History of posttransfusion hepatitis

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The risk of hepatitis virus transmission from transfusions has declined dramatically from that of the 1940s when posttransfusion hepatitis (PTH) was first appreciated. Introduction of hepatitis B surface antigen screening and conversion to volunteer donors for whole-blood donations in the late 1960s and early 1970s led to substantial reduction in PTH cases. However, up to 10% of the recipients continued to develop PTH, most cases of which were attributed to an unknown non-A, non-B viral agent. Implementation of surrogate marker testing (i.e., alanine aminotransferase and anti-hepatitis B virus core antigen) for residual non-A, non-B hepatitis in the late 1980s reduced the per unit risk of PTH from 1 in 200 to about 1 in 400. Hepatitis C virus was discovered in 1989 and quickly was established as the causative agent of >90% of non-A, non-B PTH. Introduction of progressively improved antibody assays in the early 1990s reduced the risk of PTH due to hepatitis C virus to about 1 in 100,000. Although additional hepatitis viruses exist (e.g., hepatitis G virus), these appear to be minor contributors to clinical PHT, which has been virtually eradicated.

EARLY HISTORY OF POSTTRANSFUSION HEPATITIS (PTH)

During World War II and the immediate postwar period the demand for blood and blood components in the US increased substantially. This resulted in the establishment and growth of blood banks, transfusion services, and other blood and laboratory support services. The technology for collection, processing, and storage of whole blood and blood components materialized rapidly. By 1971, >5400 organizations were involved in the field of transfusion medicine with >12 million annual whole-blood donations and a nearly equal number of plasmapheresis donations (Table 1) [1].

PTH was first reported in the US by Beeson in 1943 [2]. Seven cases of PTH occurring 1–4 months after transfusion of blood or plasma were reported. The author commented that the expanding number of blood and plasma transfusions could lead to the occurrence of a considerable number of PTH cases. In 1964 Grady and Chalmers [3] reported the results of a retrospective study of PTH in nine Boston teaching hospitals, 1952–1962. In one of the hospitals 29% of the blood transfused was from commercial sources, while in the other eight hospitals blood only from volunteer blood donors was transfused. The incidence of clinically overt (i.e., symptomatic or icteric) PTH in recipients of blood products from volunteer blood donors was 0.6 cases/1000 units compared with 2.8 cases/1000 units in recipients of blood products from a mixture of volunteer and commercial blood donors.

In 1970 scientists at the NIH reported the results of a prospective study to determine the incidence of icteric and anicteric hepatitis in patients undergoing open-heart surgery [4]. During surgery the patients were given blood from either commercial or volunteer blood donors. Icteric and anicteric hepatitis developed in 51% of the recipients of commercial blood, whereas no hepatitis occurred in patients who received blood from volunteer donors. These authors estimated the hepatitis carrier rate for commercial blood donors to be 6.3% and for volunteer donors to be <0.6%. By 1971, the association between a paid blood donor and an increased risk of PTH was accepted by most, but not all, experts in the field. In early 1972, two states (California and Illinois) considered legislation to specifically eliminate payment for blood donors. The first law effective in eliminating paid blood donations was passed on October 1, 1972 (i.e., The Blood Labeling Act of Illinois). By the end of 1975, the Food and Drug Administration (FDA) mandated an all-voluntary blood donor system in which blood donors could not receive monetary payment for donations (Fig. 1) [1].

ROLE OF HEPATITIS B VIRUS (HBV) IN PTH

A viral etiology for PTH was long suspected. In 1965, Blumberg et al. [5] first described the Australia antigen and stated that this antigen could be identified in the sera of healthy individuals.
of many hemophiliacs who had received multiple transfusions. Subsequently retrospective studies indicated that the presence of the Australia antigen in donor blood seemed to be clearly associated with the occurrence of PTH. During an NIH Conference in 1970, additional evidence was presented that linked the presence of the Australia antigen to the occurrence of PTH. Furthermore, during this conference it was stated that the Australia antigen was part of an infectious agent, presumably a hepatitis virus. Nevertheless, the screening of blood donors was not recommended. In a position paper written by Alter et al., the authors recommended testing and deferral of blood donors based on the presence of the Australia antigen. (The Australia antigen is now referred to as hepatitis B surface antigen, HBsAg; ultimately the HBV was classified as a DNA virus in the Hepadnaviridae.) These recommendations were not implemented because of lack of consensus and concern over the general availability and variable detection limits of early-generation HBsAg assays.

