table 1. Plasma or serum samples had been stored at −80°C.

Transfused blood

Blood was obtained from two commercial and two volunteer sources. Because of the large volume of blood required, it was distributed as needed and most patients received a mixture of commercial and volunteer blood. The majority of the blood transfused was from commercial sources.

Plasma

Two separate commercial lots of dried pooled NHP prepared contemporaneously with the study of the heart surgery patients were reconstituted, aliquoted and stored at −80°C since 1969. These were not administered to any of the patients but at least one was commercially distributed.

Tests

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured in serial sera from the patients as described previously.[15] A diagnosis of hepatitis was made when one or both enzymes exceeded 100 Karmen units (2.5 times the upper limit of normal) on one or more occasions and an elevation of higher than 80 units was demonstrated for 2 or more weeks between Week 2 and Week 26 after operation. Icteric hepatitis was diagnosed when clinically apparent jaundice or a total serum bilirubin level of more than 2 mg/dL was detected. Liver biopsies were not obtained.

The following tests for viral markers were employed: antibody to HAV (anti-HAV), ETI-AB-HAVK; hepatitis B surface antigen (HBsAg), ETI-MAK-4; antibody to HBsAg (anti-HBs), ETI-AB-AUK; antibody to hepatitis B core antigen (anti-HBc), ETI-AB-COREK; hepatitis D (delta) antigen (HDAg), ETI-DELTAK-2; and antibody to hepatitis D antigen (anti-HD), ETI-AB-DELTAK-2 were all measured by the listed commercial assays manufactured by DiaSorin (Saluggia, Italy). Antibody to HCV (anti-HCV) was measured by a commercial assay (HCV Version 3.0, manufactured by Ortho, Raritan, NJ). Antibody to HEV (anti-HEV) immunoglobulin (Ig)M and IgG were measured as described previously.[27,28] HAV and HDV RNA were measured by probe-based gene expression analysis (TaqMan, Applied Biosystems, Foster City, CA) with cycling conditions based on the manufacturer's recommendations; the forward and reverse primer and probe concentrations were 900, 900, and 200 (HAV) or 225 (HDV) nmol/L, respectively. For HAV, the primers and probe matched a region in VP1, with forward primer AGATGGCATGGCCTGGTTCA (Position 2690) and reverse complementary primer GTTCTCCTTGTGTTAAATCTGAC AGC (Position 2815) and probe (6-carboxyfluorescein [FAM]-TCTACCCAAGGAGTATCAACGGCAAGACCT-6-carb oxytetramethylrhodamine [TAMRA]). The quantification line was based on an in-house HAV standard. For HDV RNA, forward primer GACCCGAAGAGGAAAGAAGGA (Position 894), reverse complementary primer AGAGT TGTCGACCCCAGTGAATAA (Position 971) and MGB probe 6-FAM-CGAGACGCAAACCTGTGA (Position 917). A secondary standard was developed based on a plasmid generously provided by Dr John Taylor (Fox Chase Cancer Center, Philadelphia, PA). Sample total RNA for each HDV and HAV TaqMan procedure was extracted from 140 μL of serum. Reaction mixtures included 10 μL of RNA. The dynamic ranges for the tests were 3.7 to 8.7 (HDV) and 2.8 to 8.8 (HAV) log genome equivalents (GEQ)/mL. Negative and no-template controls were included in every test. HBV DNA, HCV RNA, and HEV RNA were measured by TaqMan as described previously.[29–32]

Genotyping of HBV and HCV strains

HBV genotypes were determined with a genotyping assay (INNO-LiPA HBV Multi-DR and genotype test, Innogenetics, Ghent, Belgium), according to the manufacturer's instructions. HCV genotypes were determined after amplification by reverse transcription–nested polymerase chain reaction and sequencing the C-E1 region as described previously.[33]

Algorithm and definitions for diagnosing hepatitis virus infection in heart surgery patients

