Rationale for Surrogate Testing to Detect Non-A, Non-B Hepatitis

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THE TERM non-A, non-B (NANB) hepatitis was introduced in the mid-1970's to classify an illness of presumed viral etiology that occurred following blood transfusion.[1-3] Serologic studies eliminated other viral causes of hepatitis such as hepatitis A, hepatitis B, cytomegalovirus, and Epstein-Barr virus as the etiologic agent. The transfusion was the presumed vector of transmission.

Approximately a dozen years later, characterization of the precise agent remains elusive. However, evidence accumulated during this period indicates that it is prudent to routinely test units of donated blood with nonspecific or surrogate tests in an effort to reduce the occurrence of NANB posttransfusion hepatitis. The rational for this decision is based on published studies showing 1. infection is transmitted by blood transfusion; 2. infection is associated with illness in some patients; and 3. nonspecific tests identify a subset of donors whose blood is considered infectious. The clinical features of NANB hepatitis and the reasons that persuaded blood banking organizations in the United States to adopt policies requiring alanine aminotransferase (ALT) and antihepatitis B core (antiHB$_c$) testing of all blood intended for homologous transfusion will be reviewed.

CLINICAL FEATURES OF NANB HEPATITIS

Etiology

The agent(s) responsible for NANB hepatitis remains unidentified despite scores of preliminary leads that proved unrevealing.[4] Evidence suggesting that NANB hepatitis is caused by more than one virus relates to observations of multiple attacks of hepatitis in chronically transfused patients including hemophilic patients, different patterns of ALT elevations in infected patients, cross challenge studies in experimentally infected chimpanzees, varying ultrastructure changes observed in liver biopsy specimens of infected subjects and multiple episodes of hepatitis in chimpanzees inoculated with infectious material treated by chemical or physical agents of varying sensitivities.[5-7] However, acceptance that multiple agents are involved is not universal.[8]

It is likely that this issue will remain unresolved for the time being, in part, because the usefulness of the chimpanzee model has been questioned. Apparently NANB hepatitis occurred in humans following transfusion of heat treated factor VIII concentrates that did not cause hepatitis in chimpanzees receiving the same preparation.[9] A water borne epidemic form of NANB hepatitis has also been described.

Clinical Features

Signs and symptoms associated with NANB hepatitis are generally less severe than those associated with hepatitis B. Symptoms are reported in roughly one-half of the infected patients and consist of fatigue, anorexia, nausea and/or vomiting, abdominal pain and weight loss. The majority of cases occur 7 to 8 weeks after transfusion, but there is considerable variation. Approximately 25% of patients with NANB hepatitis become icteric. ALT levels are elevated approximately 20 times greater than the upper limit of normal (range 2 to 82 times). Fewer than one-third of patients have ALT levels in excess of 800 International Units per liter (IU) and the bilirubin is usually lower than 10 mg/dL. The acute illness may persist for up to 10 weeks.[10-13] Extra hepatic manifestations associated with hepatitis B occur infrequently with NANB hepatitis.

A disturbing feature of this illness is that it becomes chronic in at least 50% of patients infected through transfusion.[10,11,14-22] ALT elevations persist or fluctuate. Among patients with chronic ALT elevations who undergo liver biopsy, it is not unusual to find evidence of chronic active hepatitis or cirrhosis.

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0887-7963/88/0202-0001$03.00/0

Modes of Transmission

NANB hepatitis represents 20% to 40% of acute viral hepatitis cases. Approximately 16% of NANB hepatitis cases occur in transfusion recipients, 26% in intravenous (IV) drug abusers, 1% among health care workers with blood contact, 1% among those in hemodialysis units, 1% among persons having contact with someone who has NANB hepatitis, and in 55% the route of exposure is unknown.[4,13,23] Currently 90% of posttransfusion hepatitis is thought to be caused by NANB hepatitis. Parenteral transmission is considered the route of transmission among IV drug abusers and health care workers.[4,13] Nonparenteral routes of transmission are suspected, but documentation is circumstantial. Sexual transmission probably occurs, but this mechanism is inefficient and uncommon.[4] Because 15% to 30% of sporadic cases of acute hepatitis are considered NANB and since many of those affected provide no history of percutaneous exposure, it is suspected that NANB hepatitis transmission occurs by nonpercutaneous and covert percutaneous routes.[4]

NANB HEPATITIS ATTRIBUTED TO BLOOD TRANSFUSION

Reliable tests for hepatitis B surface antigen (HB_sAg) and elimination of paid blood donors resulted in a dramatic decline of posttransfusion hepatitis B cases in the early 1970s.[24] Subsequently, when a marker for detecting hepatitis A became available, it was evident that up to 90% of posttransfusion hepatitis was caused by an infectious agent other than hepatitis A or hepatitis B virus, ie, NANB hepatitis.[2,3,25]

Two prospective studies were conducted in the United States during the 1970s to determine the incidence of hepatitis and to evaluate the role of potentially useful donor screening procedures for decreasing the occurrence of this illness.