In 1970, Gocke et al. [9], using retrospective studies, estimated that the exclusion of HBsAg-positive blood donors through either first- or second-generation assays (e.g., agar gel diffusion or counterelectrophoresis) would decrease the rate of PTH by ~25%. The results of a prospective study on the effectiveness of exclusion of commercial and HBsAg-positive blood donors on the incidence of PTH was reported in 1972 [10]. The exclusion of HBsAg-positive blood resulted in a 25% reduction in the PTH rate, as predicted, while the elimination of commercial donors resulted in a 70% reduction in the PTH rate. With the simultaneous exclusion of commercial and HBsAg-positive donors, the PTH rate was reduced to 7.1% of the prior rate.

On the basis of these studies, screening of blood donations for HBsAg began in 1971 and became a US federal regulation in July 1972. The initial methods used to detect HBsAg were relatively insensitive (i.e., immunodiffusion and counterelectrophoresis), and transfusion-associated HBV cases continued to occur. In 1975, federal regulations were implemented requiring the screening of all donor blood for HBsAg by one of the third-generation

### Table 1. Events in understanding and minimizing PTH.

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<td>1943</td>
<td>First US report of PTH</td>
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<td>1964</td>
<td>Retrospective study; paid vs nonpaid donors on rate of PTH</td>
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<td>1965</td>
<td>Discovery of HBV antigen (Australia antigen)</td>
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<td>Prospective study evaluating the contribution of paid vs nonpaid donors to the rate of PTH</td>
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<td>1970</td>
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<td>Prospective study: effectiveness of exclusion of commercial and HBsAg-positive blood donors on the incidence of PTH</td>
<td>10</td>
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<td>1972</td>
<td>FDA mandates screening of all blood donations for HBsAg</td>
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#### NANBH and surrogate markers

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<td>FDA mandates an all-voluntary blood donor system</td>
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<tr>
<td>1975</td>
<td>FDA mandates use of a third generation HBsAg assay</td>
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<td>1981</td>
<td>Transfusion-Transmitted Virus Study results suggest use of ALT as surrogate marker for PTH</td>
<td>16</td>
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<td>1981</td>
<td>Results of NIH study confirm association between increased ALT concentration in donor blood and the development of recipient PT-NANBH</td>
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#### HCV: primary cause of NANBH

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<tr>
<td>1995</td>
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#### Recent events post-HCV screening

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<td>1995</td>
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tests [i.e., radioimmunoassay or enzyme-linked immunosorbent assay (ELISA)], as well as the labeling of blood components with respect to the volunteer or paid status of the donor. Since that time, virtually 100% of all blood used in single-component transfusions has been collected from volunteer donors. The transition to volunteer whole-blood donors and routine third-generation HbsAg testing of all blood donations resulted in a marked decrease in HBV-PTH, although occasional cases continue to occur [11]. In contrast to whole-blood donors, donors of plasma for further manufacture into plasma derivatives continue to be paid. The Cohen fractionation procedure used to prepare plasma derivatives dramatically reduces viral infectivity, and over the last decade, additional viral inactivation procedures (e.g., heating and detergent treatment) performed on these products render contemporary plasma derivatives virtually risk-free.

NON-A, NON-B PTH AND SURROGATE MARKERS

In 1973, the causative agent of hepatitis A was detected by immune electron microscopy on stools from patients with acute food-borne hepatitis [12]. The hepatitis A virus (HAV) was later classified as a picornavirus closely related to the genus Enterovirus [13]. HAV is associated with acute resolving hepatitis without a chronic carrier state. Retrospective studies involving multiply transfused thalassemia patients indicated that these patients were at no greater risk of HAV infection than nontransfused children [14]. Similarly, analysis of donor and recipient samples from cases of PTH showed no evidence of involvement by HAV [15].