Pretransfusion and approximately 6-month (when available) serum samples were screened for total anti-HBc, total anti-HCV, total anti-HAV, and total anti-HEV. Patients

seroconverting for anti-HBc were further screened for HBsAg, quantitative HBV DNA, and total anti-HBs to delineate the duration of infection and for anti-HDV and HDV RNA to assess for HDV infection. Patients seroconverting for anti-HCV were further screened for quantitative HCV RNA to determine the duration of infection. One patient with anti-HEV seroconversion was further screened for HEV RNA. There were no anti-HAV seroconversions. We used the following diagnostic criteria and definitions. 1) HBV infection—anti-HBc seroconversion, with or without HBsAg, anti-HBs; 2) HBV viremia—HBV DNA positive; 3) HBV acute infection—anti-HBs positive with loss of HBsAg, HBV DNA less than 6 months posttransfusion; 4) HBV chronic infection—HBsAg, HBV DNA positive more than 6 months posttransfusion; 5) HBV occult infection—anti-HBc positive with low, sporadic, or absent HBV DNA; 6) HBV reexposure—anti-HBs before transfusion with increasing titer after transfusion; 7) HCV infection—anti-HCV seroconversion; 8) HCV viremia—HCV RNA positive; 9) HCV acute infection—HCV RNA positive less than 6 months posttransfusion; 10) HCV chronic infection—HCV RNA positive more than 6 months posttransfusion; and 11) HCV reexposure—anti-HCV positive, HCV RNA negative before transfusion and RNA positive after transfusion.

RESULTS

Hepatitis

Thirty of the 66 patients (45%) developed biochemical evidence of hepatitis as defined under Materials and Methods (Table 2, Fig. 1). Thirteen (43%) of the 30 patients with hepatitis were icteric. Patients who developed hepatitis received a mean of 22.6 units of blood, 88% of which was commercial (Table 2). The patients who did not develop hepatitis received a mean of 19.8 units of blood, 75% of which was commercial. Overall, 1392 units of blood were transfused into 66 patients (mean, 21.1 units). Only four patients received predominantly volunteer blood and none of these developed hepatitis or were infected with a hepatitis virus.

Infections with HBV and HCV

Of the 30 patients with hepatitis, 20 (67%) were acutely infected with HCV alone, four (13%) were acutely infected with HBV alone (as determined by HCV RNA and HBV DNA TaqMan, respectively), and six (20%) were infected with both HCV and HBV (Table 3, Fig. 1). Among the 36 patients who did not have biochemical evidence of hepatitis, four (11%) were infected with HCV alone, nine (25%) were infected with HBV alone, and one (3%) was infected with both viruses. Thus, in the total population of 66 transfused patients, 31 (47%) were infected with HCV and 20 (30%) were infected with HBV. Amazingly, even the two patients rejected from the study for insufficient serum samples were infected with HCV but could not be further evaluated. One HCV infection among the 66 evaluated patients was in a nonhepatitis patient who had antibody to HCV before transfusion and thus represented a reinfection. Two hepatitis patients and three nonhepatitis patients had anamnestic antibody responses to HBV, indicating reexposure to this virus. Of these, one patient in the hepatitis group (also infected with HCV) and two in the nonhepatitis group were reinfected with HBV, as evidenced by viremia (the remaining two were considered to have had exposures to

HBV, but not infections). Thus, a total of 10 hepatitis patients and 10 nonhepatitis patients were infected with HBV.

Viral genomic RNA was recovered from all 31 HCV-infected patients. Acute-phase virus titers ranged from $10^{4.2}$ to $10^{8.0}$ IU/mL (median, $10^{6.3}$; geometric mean, $10^{6.2}$). Twenty-five of the patients were infected with HCV Genotype 1b, four were infected with Genotype 1a, and two were infected with Genotype 2a (Fig. 2). This distribution of genotypes is similar to the distribution of genotypes today. There was no clustering of genomic sequences within genotypes and no particular relationship between genotype and date of infection during the 3.5 years of the study. HBV was present in sufficient titer to Genotype 12 of the isolates. Eleven were Genotype A and one was Genotype D; these continue to be relatively common HBV genotypes in the United States.

Thus, all 30 of the hepatitis patients were infected with HBV and/or HCV and 14 (39%) of the patients without hepatitis were infected with one or both of these viruses. Overall, two-thirds of the heart surgery patients were infected with HBV and/or HCV. Primary HBV monoinfections resulted in hepatitis less than half as frequently as comparable HCV infections (36% vs. 83%), but the difference was not significant ($p = 0.39$). However, when HBV caused hepatitis, it was twice as likely to be icteric. In contrast, HCV infections were 1.8 times more likely to be anicteric than icteric. All five of the patients with primary dual infections of HBV and HCV developed hepatitis. At least 17 HBV infections were acute and self-limiting, one became chronic, and two were of indeterminate duration, a ratio one would expect for infection of adults (Table 4). In contrast, 11 HCV infections resolved and 15 became chronic (the duration of five infections could not be determined).