One, the Transfusion Transmitted Virus (TTV) Study was a multicenter cooperative investigation sponsored by the National Heart, Lung, and Blood Institute.[26-28] The other evaluated transfused patients who underwent open heart surgery at the National Institute of Health (NIH) Clinical Center.[22,29] Hepatitis developed in 10.3% to 12.7% of the recipients, respectively. The attack rate varied significantly among the participating centers in the TTV Study. The rate was 7.9% in New York, 4.3% in St. Louis, 17.4% in Houston and 15.5% in Los Angeles. Overall, 10.2% of recipients developed NANB hepatitis posttransfusion. A control group consisting of patients for whom blood was ordered but not transfused had a 2.9% rate of NANB hepatitis resulting in a net 7.3% incidence of posttransfusion NANB hepatitis.[27] A 7.3% incidence of NANB posttransfusion hepatitis was reported at the NIH.[22] The background incidence of hepatitis in the nontransfused population at the NIH was .5% Hence, during the 1970s, approximately 7% of transfused patients developed hepatitis that could be attributed to receiving homologous blood. NANB hepatitis accounted for 91% of posttransfusion hepatitis cases in the TTV study and 76% to 97% of those in the NIH reports.

It should be noted that these large prospective studies were completed more than seven years ago. Since that time, significant changes affecting blood donor qualifications occurred in the United States. Specifically, those at high risk for the Acquired Immunodeficiency Syndrome (AIDS) were asked to self defer from donating blood. Presumably this includes some who are also at risk for transmitting hepatitis. Hence, current data for estimating the risk of NANB posttransfusion hepatitis are not available. It is estimated that 2% of transfusion survivors develop acute NANB posttransfusion hepatitis annually in the United States.[23] This compares with a .11% incidence of NANB posttransfusion hepatitis reported by the American Association of Blood Banks-College of American Pathologists (AABB-CAP) survey in 1985. The latter statistic is undoubtedly influenced by under reporting, incomplete follow-up, lack of symptoms in the majority of cases, etc.

Prospectively designed investigations similar to the TTV and National Institute of Health (NIH) studies were performed in Europe, Australia and Asia during the 1980's. The incidence of NANB posttransfusion hepatitis was 3.4% among 380 recipients in the Netherlands where 87% of the posttransfusion hepatitis detected was attributed to NANB.[30] In Sidney, Australia, 78% of posttransfusion hepatitis was NANB involving 16.6% of 842 cardiac surgery patients studied.[31] Of 65 open heart surgery patients followed in Finland, three (4.6%) were found to develop hepatitis; all NANB.[32] Among 246 open heart surgery recipients of blood from volunteer donors in Pa-

dova, Italy 13.8% developed hepatitis (84% NANB).[33] Two reports from Spain indicate a 12.6% and a 10.7% incidence of NANB posttransfusion hepatitis.[20,34] The incidence was 8% among 50 cardiac surgery patients in Jerusalem, Israel 6.3% of 64 cardiac surgery patients in Nancy, France;[35] and 10.7% in Japan.[36] It is interesting to note that blood was screened for aspartic amino transferase (AST) and HB_sAg in the latter study.

Overall, 3% to 16% of blood transfusion recipients studied in the United States and parts of Europe, Australia, and Asia developed NANB posttransfusion hepatitis.

DOES INFECTION WITH NANB HEPATITIS RESULT IN SIGNIFICANT DISEASE?

Shortly after NANB hepatitis became a recognized entity, elevated transaminase levels were noted in affected patients for as long as 6 months to a year following the acute illness. There was considerable debate whether this was a herald of serious illness or merely chronic persistent hepatitis that was not clinically detrimental. At the current time, most believe at least 50% of patients with acute NANB hepatitis who have chronically elevated transaminase levels are also associated with serious liver disease including cirrhosis (Table 1).