After implementation of specific screening tests for HBV and exclusion of HAV as a cause of PTH, it became clear that a substantial proportion of PTH cases continued to occur that were not caused by infections with HBV or other known viral agents. Termed non-A, non-B hepatitis (NANBH), this entity represented 90% of residual PTH cases in the US. In the late 1970s and early 1980s, rates of NANBH in multiply transfused patients were reported to be as high as 10%.

The results of the multicenter prospective Transfusion-Transmitted Viruses Study were published in 1981 [16]. Two major goals of this study were to define the incidence of PTH and to evaluate the factors influencing its occurrence. The data reported indicated a substantial association between recipients with NANBH and donor alanine aminotransferase (ALT) concentrations. Another independent study conducted at NIH confirmed an important association with an increased ALT concentration in donor blood and the development of NANBH in recipients of that blood [17]. Furthermore, in 1984 Stevens et al. [18], representing the Transfusion-Transmitted Viruses Study, reported an important association between the occurrence of NANBH in recipients of blood that tested positive for antibody to the core protein of HBV (anti-HBc). The research group at NIH confirmed this observation [19]. This study showed that anti-HBc testing of donors, in concert with ALT testing, might eliminate 30–50% of recipient NANBH. Primarily on the basis of these studies, in 1986–87 blood collection agencies began screening donated blood for surrogate markers of NANBH (anti-HBc and ALT) [20]. The rate of PTH among recipients dropped subsequently to as low as 2–3% [21].

HCV AS MAJOR ETIOLOGICAL AGENT OF NANBH

Extensive research was conducted in the 1970s and 1980s to identify the etiological agent(s) of NANBH. More than 25 preliminary reports of associated agents were determined to be false. Then, in 1988, the hepatitis C virus (HCV) was identified with molecular biology techniques...
by M. Houghton and associates at Chiron, in collaboration with D. W. Bradley of the Hepatitis Branch of the Centers for Disease Control [22]. The process of virus discovery involved construction of a cDNA expression library in the bacteriophage Agt11 by using high-titer plasma from a chimpanzee inoculated with PTH plasma. The library was then screened for rare clones expressing viral antigen with serum from a chronic NANBH patient as a presumed source of viral antibodies. Screening of this library led to the identification of the positive cDNA clone 5-1-1 (Fig. 2) [23].

HCV is a single-stranded positive-sense RNA virus with a genome of ~9500 bases coding for ~3000 amino acids. This small RNA lipid-envelope [24] virus has been classified in the family Flaviviridae. The entire viral genome was sequenced within 1 year, and antigens were expressed for development of antibody detection assays. Early studies established that HCV was the etiological agent of at least 80–90% of residual NANBH [21].

HCV SCREENING ASSAYS AND RESIDUAL RISK OF HCV-PTH

Blood collection agencies in the US implemented donor screening immediately after licensure of the first-generation anti-HCV enzyme immunoassay (HCV 1.0 EIA) in 1990. This EIA detected antibodies to an antigenic protein (c100-3) of HCV (Fig. 2). Even though this assay facilitated the screening of blood donors for anti-HCV antibodies, it did not detect all infectious blood donations [25] and had a protracted window of infectivity ranging from 12 weeks to >26 weeks postinfection [26]. Nevertheless, a prospective study of patients receiving transfusions before and after mid-1990 found that the risk of transfusion-associated HCV infection per unit dropped from 0.36% (1 in 274) before anti-HCV screening to 0.07% (1 in 3300) for donations screened with both surrogate markers and first-generation anti-HCV tests [27, 28]. It has since been estimated that this test prevented transmission of HCV to 40 000 patients per year in the US [21].