Of eight patients with dual HBV and HCV exposures, one was an HBV reexposure with an anamnestic response but no viremia. Among the other seven, one did not develop hepatitis, but HCV in this patient was a reinfection, which may have modified the clinical response. The remaining six all developed hepatitis. Based on ALT patterns, four had monophasic hepatitis (one was an HBV reinfection) and two had biphasic hepatitis. One of the latter patients had been monitored previously for “hepatitis-associated antigen” (HBsAg) by complement fixation (Fig. 3) and it was suggested at the time that the data were consistent with infection with two hepatitis viruses, although the identity of the second was not known.[22] This was among the earliest evidence that another transfusion-associated virus (“non-A, non-B hepatitis virus”) existed. Herein we confirm the presence of two hepatitis viruses and identify the second as HCV.

Other hepatitis virus infections

Of the patients who could be evaluated, 75% (44/59) had existing antibody to HAV and 29% (18/62) had antibody to HEV. There were no seroconversions or anamnestic responses to HAV, but several patients did acquire passively transfused antibody that was detectable for up to 6 months. One nonhepatitis patient, seronegative before transfusion, had a brisk IgG anti-HEV response in the absence of IgM anti-HEV. In addition, the patient was transiently positive for HEV RNA (Genotype 3) 4 weeks after transfusion. The patient was also coinfected with HBV. None of the patients infected with HBV had evidence of coinfection

with HDV. We did not detect any acute hepatitis cases that could not be diagnosed as hepatitis B, C, or E.

Plasma

Both commercial lots of plasma were positive for HBsAg and anti-HBc but not anti-HBs (Table 5). One was also positive for HBV DNA ($10^{5.4}$ IU/mL) and was also weakly positive for HDV RNA ($10^{2.1}$ GEQ/mL). They were both negative for HDAg, but one was positive for anti-HD. Both lots were also positive for anti-HCV and HCV RNA ($10^{3.5}$ and $10^{4.1}$ IU/mL, respectively). Both lots were positive for anti-HAV and one was also positive for anti-HEV. They were negative for HEV RNA.

DISCUSSION

Hepatitis associated with the transfusion of blood and blood products was well recognized 50 years ago. A number of large prospective studies of transfused patients in the United States found incidence rates of 9% to 18% and calculated donor carrier rates of 1% to 9%.[5,9,12,14] However, the most sensitive tests for detecting the various hepatitis viruses were developed after populations of donors had changed markedly from the unscreened populations of 50 years ago. This study is an attempt to link cutting-edge tests for viral markers with a virgin population of blood recipients whose serum had been stored frozen for approximately 50 years. The results revealed a surprisingly high rate of exposure to HBV and HCV: every one of the 30 patients with hepatitis was exposed to one or both of these viruses, but so were 15 of the 36 patients who did not develop hepatitis. Thus, 45 (68%) of the patients had a total of 53 exposures to HBV and/or HCV. Based on the calculations proposed by Senior,[9] the donor carrier rate was approximately 3% for all hepatitis, 2% for HBV, 3% for HCV, and 6% for either or both viruses. For comparison, carrier rates among current blood donors are 0.012 and 0.032% for HBV and HCV, respectively,[34] and the risk of HBV and HCV transmission is less than one in 500,000 and one in 2 million transfused units, respectively, for these two viruses.

Six patients had existing antibody to HBV. Five of these had anamnestic responses after transfusion and three (60%) were reinfected, as measured by transient viremia. Thus, prior infection with HBV in this group did not provide sterilizing immunity: the three patients with the lowest pretransfusion anti-HBs values (23, 568, and 1488 mIU/mL) were all viremic whereas the two with higher values (10,880 and 26,112) were not viremic.

Six patients had irregular expression or consistently low-level expression of HBV markers during the observation period. It is probably not coincidental that five of the six patients were coinfected with HCV, since an HCV infection, which stimulates a strong innate immune response,[35,36] can suppress viral replication of a coincident HBV infection.[37] Suppression of HBV replication in the occult hepatitis B case without coincident HCV infection could conceivably have been caused by a putative non-A, non-E hepatitis virus infection. No non-A, non-E infections were detected in this study, although a number of unexplained liver enzyme elevations were detected in subsequent studies of transfused patients at the NIH.[38] Their etiology remains unknown. We did not search for HGV/GBV-C

(Pegiviruses) or TTV (Anelloviruses), both originally thought to be hepatitis viruses; their role in viral hepatitis has been challenged.[38,39]

We did not detect HDV among the many HBV infections. This study preceded the peak of HDV transmission among HBV-infected populations, and thus, this population may have been spared. However, one of the lots of contemporaneously prepared commercial NHP contained HDV, as well as anti-HD. This may be the earliest detection of HDV in the United States, if not elsewhere.