Transfusion Recipients

Knodell, et al prospectively followed 44 patients at the Walter Reed and Letterman Army Medical Centers who developed NANB posttransfusion hepatitis during the mid-1970s.[14] Ten (23%) had abnormalities of liver enzymes consistent with chronic hepatitis 12 to 36 months after the acute illness. Liver biopsy was performed in each of these patients; one showed cirrhosis, one chronic, persistent hepatitis, and eight chronic active hepatitis. That is, nine of 44 (20%) had evidence of significant liver disease. Berman, et al observed 26 patients at the NIH Clinical Center for at least one year following an acute episode of NANB posttransfusion hepatitis. Twelve (46%) had evidence of chronicity. Liver biopsy was performed in eight patients, and six showed chronic active hepatitis including one with early cirrhosis; at least six of 26 (23%) had significant liver disease.[10] Hernandez, et al prospectively followed 29 patients who developed NANB posttransfusion hepatitis in Barcelona, Spain.[20] Sixteen (55%) had transaminase elevations after 1 year. Two patients underwent liver biopsy, and one had chronic, active hepatitis.

Koretz, et al prospectively followed patients transfused in Los Angeles between 1972 and 1983.[21] Sixty-nine patients with NANB posttransfusion hepatitis were identified. They found an 82% incidence of chronic hepatitis in patients transfused before 1976 compared with 29% among patients transfused after 1976 when only volunteer donor blood was used. Twenty-one patients underwent liver biopsy; four had cirrhosis. Hence, cirrhosis occurred in at least four of 69 (6%) patients. Two died with complications of liver failure.

Alter and Hoofnagle prospectively followed 75 patients who developed NANB posttransfusion hepatitis at the NIH Clinical Center.[37] Sixty-eight percent had ALT elevations for more than 1 year after transfusion. Thirty-two were selected for liver biopsy on the basis of clinical symptoms or laboratory abnormalities. Of the 32, 69% had chronic active hepatitis, 9% cirrhosis, 3% nonspecific lesions, and 19% chronic persistent hepatitis. Thirteen patients underwent repeat liver biopsy 1 to 3 years following the first. Histologic evidence of improvement occurred in 46%, 15% were stable, but 39% had evidence of deterioration. One patient developed more severe chronic active hepatitis, and four developed cirrhosis. Overall, cirrhosis developed in seven (9%) of the 75 patients enrolled in the study. Two were severely incapacitated by hepatic insufficiency.

Table 1. NANB Posttransfusion Hepatitis: Long-Term Sequelae

Patients with NANB Post-transfusion Hepatitis	Patients with Prolonged Liver Function Abnormality	Biopsy Evidence of Chronic Active Hepatitis/ Cirrhosis	Reference
44	10 (23%)	20%	14
26	12 (46%)	23%	10
29	16 (55%)	3%	20
69	46 (67%)	6%	21
75	51 (68%)	33%	37
13	7 (54%)	31%	15
15	6 (40%)	27%	16
21	14 (67%)	48%	19
70	32 (46%)	7%	17
Hemophilic Patients			
79	—	22%	39
115	—	22%	40

NOTES: Selected patients only underwent biopsy.

There are several published reports of long term sequelae of patients with acute NANB posttransfusion hepatitis who were followed after resolution of initial symptoms. Rakela, et al observed 13 patients in southern California with NANB posttransfusion hepatitis.[15] Seven (54%) developed chronic hepatitis. Liver biopsy was performed in six; four had chronic active hepatitis. One of these patients died with hepatic failure 42 months after acute hepatitis.

Of 15 patients with NANB posttransfusion hepatitis in Catania, Italy in the late 1970s, six (40%) were found to have persistence of clinical and serologic abnormalities during an 18 month follow up.[16] Four of the six had changes consistent with chronic active hepatitis on liver biopsy. In another Italian study, 21 patients were observed in Padova for up to 5 years, 14 developed chronic hepatitis including five with biopsy evidence of chronic active hepatitis and five with biopsy evidence of cirrhosis.[19]

A follow-up study of 70 patients with acute NANB hepatitis in Japan found elevated transaminase levels for more than 6 months in 66% and 1 year in 46%.[17] Fourteen patients underwent liver biopsy. Five had chronic active hepatitis.

Hence, the cumulative evidence indicates that 23-68% of patients with acute hepatitis develop biochemical changes indicating chronicity. The liver biopsy reports are more difficult to interpret. It is presumed that there is a bias toward selecting the sickest patients in each study for this invasive procedure. Nevertheless, they indicate that perhaps as many as 10% of transfused patients develop cirrhosis and chronic active hepatitis as a long-term complication of transfusion.

