

Haematologia 15 (2), pp. 153-173 (1982)

Non-A, Non-B Hepatitis: A Contemporary Assessment

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(Received November 2, 1981)

The current status of non-A, non-B (NANB) hepatitis is reviewed with respect to: (i) epidemiology and incidence of posttransfusion and sporadic NANB hepatitis; (ii) experimental transmission of NANB hepatitis; (iii) serologic tests and the evidence for virus-like NANB agents; (iv) clinical features of NANB hepatitis as compared to hepatitis B; (v) indication and impact of therapeutic and preventive measures against NANB hepatitis. Without specific markers for NANB infection, non-A, non-B hepatitis continues to be a diagnosis by exclusion of infection with hepatitis A, hepatitis B or other viruses. Recent observations suggest that NANB hepatitis may in fact be caused by a group of viruses immunologically different from but genetically similar to the hepatitis B virus.

Key words: chronic and acute hepatitis, commercial and volunteer donor blood, non-A, non-B hepatitis, posttransfusion hepatitis, prevention of non-A, non-B hepatitis

Introduction

Diseases of the liver and particularly jaundice have been described since ancient times, e. g. in the Babylonian Talmud (5th century B.C.) and in the writings of Hippocrates (late 5th century B.C.), and numerous epidemics of jaundice were recorded, especially during times of war [1]. The misconception that epidemic jaundice was caused by a swelling of the ostium of the common bile duct ('catarrhal jaundice') [2, 3] prevailed until the beginning of this century, when it was discarded in favor of a proposed viral etiology of hepatitis [4, 5]. It was not until the second World War, however, that the viral etiology of hepatitis was finally established by experimental transmission to human volunteers [6-10]. Based on the work of MacCallum et al. [8, 9], Havens et al. [10], and Krugman et al. [11], the concept of two types of viral hepatitis emerged:

(i) 'Infectious' hepatitis or hepatitis A with a brief incubation period of 15-45 days and transmission predominantly by the fecal-oral route, and

(ii) 'Serum' hepatitis or hepatitis B with a longer incubation period of 45 to 150 days and a predominantly parenteral mode of transmission through contaminated blood or instruments.

* Recipient of a Heisenberg Award from the Deutsche Forschungsgemeinschaft

viruses. From the evaluation of these findings the concept of non-A, non-B hepatitis has evolved. Hepatitis cases that are serologically unrelated to HAV and HBV or to other known viruses, and for which other causes of hepatocellular damage are clinically excluded, are currently referred to as non-A, non-B (NANB) hepatitis. A specific nomenclature must await further etiologic elucidation of NANB hepatitis, including definition of serologic markers and demonstration of the presumed causative virus(es).

Non-A, Non-B Hepatitis: A Dominant Cause of Posttransfusion Hepatitis

The existence of NANB hepatitis as a disease entity distinct from hepatitis A or B was mostly derived from studies of posttransfusion cases [21, 22, 25-33, 35]. To evaluate the incidence of posttransfusion hepatitis (PTH) and the factors influencing its occurrence, a large number of prospective studies have been carried out. These studies clearly defined three groups of blood donors who are associated with an increased likelihood of transmitting PTH: (i) commercial blood donors, (ii) donors positive for HBV markers (HBsAg or anti-HBc), and (iii) donors with an elevated serum alanine aminotransferase.

(i) *Commercial blood donors.* Blood from commercial donors carries a higher risk of transmitting hepatitis than blood from volunteer donors as first recognized by Allen et al. [36], and by Kunin [37]. The development of assays for HBsAg and their application provided the first direct evidence that commercial blood donors had a 3.5 times higher carrier rate for this HBV marker than volunteer donors [38]. Similarly, commercial donors were found to have an antibody prevalence (anti-HBs) three times as high as volunteer blood donors [39]. With increasingly sensitive assays for the markers of HBV infection it became evident that PTH was not caused by HBV alone and thus the existence of NANB hepatitis was strongly suggested. The relative risk of contracting NANB hepatitis following transfusion of commercial blood was found to be 3.3 times greater than the risk of developing hepatitis from volunteer blood, a figure virtually identical with the relative risk of contracting hepatitis B [26, 27, 40-42]. Based on an analysis of single-donor transfusions, a minimum carrier rate for NANB hepatitis of 1.6 per cent among volunteer donors and of 5.4 per cent among commercial donors was calculated, indicating again that the risk of acquiring NANB hepatitis from commercial blood is 3.3 times greater than from volunteer blood [27].