The detection of a case of transfusion-associated HEV infection was surprising. This is probably the earliest HEV infection to be identified in the United States and the first transfusion-associated case to be reported in this country, although a few cases of transfusion-associated HEV infection have been reported from Asia and Europe.[40,41] The virus was typed as a Genotype 3, one of the two types (Genotype 4 being the other) that regularly infect swine and can be transmitted to humans. Genotype 3 is the only swine HEV genotype regularly found in the United States. Although common in most herds of domestic swine, it is only occasionally recovered from human cases of hepatitis in the United States.[42,43] This pattern of HEV viremia, plus a brisk IgG (but absent IgM) anti-HEV response suggests an HEV reinfection, a phenomenon suspected but not fully documented elsewhere.

We have confirmed the presence of multiple hepatitis viruses in pooled NHP as it was prepared more than 50 years ago. Both lots contained HCV and one also contained HBV and HDV. This lot was withdrawn in 1961 by the CDC because of its association with eight cases of hepatitis, including four deaths, despite its having been irradiated. The severity of the hepatitis may have resulted from coinfection with two or more viruses, including HDV. Our analysis extends the finding of HBsAg in 30-year-old lyophilized NHP reported in 1977.[44]

In conclusion, TAH among heart surgery patients at the NIH has been a surrogate and predictor of such disease in the larger arena of public health for the past 50 years. From an incidence of approximately 30% among recipients receiving approximately 20 units of blood and approximately 50% among those receiving blood predominantly from commercial sources, TAH has diminished with each introduced intervention. The last case of transfusion-transmitted HBV infection at the NIH was recorded between 1981 and 1985. The last case of HCV was recorded between 1990 and 1992. Surprisingly, the last case of TAH at the NIH occurred between 1992 and 1994 and was classified by exclusion as a case of non-A, non-E hepatitis. To the present, we do not know what those presumed non-A, non-E cases represent, but in retrospect the transaminase elevations may have been related to medication or surgery or to the effect of steatohepatitis. No established human non-A, non-E hepatitis agent has been identified in the past two decades despite intensive efforts aimed at their discovery and the retrospective virologic analysis presented herein provides no evidence to support the existence of a non-A, non-E agent. The absence of a single identifiable case of TAH in prospectively followed recipients at the NIH over the past two decades highlights the extraordinary success of successive interventions in donor screening

to control blood-borne hepatitis agents in the United States and other industrialized countries.

In summary, we have provided the first complete analysis of transfusion-associated viral hepatitis as it occurred at the NIH before the screening of blood for hepatitis risk factors. We have shown that HBV and HCV accounted for virtually all of the TAH in these patients and that the viruses were more prevalent than recognized at the time. This is the earliest example of HCV and of HCV sequences in the United States and it demonstrates that the same genotypes of HCV and HBV were circulating then as now. We report the earliest example of HEV in the United States and the only transfusion-associated case of HEV infection in the United States reported to date and demonstrate that the virus was Genotype 3, the most common genotype infecting humans and pigs in the United States. Further, we demonstrate that exposure to HEV was common among this population as early as the 1960s with anti-HEV prevalence approaching 30%. We report the earliest example of HDV in the United States and probably elsewhere and show that NHP implicated in highly fatal blood-borne hepatitis contained HBV, HCV, and HDV, suggesting that its virulence was related to multiple simultaneous infections with hepatitis viruses. We show that occult hepatitis B among blood recipients was associated with coinfection with HCV in most cases. We could not detect additional hepatitis agents beyond the presently recognized five. Finally, we have contrasted the serious health risk of transfusion before the screening of blood with its extraordinary safety today.

Acknowledgments

The HCV and HEV RNA sequences described herein have been deposited in GenBank under Accession Numbers KJ605354, KJ605355, KJ605356, KJ605357, KJ605358, KJ605359, KJ605360, KJ605361, KJ605362, KJ605363, KJ605364, KJ605365, KJ605366, KJ605367, KJ605368, KJ605369, KJ605370, KJ605371, KJ605372, KJ605373, KJ605374, KJ605375, KJ605376, KJ605377, KJ605378, KJ605379, KJ605380, KJ605381, KJ605382, KJ605383, KJ605384.