Hemophilic Patients

Hemophilic patients have a high incidence of liver function test abnormalities presumably as a result of hepatitis associated with use of factor VIII concentrates. Before routine implementation of hepatitis B vaccination, hepatitis B infection was common. NANB hepatitis continues to be endemic among this patient population. Therefore, interpretation of studies aimed at defining the spectrum of liver disease in hemophilic patients suffers from the likelihood that multiple infectious etiologies may be the cause of hepatic injury. However, features of NANB hepatitis can be discerned and form the basis of estimating the risk of serious liver disease attributed to this disorder.

Mannucci reported the result of serial liver biopsies performed in 11 patients who were assumed to have NANB hepatitis in Milan.[38] Following a 3 year interval at which time biopsies were repeated, six patients continued to have chronic persistent hepatitis, and four with evidence of chronic active hepatitis on initial biopsy improved to chronic persistent hepatitis. One patient with cirrhosis had deteriorated clinically. They concluded that NANB chronic liver disease is nonprogressive in hemophilic patients.

Contradictory findings were subsequently reported by Hay, et al in a study of 79 unselected hemophilic patients in England.[39] Seventeen (22%) had evidence of significant liver disease: nine had cirrhosis and eight chronic active hepatitis. Serial liver biopsies showed progression of chronic persistent hepatitis to chronic active hepatitis within a 2 to 6 year follow-up period. None of these patients had histologic or serologic evidence to indicate they were hepatitis B carriers. They were considered to have NANB hepatitis. Two patients with evidence of cirrhosis died from intracerebral hemorrhage.

A multicenter study was conducted in the United States to determine the extent of liver disease in 115 hemophilic patients.[40] The incidence of cirrhosis was 15% and that for chronic active hepatitis 7%. Features considered suggestive of NANB hepatitis were frequently noted.

Hence, evidence indicating that approximately 20% of hemophilic patients have serious liver disease attributable to NANB hepatitis is presented in these two large series.

Other Chronic Sequelae

Patients with persistent biochemical abnormalities are at risk for morphologic liver changes. It should be recognized that they may also be chronic carriers of the virus and their blood may be infectious to others.[41,42]

Severe aplastic anemia is a reported complication of hepatitis. Most cases of hepatitis preceding aplastic anemia are probably NANB.[43]

There is a strong correlation between the hepatitis B virus and hepatocellular carcinoma. In addition, there are case reports indicating that patients have developed hepatocellular carcinoma

as a consequence of acute NANB posttransfusion hepatitis that occurred 9 to 19 years earlier.[44]

METHODS FOR IDENTIFYING BLOOD DONORS POTENTIALLY CAPABLE OF TRANSMITTING NANB HEPATITIS

Because specific tests for detecting those infected with NANB hepatitis do not exist, we must rely on nonspecific or surrogate tests to identify blood donors capable of transmitting this virus through transfusion. Two comprehensive, prospectively designed studies to determine factors in donors that might reduce the occurrence of NANB posttransfusion hepatitis were conducted in the United States. They are the multicenter TTV Study conducted between 1974 and 1979 and the NIH Clinical Center Study conducted between 1973 and 1980.[19,26,28,29] Both groups found an association between elevated ALT level and the presence of antibody to hepatitis B core antigen (anti-HB_c) in blood donors, with the incidence in recipients of NANB posttransfusion hepatitis.

ALT Testing

It is somewhat logical to use ALT as a nonspecific screening test because elevated transaminase levels are required to make a diagnosis of hepatitis, because this marker persists in those with chronic hepatitis, and because it is reasonable to assume that asymptomatic carriers of the virus have elevated levels. ALT is favored over AST because the former is present primarily in the cytosol of hepatocytes, whereas the latter is present in high concentrations in a variety of tissues including heart, liver, skeletal muscles, kidney, and pancreas (Table 2).

The TTV study included 1,513 patients followed for a minimum of 21 weeks and a median of 40 weeks.[26] One hundred fifty-six (10%) developed evidence compatible with NANB hepatitis. Donor ALT levels were such that 96.9% had levels <45 IU, 1.5% between 45 to 59 IU and 1.6% between 60 to 284 IU. The frequency of donors involved in NANB posttransfusion hepatitis cases progressively increased from 9% among those with levels of 1 to 14 IU to 13% in the 15 to 29 IU group, 19% in the 30 to 44 IU group, 35% in the 45 to 59 IU group, and 47% in the group with values of 60 IU and higher. This correlation was further strengthened when the relationship between ALT level of donor and incidence of hepatitis in recipients of single units was analyzed. Only 5% of recipients of single units of blood from donors with ALT levels less than 45 IU developed hepatitis compared to 42% of recipients of blood from donors whose ALT levels were 45 IU or greater.