(ii) *Blood donors positive for HBsAg or anti-HBc.* The risk of transmitting hepatitis from HBsAg positive blood is more than 4 times that from blood which is HBsAg negative [41, 43-48]. While transfusion of blood containing anti-HBs or anti-HBs and anti-HBc did not transmit hepatitis more often than blood which lacked both antibodies [26, 47-50, 53], blood containing anti-HBc alone carries a greater risk of leading to PTH than blood negative for anti-HBc [51-53]. Even though the identification of anti-HBc positive, anti-HBs negative donors could

NANB, ranging from 89 to 100 per cent. No case of posttransfusion hepatitis A was observed and only one case of transfusion transmitted EBV infection was reported [31]. An exceptional case of posttransfusion hepatitis A in two babies transfused with blood from a single donor, retrospectively proven to have IgM anti-HAV, was recently reported to the California State Department of Public Health (personal communication by Dr. S. Hoag). The role of CMV in posttransfusion hepatitis, however, is more difficult to evaluate. Even though serologic evidence of CMV infection was reported in a number of prospective studies [28, 31, 33, 57, 58, 60] and CMV seroconversion was associated with the development of PTH, an etiologic role of this virus in the pathogenesis of PTH could not be established. Except for neonates and patients with acquired or hereditary immune deficiency, transfusion related CMV infection with its mild and transient clinical course does not appear to be a clinically relevant entity [60].

Sporadic Non-A, Non-B Hepatitis

There is now increasing evidence that NANB hepatitis also occurs without prior blood transfusion indicating other modes of transmission of the disease. NANB hepatitis occurring in the absence of a well-defined epidemiologic setting accounts for 15 to 20 per cent of sporadic cases of hepatitis (Table 2) with, except for one study [120], little variation in the percentage of cases classified as NANB [61-69].

Table 2
Type of hepatitis in patients with symptomatic disease

| No. of subjects | Type of hepatitis, % of total hepatitis | | | Reference |
|-----------------|---|----|------|-----------------------------|
| | A | B | NANB | |
| 103 | | | 12 | Villarejos et al. [61] |
| 175 | | 55 | | Hadziyannis [63] |
| 845 | | 55 | | Mueller [62] |
| 417 | 23 | 54 | 23 | Dienstag et al. [64] |
| 329 | 17 | 64 | 19 | Mueller et al. [67] |
| 480 | 23 | 62 | 15 | Norkrans [65] |
| 148 | 27 | 57 | 16 | Norkrans et al. [66] |
| 73 | 37 | 49 | 14 | Kryger et al. [68] |
| 368 | 58 | 27 | 13 | Farrow et al. [69] |
| 1284 | | | 2 | Villarejos and Visona [120] |

Mode of Transmission of Non-A, Non-B Hepatitis

Although discussion of the mode of transmission of NANB hepatitis must be considered tentative until specific serologic markers are identified, it is probable that its transmission pattern will be similar to hepatitis B. Certainly, NANB hepa-

- (ii) Sera from patients with chronic NANB hepatitis were infectious.
- (iii) The incubation period in chimpanzees was similar to that in humans, both short (2 to 6 weeks) and long (10 to 20 weeks) incubation periods being observed.
- (iv) The illness was relatively mild, almost invariably anicteric and asymptomatic, and self-limited in most cases.
- (v) The histologic changes on liver biopsy were typical of viral hepatitis, resembling those seen in chimpanzee hepatitis B. The degree of histologic change paralleled the extent of transaminase abnormality.
- (vi) The transmissible agent could be filtered through a 220 nm filter consistent with its presumed viral nature [93]. Furthermore, it could be inactivated by formalin [97].
- (vii) Chronicity and carrier state of NANB hepatitis were demonstrated by transmitting hepatitis to chimpanzees from asymptomatic patients and donors implicated in transmitting NANB hepatitis to human recipients as long as 1 to 5 years ago [88, 90, 91]. The frequency of this carrier state may be in the range of 1 to 2 per cent [54].

The transmission studies mentioned above document two important points in the epidemiology of NANB hepatitis: (i) NANB hepatitis is caused by a transmissible agent and blood-borne transmission is possible from human to human, from human to chimpanzee and from chimpanzee to chimpanzee. (ii) As demonstrated for hepatitis B, there is a chronic carrier state for NANB agent(s) with no demonstrable abnormalities of liver function as well as clinical evidence of chronic NANB induced liver disease.