A second-generation anti-HCV EIA (HCV 2.0 EIA) was licensed and rapidly implemented in 1992. This test incorporated two additional proteins, one structural (c22-3) and one nonstructural (c33c) (Fig. 2). This test was substantially more sensitive than HCV 1.0 EIA in detecting acute and chronic HCV infections. Antibodies to these proteins generally appear much earlier than those to c100-3, so the seroconversion window period could be shortened by 10–20 days. In addition, parallel studies comparing first- and second-generation tests showed that HCV 2.0 EIA detected additional HCV chronically infected donors at a rate of 1 in 1000 [25]. This increased yield translated into prevention of an additional 13 000 HCV transmissions per year by transfusion [21].

HCV 2.0 EIA has consequently narrowed the seroconversion window by about 10 days relative to the HCV 2.0 EIA [31, 32]. This window period reduction is projected to detect 1–2 additional seroconverting donors per million units screened. Although the impact of the HCV 3.0 EIA on residual HCV risk has not been prospectively quantified, the ongoing NIH prospective study of PTH has failed to detect any HCV transmissions among >650 patients transfused with >2500 units of blood screened by second-generation HCV EIA since late 1992 (H. Alter, personal communication).

HCV CONFIRMATORY ASSAYS

Given the low prevalence of viral infection in preselected volunteer donors, it is important that confirmatory assays are developed and used to discriminate between true and falsely positive EIA-reactive donors. The Chiron RIBA™ HCV 2.0 strip immunoassay (RIBA 2.0) was licensed on June 27, 1993. The RIBA 2.0 is an immunoblot assay in which four recombinant HCV-encoded antigens fused to human superoxide dismutase are immobilized on nitrocellulose strips. A negative, indeterminate, or positive interpretation is based on the reaction pattern present on the strip. About 89% of RIBA 2.0-positive specimens are HCV RNA-positive by PCR tests, while ~19% of RIBA 2.0-indeterminate specimens are HCV RNA-positive by PCR tests [25].
The HCV 3.0 EIA companion supplemental test, Chiron RIBA HCV 3.0 SIA (RIBA 3.0), is now pending licensure from the FDA. This assay uses a mixture of peptides, rather than recombinant antigens, for the 5-1/1-c100 and c22-3 regions of the HCV genome. The use of peptides, when appropriate, eliminates those amino acid sequences that are a source of nonspecific cross-reactivity with other antigens [33, 34]. On the basis of data from Europe [35], these changes have substantially improved this assay’s lowest detection limits and specificity.

The prevalence of anti-HCV antibody in US blood donors, as determined with HCV 2.0 and 3.0 EIA confirmed by RIBA 2.0 and 3.0, respectively, ranges from 0.2% to 0.4% [21]. At least 70% of seropositive donors are viremic [25]. Similar rates have been observed in Europe, and higher rates in Japan and other Asian countries. Importantly, these rates are in highly selected blood donors [26]. The prevalence of HCV infection in the general population of the US is currently estimated to be between 1% and 2% [36].

RETENTION OF SURROGATE TESTS

With virtual elimination of NANBH by HCV screening, the issue of whether surrogate tests should be retained has surfaced. Although the direct cost of ALT testing is low, the indirect cost is very high because >1% of units test positive and are discarded. Anti-HBc screening has a higher direct cost and results in ~0.5% unit loss. Furthermore, the donors of these units are either temporarily or permanently deferred. The preponderance of available data indicates that, in the presence of anti-HCV testing, retention of ALT testing prevents few if any residual PTH cases [30, 37]. Therefore, a 1995 NIH Consensus Statement recommended that ALT testing of volunteer blood donors be discontinued. On the other hand, the consensus conference recommended that anti-HBc testing be retained, at least temporarily, for the following reasons: possible prevention of some PTH HBV cases and possible prevention of some cases of transfusion-transmitted HIV from donors who test negative for anti-HIV during the window phase of infection [20]. Recent studies suggest that these applications of anti-HBc have a very low predictive value and very poor cost effectiveness [37]. Therefore, discontinuation of anti-HBc is being actively reconsidered.