When the 1,513 recipients were grouped according to the donor whose blood had the highest ALT value, a clear difference emerged. Sixty of 160 (37.5%) recipients of blood from a donor whose ALT activity was 45 IU or higher developed hepatitis compared with only 96 of 1,353 (7.1%) recipients of blood from donors with levels of 44 IU or below. Overall, 60 of 156 (38.5%) posttransfusion hepatitis cases occurred in recipients of blood with ALT levels of 45 IU or greater and 38 of 156 (24.3%) occurred in recipients of blood with levels of 60 IU or higher.

The NIH investigators arranged their ALT data according to standard deviations (SD) from the mean log value in an effort to make the results applicable to other laboratories.[29] A logarithmic transformation was made because the ALT values were found to follow a log normal rather than a normal distribution. Two hundred thirty-eight patients undergoing open heart surgery were included in the study.

Thirty-five (12.4%) developed NANB posttransfusion hepatitis. The hepatitis incidence was 9.1% (21 of 231) among recipients of blood with ALT values ≤2.25 SD above the mean log value. The incidence increased to 26.3% when at least one donor had an ALT level up to 3.0 SD above the mean log value and 35.7% when there was a donor with an ALT level greater than 3.0 SD beyond the mean log value. The cutoff determined to have the most specific association between donor ALT and recipient hepatitis was 2.25 SD

Table 2. NANB Posttransfusion Hepatitis Related to Donor ALT Status: Results of TTV and NIH Studies

	No. of Patients in Study	Recipients of Blood Below Cutoff With Hepatitis	Recipients of Blood Above Cutoff With Hepatitis
TTV Study	1,513	96/1,353 (7.1%)	60/160* (37.5%)
NIH Study	283	21/231 (9.1%)	15/52† (28.8%)

* 38.5% (60/156) hepatitis cases occurred in recipients of blood with elevated ALT levels.

† 41.7% (15/36) hepatitis cases occurred in recipients of blood with elevated ALT levels.

above the mean log ALT level. This reflected a loss of 1.4% to 1.6% of the donor population and included 15 of 36 (41.7%) of the posttransfusion hepatitis cases.

In summary, the two studies found 1. the higher the ALT level in the donor, the more likely hepatitis would occur in the recipient; 2. the hepatitis attack rate in the recipients correlated with the highest donor ALT unit administered (Table 3); 3. a similar relationship between donor ALT and recipient hepatitis occurred among recipients of single unit transfusions; 4. the hepatitis risk increased significantly if more than one unit with an elevated ALT level was transfused; and 5. approximately 30% of NANB posttransfusion hepatitis could be eliminated by instituting routine ALT testing. The 30% reduction estimate is lower than the 38% to 42% incidence found in the TTV and NIH studies, because donors with normal ALT levels were involved in hepatitis cases and their blood would be used to replace that not used from donors with elevated levels. These studies, when reported in 1981, raised questions about requiring all blood to be screened for ALT. This did not occur until several years later when questions posed by these studies were resolved or placed indefinitely in abeyance.

INITIAL CONCERNS ADVISING AGAINST ROUTINE ALT TESTING

The technical and administrative concerns about routine ALT testing that emerged after publication of the TTV and NIH studies in 1981 included: 1. no randomized prospective studies demonstrating efficacy of ALT testing had been performed; 2. the test was nonspecific. Seventy percent of the cases of posttransfusion hepatitis were not prevented, and 72% of the donors with elevated ALT levels were not associated with hepatitis cases (NIH study); 3. an unacceptable loss of donors would occur that might exacerbate existing blood shortages; 4. the benefit of testing compared with the cost was uncertain; 5. the significance of ALT elevations following transfusion were unknown; 6. the information and deferral practice for donors with elevated ALT levels was unclear; 7. technical questions regarding the cutoff, standardization of testing, etc were unanswered.[45]

A randomized, prospective study to determine the efficacy of ALT testing to reduce the occurrence of non-A, non-B posttransfusion hepatitis was not conducted and is not planned currently. A study was conducted at the NIH Clinical Center in which blood from donors with elevated ALT levels were excluded, and recipients were prospectively followed. It showed no impact of ALT testing on the incidence of transfusion-associated hepatitis compared with historic data.[37] Unfortunately the lack of a simultaneously studied control group significantly flawed this report. During the early 1980s, it became apparent that a controlled, randomized prospective study would not be performed in the United States. Hence, this argument for delaying ALT test implementation became untenable.