This work was supported in part by the Intramural Research Programs of the Clinical Center Department of Transfusion Medicine and the National Institute of Allergy and Infectious Diseases.

ABBREVIATIONS

HDAg	hepatitis D (delta) antigen
NHP	normal human plasma
TAH	transfusion-associated hepatitis

References

1. MacCollum FO. Homologous serum hepatitis. Lancet. 1947; 2:691.
2. Allen JG, Sayman WA. Serum hepatitis from transfusions of blood. Epidemiologic study. JAMA. 1962; 180:1079–85. [PubMed: 13860548]
3. Grady GF, Chalmers TC. Risk of post-transfusion viral hepatitis. N Engl J Med. 1964; 271:337–42. [PubMed: 14171801]
4. Kunin CM. Serum hepatitis from whole blood: incidence and relation to source of blood. Am J Med Sci. 1959; 237:293–303. [PubMed: 13626970]

5. Mirick GS, Ward R, McCollum RW. Modification of post-transfusion hepatitis by gamma globulin. N Engl J Med. 1965; 273:59–65. [PubMed: 14301199]
6. Shimizu Y, Kitamoto O. The incidence of viral hepatitis after blood transfusions. Gastroenterology. 1963; 44:740–4. [PubMed: 13988539]
7. Hampers CL, Prager D, Senior JR. Post-transfusion anicteric hepatitis. N Engl J Med. 1964; 271:749–54. [PubMed: 14186196]
8. Karmen A, Wroblewski F, Ladue JS. Transaminase activity in human blood. J Clin Invest. 1955; 34:126–31. [PubMed: 13221663]
9. Senior JR. Reflections upon the incidence of posttransfusion hepatitis in various parts of the world. Am J Gastroenterol. 1968; 49:298–303. [PubMed: 5658857]
10. Aach RD, Alter HJ, Hollinger FB, et al. Risk of transfusing blood containing antibody to hepatitis-B surface antigen. Lancet. 1974; 2:190–3. [PubMed: 4135609]
11. Gocke DJ. A prospective study of posttransfusion hepatitis. The role of Australia antigen. JAMA. 1972; 219:1165–70. [PubMed: 5066869]
12. Grady GF, Bennett AJ. Risk of posttransfusion hepatitis in the United States. A prospective cooperative study. JAMA. 1972; 220:692–701. [PubMed: 5067145]
13. Knodell RG, Conrad ME, Ginsberg AL, et al. Efficacy of pro-phylactic gamma-globulin in preventing non-A, non-B post-transfusion hepatitis. Lancet. 1976; 1:557–61. [PubMed: 55838]
14. Seeff LB, Zimmerman HJ, Wright EC, et al. A randomized, double blind controlled trial of the efficacy of immune serum globulin for the prevention of post-transfusion hepatitis. A Veterans Administration cooperative study. Gastroenterology. 1977; 72:111–21. [PubMed: 318578]
15. Walsh JH, Purcell RH, Morrow AG, et al. Posttransfusion hepatitis after open-heart operations. Incidence after the administration of blood from commercial and volunteer donor populations. JAMA. 1970; 211:261–5. [PubMed: 5466902]
16. Alter HJ, Holland PV, Morrow AG, et al. Clinical and serological analysis of transfusion-associated hepatitis. Lancet. 1975; 2:838–41. [PubMed: 53329]
17. Alter HJ, Holland PV, Purcell RH, et al. Posttransfusion hepatitis after exclusion of commercial and hepatitis-B antigen-positive donors. Ann Intern Med. 1972; 77:691–9. [PubMed: 4628213]
18. Alter HJ, Purcell RH, Holland PV, et al. Donor transaminase and recipient hepatitis. Impact on blood transfusion services. JAMA. 1981; 246:630–4. [PubMed: 6788964]
19. Alter HJ, Purcell RH, Shih JW, et al. Detection of antibody to hepatitis C virus in prospectively followed transfusion recipients with acute and chronic non-A, non-B hepatitis. N Engl J Med. 1989; 321:1494–500. [PubMed: 2509915]
20. Holland PV, Walsh JH, Morrow AG, et al. Failure of Australia antibody to prevent post-transfusion hepatitis. Lancet. 1969; 2:553–5. [PubMed: 4185530]
21. Koziol DE, Holland PV, Alling DW, et al. Antibody to hepatitis B core antigen as a paradoxical marker for non-A, non-B hepatitis agents in donated blood. Ann Intern Med. 1986; 104:488–95. [PubMed: 3006567]
22. Purcell RH, Walsh JH, Holland PV, et al. Seroepidemiological studies of transfusion-associated hepatitis. J Infect Dis. 1971; 123:406–13. [PubMed: 4329346]
23. Allen JG, Sykes C, Enerson DM, et al. Homologous serum jaundice and its relationship to methods of plasma storage. J Lab Clin Med. 1950; 36:796–7.
24. Sayman WA, Gauld RL, Star SA, et al. Safety of liquid plasma: a statistical appraisal. J Am Med Assoc. 1958; 168:1735–9. [PubMed: 13587253]
25. Redeker AG, Hopkins CE, Jackson B, et al. A controlled study of the safety of pooled plasma stored in the liquid state at 30–32 C for six months. Transfusion. 1968; 8:60–4. [PubMed: 5643633]
26. Committee on Plasma and Plasma Substitutes of the Division of Medical Sciences NRC. Statement on normal (whole, pooled) human plasma prepared by Committee on Plasma and Plasma Substitutes of the Division of Medical Sciences, National Research Council. Transfusion. 1968; 8:57–9. [PubMed: 5643632]