Cross-challenge studies with different infectious human sera showed that after one NANB inoculum chimpanzees were protected from infection with two unrelated inocula, suggesting that a single agent was involved in NANB hepatitis [98]. However, there is now evidence from several sources pointing toward the existence of more than one NANB agent: (i) NANB hepatitis presents itself clinically with both long and short incubation periods. The most marked difference is found between PTH cases with a mean incubation period of 7 to 8 weeks [27, 35, 58, 99] and NANB hepatitis with an incubation period of 1 to 4 weeks after infusion of factor VIII concentrates or cryoprecipitates [100] and in a nosocomial outbreak [101]. Based on different incubation periods and clinical/biochemical presentation of the disease two [102] or even three types [30] of NANB hepatitis are suggested. (ii) Multiple attacks of NANB hepatitis have been reported in drug addicts [20, 70, 71], in hemophiliacs [19, 100] and in renal transplant recipients [75-77]. (iii) The most convincing evidence for the existence of two different NANB agents, however, is derived from cross-challenge studies in chimpanzees, demonstrating two types of NANB agents inducing two different types of NANB hepatitis [103-105]. (iv) Plasma from a patient with acute NANB hepatitis and from a patient with chronic NANB hepatitis, when inoculated into chimpanzees lead to different ultrastructural changes in liver biopsies [106], further suggesting the existence of two distinct etiological agents.

Table 3
Virus-like particles in non-A, non-B hepatitis

| Localization | Diameter nm | Buoyant density g/cm ³ | Reference |
|----------------------|----------------------------------|-----------------------------------|-----------------------------|
| Plasma/serum | 60 (40 nm inner core) | 1.31 1.30 1.28 | Coursaget et al. [116] |
| | 27 | | Bradley et al. [94] |
| | 32 | | Mori et al. [117] |
| | 27 | | Yoshizawa et al. [118] |
| | 15-25 (spheres and filaments) | | Hantz et al. [119] |
| | 35-40 (double-shelled particles) | | Hantz et al. [119] |
| | 27/25 (NANB 1/NANB 2) | | Yoshizawa et al. [105] |
| | 38-40 (double-shelled particles) | | Villarejos and Visona [120] |
| Hepatocyte nucleus | 20-27 | | Shimizu et al. [106] |
| | 15-27 | | Tsiquaye et al. [121] |
| | 25-30 | | Hantz et al. [119] |
| | 27 | | Gmelin et al. [122] |
| | 20 | | Burk et al. [123] |
| | 15-20 | | Tsiquaye et al. [124] |
| | 15-27 | | Busachi et al. [125] |
| 20-27 | De Wolf-Peeters et al. [126] | | |
| Hepatocyte cytoplasm | 37 | | Burk et al. [123] |

ously, the virus specificity of such cytoplasmic changes remains to be determined and more information is needed on the immunologic and biochemical properties of these membranous structures, as compared to normal endoplasmic reticulum. In addition, immunohistochemical confirmation is essential to lend meaning to these histologic observations.

Clinical Characteristics of Non-A, Non-B Hepatitis

Even though NANB hepatitis is a relatively recently recognized entity, its clinical pattern displays several remarkable features.

(i) *Incubation period.* The average incubation period of NANB hepatitis following blood transfusion is about 8 weeks (5 to 10 weeks) as compared to about 11 weeks for hepatitis B (Table 4) [27, 28, 35, 42, 55, 58-60, 99]. In about 10 per cent of patients, however, the incubation period may be as short as 1 to 2 weeks, especially after administration of factor VIII to hemophiliacs [100, 130], and after percutaneous inoculation with contaminated blood products [88, 89], or as long as 26 weeks. In general, however, the incubation period of NANB hepatitis describes a sharp unimodal curve with a peak at about 8 weeks after exposure.

Table 6
Etiology of fulminant viral hepatitis

| No. of subjects ^a | Type of hepatitis, % of total hepatitis | | | Reference |
|------------------------------|---|----|--------|---|
| | A | B | NANB | |
| 71 | 8 | 41 | 51 | Rakela et al. [131] |
| 173 | 2 | 61 | 37 | Acute Hepatic Failure Study Group [132] |
| 21 | 19 | 43 | 38 | Mathiesen et al. [133] |
| 63 | 30 | 27 | 43 | Wyke and Williams [134] |
| Mortality, % | 66-75 | 66 | 87-100 | Acute Hepatic Failure Study Group [132] Mathiesen et al. [133] |

^a Patients with evidence for both hepatitis A and B excluded

[131-134] with a mortality rate of 87-100 per cent, exceeding that of fulminant hepatitis A or B [132, 133].