The thought of losing 1% to 2% of the blood supply in the light of chronic blood shortages during the late 1970s and early 1980s signaled alarm when the TTV and NIH studies were reported in 1981. However, issues relating to safety of the blood supply, became engulfed in emotional as well as scientific discourse as a result of the AIDS epidemic. This led to acceptance that the information was sufficiently compelling to

Table 3. Relationship of Donor ALT Level and Post-Transfusion Hepatitis

Maximum Donor ALT Level	No. of Recipients	Average No of Units Transfused	Recipient Hepatitis	
			No (%)	No/1,000 Units
≤1.35 SD*	1,115	3.4	61 (5.5%)	16
>1.35 ≤ 1.86 SD*	238	4.7	35(14.7%)	32
>1.86 ≤ 2.24*	76	4.8	22(28.9%)	60
>2.24* SD*	84	4.5	38(45.2%)	101
≤1.5 SD†	162	11.2	14 (8.6%)	7.7
>1.5 ≤ 2.25† SD†	69	12.9	7(10.1%)	7.9
>2.25 SD†	52	14.0	15(28.8%)	20.7

* TTV Study.
† NIH Study.

justify testing despite concern that the data regarding ALT efficacy were not definitive.

The cost/benefit ratio for preventing NANB posttransfusion hepatitis has been estimated.[46,47] However, this issue was always one of variable relevance. The real concern was not cost effectiveness but whether significant disease followed the abnormal liver function laboratory test results observed in transfusion recipients.

By the mid-1980s, the evidence indicated that at least one-half of patients with NANB posttransfusion hepatitis had chronic liver dysfunction. Approximately 10% of these patients developed cirrhosis. Although ALT testing might reduce the incidence by only 30%, it became important to attempt some reduction in NANB hepatitis occurrence rather than take no action at all.

The deferral procedure and information given to rejected donors remains particularly unsettling because there are multiple reasons for abnormal ALT results (Table 4). Moreover, the elevated levels fluctuate or are transient. Friedman, et al concluded from their analysis of 100 consecutive blood donors with elevated ALT levels that an abnormal level on a single occasion may be as significant as several elevated levels for identifying donors likely to transmit NANB hepatitis.[48] These authors recognize that approximately two-thirds of donors with elevated ALT levels do not transmit NANB hepatitis. Nevertheless, they advised excluding all donors with elevated levels hoping the donors understand that ALT is a marker, despite shortcomings, of potentially infectious blood. An approach such as this was deemed unacceptable in 1981. However, heightened concern about transfusion safety and an appreciation that blood collection agencies must not be paternalistic in their approach to providing test result information to donors decreased the opposition to non-specific testing by the mid-1980s.

Table 4. Possible Causes of Elevated ALT Levels

Obesity
Some prescription medications
Alcohol use
Hepatitis
Biliary tract disease
Hemachromatosis
Wilson's disease
Alpha$_1$-antitrypsin deficiency
Autoimmune disorders
Hypothyroidism
Psoriasis

The final major objection to ALT testing raised in 1981 involved selecting an appropriate cutoff for excluding blood for transfusion purposes and for deferring donors. The NIH data supported a cutoff that is 2.25 SD above the mean log value of the population tested. Subsequently the TTV data were analyzed in a similar fashion. The peak benefit in that study occurred at 2.0 SD above the mean log value. This value was eventually chosen as the cutoff for determining acceptability for transfusion purposes pending availability of nationwide reference standards. Donor notification procedures were devised to identify donors who might be at risk for liver disease to encourage them to undergo medical evaluation from their personal physician. Included are donors whose ALT test result is $\geq$ two times the upper limit of normal or donors with test results above the cutoff level for using blood for transfusion on two occasions.

Another issue that tipped the scales in favor of ALT test implementation in the mid-1980s was the realization that availability of a specific test for detecting NANB hepatitis was unlikely in the foreseeable future. Hence, it was unrealistic to continue to delay making a decision about adopting nonspecific tests for detecting NANB hepatitis carriers.