27. Engle RE, Yu C, Emerson SU, et al. Hepatitis E virus (HEV) capsid antigens derived from viruses of human and swine origin are equally efficient for detecting anti-HEV by enzyme immunoassay. J Clin Microbiol. 2002; 40:4576–80. [PubMed: 12454155]
28. Yu C, Engle RE, Bryan JP, et al. Detection of immunoglobulin M antibodies to hepatitis E virus by class capture enzyme immunoassay. Clin Diagn Lab Immunol. 2003; 10:579–86. [PubMed: 12853389]
29. Engle RE, Russell RS, Purcell RH, et al. Development of a TaqMan assay for the six major genotypes of hepatitis C virus: comparison with commercial assays. J Med Virol. 2008; 80:72–9. [PubMed: 18041021]
30. Payette PJ, Ma X, Weeratna RD, et al. Testing of CpG-optimized protein and DNA vaccines against the hepatitis B virus in chimpanzees for immunogenicity and protection from challenge. Intervirology. 2006; 49:144–51. [PubMed: 16428890]
31. Shukla P, Nguyen HT, Torian U, et al. Cross-species infections of cultured cells by hepatitis E virus and discovery of an infectious virus-host recombinant. Proc Natl Acad Sci U S A. 2011; 108:2438–43. [PubMed: 21262830]
32. Yu C, Boon D, McDonald SL, et al. Pathogenesis of hepatitis E virus and hepatitis C virus in chimpanzees: similarities and differences. J Virol. 2010; 84:11264–78. [PubMed: 20739520]
33. Corbet S, Bukh J, Heinsen A, et al. Hepatitis C virus subtyping by a core-envelope 1-based reverse transcriptase PCR assay with sequencing and its use in determining subtype distribution among Danish patients. J Clin Microbiol. 2003; 41:1091–100. [PubMed: 12624035]
34. Dorsey KA, Moritz ED, Steele WR, et al. A comparison of human immunodeficiency virus, hepatitis C virus, hepatitis B virus, and human T-lymphotropic virus marker rates for directed versus volunteer blood donations to the American Red Cross during 2005 to 2010. Transfusion. 2013; 53:1250–6. [PubMed: 23003320]
35. Bigger CB, Brasky KM, Lanford RE. DNA microarray analysis of chimpanzee liver during acute resolving hepatitis C virus infection. J Virol. 2001; 75:7059–66. [PubMed: 11435586]
36. Su AI, Pezacki JP, Wodicka L, et al. Genomic analysis of the host response to hepatitis C virus infection. Proc Natl Acad Sci U S A. 2002; 99:15669–74. [PubMed: 12441396]
37. Dienes HP, Purcell RH, Popper H, et al. The significance of infections with two types of viral hepatitis demonstrated by histologic features in chimpanzees. J Hepatol. 1990; 10:77–84. [PubMed: 2106549]
38. Alter HJ, Nakatsuji Y, Melpolder J, et al. The incidence of transfusion-associated hepatitis G virus infection and its relation to liver disease. N Engl J Med. 1997; 336:747–54. [PubMed: 9052652]
39. Matsumoto A, Yeo AE, Shih JW, et al. Transfusion-associated TT virus infection and its relationship to liver disease. Hepatology. 1999; 30:283–8. [PubMed: 10385668]
40. Boxall E, Herborn A, Kochethu G, et al. Transfusion-transmitted hepatitis E in a “nonhyperendemic” country. Transfus Med. 2006; 16:79–83. [PubMed: 16623913]
41. Matsubayashi K, Kang JH, Sakata H, et al. A case of transfusion-transmitted hepatitis E caused by blood from a donor infected with hepatitis E virus via zoonotic food-borne route. Transfusion. 2008; 48:1368–75. [PubMed: 18651907]
42. Davern TJ, Chalasani N, Fontana RJ, et al. Drug-Induced Liver Injury Network. Acute hepatitis E infection accounts for some cases of suspected drug-induced liver injury. Gastroenterology. 2011; 141:1665–72. e1–9. [PubMed: 21855518]
43. Meng XJ, Purcell RH, Halbur PG, et al. A novel virus in swine is closely related to the human hepatitis E virus. Proc Natl Acad Sci U S A. 1997; 94:9860–5. [PubMed: 9275216]
44. Fu P, Myhre B. Chemical analysis of a 30-year-old bottle of lyophilized plasma. Transfusion. 1977; 17:73–4. [PubMed: 841680]