HEPATITIS G VIRUS (HGV) AND OTHER PUTATIVE PTH AGENTS

Evidence indicates that additional viruses that explain rare residual PTH cases may exist. Indeed, before HCV was identified there was strong suspicion that more than one blood-borne viral agent was causing NANBH [26]. However, it has now become clear that reinfection by different HCV strains is not uncommon, which explains many of the cases of recurrent PTH that led to speculation regarding multiple agents. In addition, the rate of non-ABC hepatitis in recipients is now comparable with that in nontransfusion controls (i.e., <0.8% in both US and Canadian studies, similar to background values) [20]. Nevertheless, the search for additional agents has continued.

In 1995, researchers at Abbott Laboratories reported the isolation of three viral agents [GB viruses (GBV) A, B, and C] from tamarins inoculated with serum from a surgeon “GB” who had contracted acute icteric hepatitis 20 years earlier [38, 39]. Only one of the isolates (GBV-C) proved to be a human viral hepatitis candidate; the other two were tamarin agents incidentally infecting the animals at the time of the experiments. A second independent group of researchers from GeneLabs performed molecular cloning with plasma from a patient designated PNPF161, who was originally identified by the CDC as having NANBH. The virus cloned by these researchers was designated HGV [40]. Given the amino acid sequence matching of 95% between GBV-C and HGV, these viruses are therefore considered to be different strains of the same virus, tentatively termed GBV-C/HGV [41]. GBV-C/HGV is a positive-stranded RNA virus classified in the Flaviviridae, the same family as HCV. Only 32% amino acid homology exists between GBV-C/HGV and HCV. Therefore, although GBV-C/HGV is distantly related to HCV, this agent is not a different serotype of HCV.

GBV-C/HGV RNA has been detected at a high prevalence (2–25%) among various high-risk groups, e.g., intravenous drug users, hemophiliacs, and hemodialysis patients. About 1–2% of blood donors have also been found to be RNA-positive [42]. A recent study, using PCR technology, clarified the relationship of GBV-C/HGV and the occurrence of hepatitis [43]. PCR was performed on selected patients from a surveillance study of acute viral hepatitis in four US counties. The patients included in this study were: 45 patients with a diagnosis of non-A-E hepatitis, 116 patients with hepatitis C, 100 patients with hepatitis A, and 100 patients with hepatitis B. The results of this study indicated that HGV was implicated as a potential etiological agent in only 0.3% of cases. These HGV cases had only mild liver enzyme increases. Furthermore, coinfection by HGV did not affect the clinical course in patients with hepatitis A, B, or C.

Initial studies involving the prevalence of GBV-C/HGV infection were hampered by the absence of an antibody detection system; therefore, the diagnosis of GBV-C/HGV infection depended on the use of PCR to detect GBV-C/HGV RNA. Recently an antibody test has been developed that has demonstrated seroconversion associated with viral RNA clearance in about two-thirds of infected persons [44, 45].

GBV-C/HGV is unequivocally a highly prevalent transfusion-transmissible agent [46]. However, a causal relationship between HGV infection and hepatitis or other diseases has not been established [41, 43]. GBV-C/HGV appears to explain only a small fraction of either transfusion- or community-acquired hepatitis cases, and these cases are very mild [43, 46]. Thus at present, it is unclear
whether screening of blood donors for GBV-C/HGV will be recommended.

The overall risk of PTH has declined markedly over the past 2 decades with virtual elimination of transmission of HBV and HCV infections. The advances in prevention of PTH have been not only extraordinary with regard to recipient safety, but also highly cost-effective. Indeed, the cost of screening the blood donor population for HCV is more than offset by the reduced healthcare costs achieved by prevention of PTH cases [30]. Unfortunately, many infections did occur before the availability of screening assays. The tragedy of the transfusion AIDS epidemic also echoes loudly. Thus, the current priority of infectious disease blood banks is further enhancement of blood safety, through elimination of rare residual cases of virally transmitted hepatitis by implementation of nucleic acid screening assays [47] and avoidance of complacency through proactive surveillance of the blood supply for new or emerging infectious agents.

References
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