ANTI HB$_c$ TESTING

Anecdotal observations made during the course of studies performed in the late 1970s showed an increased incidence of NANB posttransfusion hepatitis among recipients of anti-HB$_s$-positive and anti HB$_c$-positive blood.[14,25,49] As a result, the TTV study data were reanalyzed to determine whether blood donors with anti-HB$_c$ were associated with an increased incidence of NANB posttransfusion hepatitis. Surprisingly, a positive correlation was noted. However, an editorial accompanying the published findings raised multiple questions about the author's interpretation of the data.[50] They were answered by a reanalysis of the NIH data that confirmed the TTV Study findings (Table 5).[22] The association between donor anti-HB$_c$ positivity and recipient NANB hepatitis is explained by possible cross-reactivity between hepatitis B and NANB virus(es) or an increased likelihood that someone exposed to one hepatitis

Table 5. NANB Posttransfusion Hepatitis Related to Donor Anti-HB_c Status: Results of TTV and NIH Studies

	No of Patients in Study	Recipients of Anti-HB_c-negative Blood With Hepatitis	Recipients of Anti-HB_c-Positive Blood With Hepatitis
TTV Study	1,151	69/853 (7.2%)	37/198* (18.7%)
NIH Study	481	12/288 (4.2%)	23/193† (11.9%)

* 34.9% (37/106) hepatitis cases occurred in recipients of anti-HB_c-positive blood.

† 65.7% (23/35) hepatitis cases occurred in recipients of anti-HBc-positive blood.

virus would be exposed to a second hepatitis agent. The latter explanation is accepted by most investigators currently.

The TTV study included 1,151 recipients.[28] Patients receiving blood from donors positive for anti-HB_c had an 18.7% incidence of NANB hepatitis compared to 7.2% of those receiving blood from anti-HB_c-negative donors. Overall, 34.9% of posttransfusion hepatitis cases occurred in recipients of anti-HB_c-positive blood. There was no correlation between donor anti-HB_s serostatus and posttransfusion hepatitis in the recipient.

Of interest, only 8.6% of anti-HB_c-positive donors had elevated ALT levels. That is, two overlapping but distinct donor subsets were identified by ALT and anti-HB_c testing. In addition, a correlation was noted between donor ALT and anti-HB_c result and severity of hepatitis. Recipients of blood that was anti-HB_c positive or had an elevated ALT level who developed hepatitis had more severe illness than those with hepatitis who received anti-HB_c negative or below cutoff ALT blood. The authors of the study concluded that anti-HB_c testing would reduce NANB posttransfusion hepatitis by 21.4%; and 39.2% if both ALT and anti-HB_c testing were performed. However the donor loss would be staggering: 7.5% if both tests were performed; and 5.1% if only anti-HB_c testing were performed.

In the NIH study, 4.2% of 288 recipients of anti-HB_c negative blood developed hepatitis compared to 11.9% of 193 receiving at least one unit of anti-HB_c-positive blood.[22] That is, 65.7% of the hepatitis cases occurred among recipients of anti-HB_c-positive blood. No correlation was found between donor anti-HB_s serostatus and posttransfusion hepatitis. Approximately 4% of the donors were anti-HB_c-positive. A dose/response relationship between number of anti-HB_c-positive units transfused and likelihood of hepatitis occurring could not be demonstrated. A significant association between donor anti-HB_c status and severity of posttransfusion hepatitis was not demonstrated in contrast to the TTV Study. Again, nonoverlapping subsets of donors with abnormal test results were observed. Only 8.3% of donors with elevated ALT levels were anti-HB_c-positive. When sophisticated mathematical modeling studies were performed, donor anti-HB_c status was found to be the primary contributor to hepatitis risk. The predicted efficacy of excluding anti-HB_c-positive donors was 43% and that of using both tests, 58%.

The authors concluded that the study confirmed the previously reported TTV Study results and that the advantages of performing surrogate tests to reduce NANB posttransfusion hepatitis outweighed the disadvantages.

Concerns about Anti-HB_c Testing

A number of concerns were voiced about the efficacy of screening donors for anti-HB_c following publication of the TTV and NIH reports. In addition to those raised about ALT testing, included were: 1. anti-HB_c testing has a low predictive value for preventing NANB post-transfusion hepatitis; 2. donor loss would be unacceptably high; 3. the TTV and NIH studies were performed with anti-HB_c test kits prepared using stripped Dane particle material. Current tests use core antigen synthesized by *Escherichia coli* using recombinant DNA technology. A correlation between the tests is not known; 4. there is significant nonreproducibility of test results; 5. the tests are not licensed as biologics by the FDA nor approved for donor screening; 6. the safety of immune globulin preparations may be impaired because anti-HB_s activity would be lost.