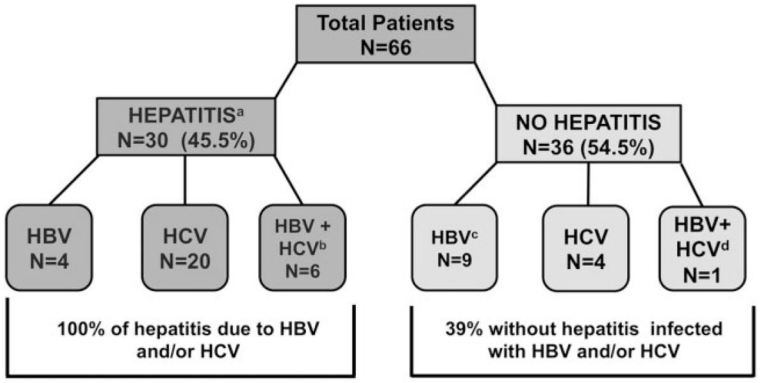

Fig. 1.

Hepatitis outcome in 66 prospectively followed patients transfused with unscreened donor blood. [a]Hepatitis based on ALT elevations meeting study criteria; [b]one HBV reinfection; [c]two HBV reinfections, one HEV coinfection; [d]one HCV reinfection.

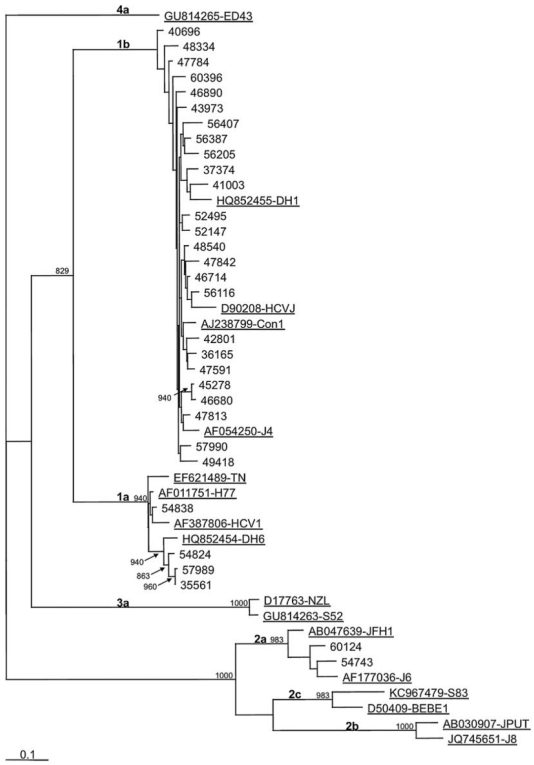

Fig. 2.

Maximum likelihood phylogenetic tree of 31 HCV nucleotide sequences (indicated by five-digit sample numbers), comprising partial core and E1 genomic regions (424 nucleotides), constructed using PHYML with nucleotide substitution model Kimura two-parameter. Reference sequences (strain name and GenBank Accession Number) for Genotypes 1a, 1b, 2a, 2b, 2c, 3a, and 4a are underlined. The tree was rooted using ED43 (4a) sequence as an outgroup. Support values of branch nodes were estimated by bootstrap analysis using 1000 replicates; only values of more than 850 are depicted. The indicated scale bar represents 0.1 substitutions per nucleotide position.

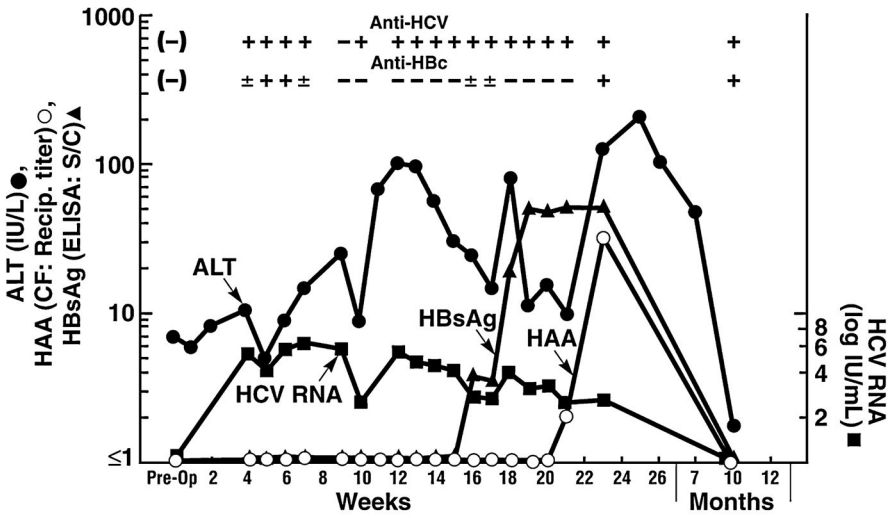

Fig. 3.

Coinfection with HBV and HCV. +, − = anti-HCV and anti-HBc (passively acquired, followed by actively acquired); HAA = CF titer of HBsAg (modified from Purcell et al.[22]). AST values were replaced with ALT values and converted from Karmen units to IU/L.

TABLE 1

Demographic characteristics of transfused patients*

Variable	
Sex	
Male	39 (59.1)
Female	27 (40.9)
Race	
White	42 (63.6)
Black	2 (3.0)
Unknown	22 (33.3)
Operation	
Valve repair	47 (71.2)
Atrial septal defect repair	10 (15.2)
Other	6 (9.1)
Unknown	3 (4.5)
Age (years)	47.3 (21–68)
Number of blood units transfused	21.1 (12–62)

*Data are reported as number (%) or mean (range).

TABLE 2

Hepatitis in transfused patients: relationship to source and volume of blood

		Number of units transfused, by source			
Patient outcome	**Number of patients**	**Commercial**	**Volunteer**	**Total**	**Units/patient**
Hepatitis	30	597	81	678	22.6
No hepatitis	36	531	183	714	19.8
Total	66	1128	264	1392	21.1

TABLE 3

HBV and HCV infections in transfused patients*

		Infections					
Patient outcome	**Number of patients**	**HBV only**	**HCV only**	**Both**	**All HBV**	**All HCV**	**Total**
Hepatitis†	30	4	20	6	10	26	36
No hepatitis	36	9	4	1	10	5	15
Total	66	13	24	7	20	31	51

*HBV infection was defined by positive HBsAg or anti-HBc (total) after transfusion. HCV infection was defined by positive anti-HCV after transfusion.

†Defined by transferase elevations meeting study criteria (see Materials and Methods).

TABLE 4

Duration of hepatitis virus infection in transfused patients

		Outcomes of viral infection		
Virus	**No.**	**Acute**	**Chronic**	**Indeterminate***
HBV	20	17	1	2
HCV	31	11	15	5

* Patients were followed less than 6 months.

TABLE 5

Biomarkers of hepatitis viruses in pooled NHP

	Biomarker											
				HBV		HCV			HDV			HEV
Plasma lot	HBsAg	Anti-HBc	Anti-HBs	DNA	Anti-HCV	RNA	HDAg	Anti-HD	RNA	Anti-HAV	Anti-HEV	RNA
1	+	+	−	5.4*	+	3.5	−	+	2.1	+	+	−
2	+	+	−	−	+	4.1	−	−	−	+	−	−

*Log IU or GEQ/mL.