Subsequent discussions addressed these problems. The issue of sensitivity, specificity and low positive predictive value, ie, the likelihood of a recipient of anti-HB_c positive blood developing hepatitis, was displaced by the desirability of preventing 43% of posttransfusion hepatitis cases.

The predicted 2% to 3% donor loss[51] (range: .5% to 6.4%) was also considered to be less important than preventing an illness that relatively new information portrayed as having significant long-term consequences.

Considerable attention was given to possible

changes in efficacy attributed to test reformulation that was made after the TTV and NIH studies were completed. However, at least one group of investigators found an excellent correlation between the two tests.[52] At Baylor College of Medicine, all donor samples from blood administered to the 57 cases of NANB posttransfusion hepatitis followed prospectively during the TTV Study were retested with the recombinant DNA-derived anti-HB_c test. All samples reactive in the initial tests were positive by current procedures.

Test result reproducibility remains an issue. One study indicated that perhaps as many as 50% of reactive tests were false positive,[53] although this is disputed by other studies.[52,54] The American Association of Blood Banks (AABB) implementation Guidelines for anti-HB_c testing addressed this problem by recommending that positive tests should be confirmed before excluding units for transfusion purposes by performing at least two additional tests (by the same or a different method or test kit). If two of the three tests are positive, the result is considered reactive, and the blood should not be used for single unit transfusion. In addition, the donor does not have to be informed of the positive result unless testing is again positive at the time of a subsequent donation.

Although the tests are not licensed as biologics by the FDA, they are classified as medical devices and must conform to acceptable standards. Hence, monitoring of manufacturing practices does occur.

The titer of anti-HB_s in immune globulin preparations will decrease if plasma from donors testing anti-HB_c-positive is excluded because most anti-HB_c-positive donors are also anti-HB_s positive. The resultant decrease in anti-HB_s titers would render immune globulin preparations less effective in hepatitis B prophylaxis. Until this issue is fully resolved, plasma from anti-HB_c-positive donors may be sent for fractionation if the manufacturer is notified and aware that anti-HB_c-positive plasma is being sent to them for fractionation purposes.

Information presented during an FDA sponsored workshop on surrogate testing in January 1987 also addressed lingering questions about anti-HB_c testing efficacy. A study was conducted in Tubingen, Germany between 1980 to 1982 in which 417 transfusion recipients were followed.[23] All received blood screened for ALT. Donor anti-HB_c status was determined later. Among those receiving anti-HB_c-negative blood, 2.1% developed hepatitis compared to 10.1% of those receiving anti-HB_c-positive blood. Anti-HB_c test sensitivity was calculated as 53%, specificity 82% and positive predictive value 10%. The overall 42% efficacy rate in reducing NANB posttransfusion hepatitis was similar to the result of the NIH study. Although this was not a randomized prospective study, it provided additional evidence attesting to anti-HB_c test efficacy.

The effect of this and other information presented at the FDA sponsored workshop was an acceleration of plans for implementing surrogate testing. Because different donor subsets are identified by ALT and anti-HB_c, both tests are performed.

SUMMARY

NANB hepatitis was initially recognized in 1975 and 13 years later, the exact etiology of this presumed viral disease remains uncertain. The acute illness is relatively mild with only about 25% of patients becoming icteric. Nevertheless, at least one half of the patients have evidence of chronic infection and, as recently recognized, 10% to 20% develop severe liver disease. Because approximately 2% of patients who receive transfusions and whose underlying medical condition permits long term follow-up develop posttransfusion hepatitis, procedures for reducing this risk are considered prudent.

Unfortunately specific tests for detecting NANB hepatitis are not available, and it is unlikely that such tests will be available in the near future. Hence, testing by surrogate or nonspecific tests (ALT and anti-HB_c) were recommended because evidence from two studies conducted during the 1970s showed these tests identify some donors thought to transmit the infection. However, randomized, controlled prospective studies to determine whether these tests will, in fact, reduce NANB posttransfusion hepatitis were not performed. By the mid-1980s it was apparent these studies would not be performed nor were studies to determine the incidence of NANB posttransfusion hepatitis in the post-AIDS screening era likely to be initiated. Therefore, surrogate testing, as the best available method for reducing posttransfusion hepatitis, was implemented in the United States in 1986-87.

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