(iii) *Fluctuation of enzyme levels.* Another clinical characteristic of NANB hepatitis is the tendency for ALT/AST levels to fluctuate markedly over relatively short time intervals and to remain abnormal for prolonged periods of time. These recurring enzyme elevations over six months and longer are typical of NANB hepatitis and are rarely, if ever, seen in other types of viral hepatitis. Due to these fluctuating enzyme patterns, the biochemical resolution of the disease is difficult to assess. Arbitrarily, a minimal criterion for resolution should be normal ALT/AST levels in at least six consecutive monthly serum samples. It should be noted, however, that even normal ALT levels do not preclude persistence of NANB hepatitis [135].

(iv) *Extrahepatic manifestations.* Unlike in hepatitis B, extrahepatic manifestations such as urticaria, purpura, arthritis or arthralgia are rarely, if ever, seen in acute NANB hepatitis despite the frequency of immune complexes in this disease [136]. Also antimitochondrial, antinuclear or antibodies against smooth muscle are usually not detectable.

(v) *Chronic sequelae.* Chronic viral hepatitis, defined as continuous or recurrent inflammation of the liver over at least 3 to 6 months [137, 138] without lasting clinical or biochemical improvement, has been observed in 6 to 82 per cent of patients with NANB hepatitis [26, 27, 42, 58, 60, 61, 65, 67, 72-74, 77, 89, 99, 139-143]. Two important factors predictive of a high frequency of chronicity emerge from these studies: (a) The incidence of chronicity is significantly higher following blood transfusions (40-60 per cent) [141-143], among drug addicts (58 per cent) [142] or among renal transplant recipients (82 per cent) [77] than in cases of sporadic NANB hepatitis (9-20 per cent) [61, 65, 142]. (b) Patients with peak ALT levels during the acute phase of NANB hepatitis of less than 300 U/l were less likely to develop chronic hepatitis (11-27 per cent) as compared to patients with peak ALT

more than 2 fold elevated over at least three months) corticosteroid therapy alone or in combination with azathioprine was shown to improve certain biochemical abnormalities (serum bilirubin, globulin and albumin levels), to control complications from portal hypertension and to increase immediate life-expectancy [146]. By contrast, more recent studies on HBsAg positive CAH have shown no apparent benefit [147] or even a detrimental effect of steroids [148], especially in HBeAg positive patients [149], raising the possibility that no form of truly chronic viral hepatitis is improved by steroid therapy. The testing of this hypothesis, however, must await the identification of the NANB agent(s) and the results of further controlled trials. While chronic hepatitis B seems to improve upon antiviral therapy (interferon and/or adenine arabinoside), with a concomitant loss of infectivity [150], similar therapeutic trials in NANB hepatitis must await the specific serologic detection of markers of NANB virus(es).

A number of measures to prevent posttransfusion hepatitis, such as pre- or posttransfusional administration of globulin preparations, transfusion of packed red cells, frozen red cells or frozen deglycerolized red cells are not convincingly effective in reducing the incidence of PTH [54]. Similarly, studies designed to determine the preventive effect of lysozyme [151] and of vitamin C [152] on PTH showed no benefit from these two compounds.

As mentioned above, the measures conclusively established to reduce the risk of transmitting viral hepatitis following blood transfusions are: (i) exclusion of commercial blood donors, (ii) exclusion of HBsAg (and anti-HBc) positive donors, and (iii) exclusion of donors with persistently elevated serum ALT levels. The development of a specific and practical test for the detection of the NANB agent(s), however, is of highest priority to effectively screen for NANB virus carriers.

Conclusions and Perspectives

(i) NANB hepatitis is a newly emerged clinical entity accounting for more than 90 per cent of cases of posttransfusion hepatitis and up to 20 per cent of cases of sporadic hepatitis. NANB hepatitis displays distinct clinical features (incubation period, biochemical characteristics) and is in general only mildly symptomatic or asymptomatic. The ALT/AST levels, however, tend to fluctuate markedly and to remain abnormal for prolonged periods of time. Depending on the epidemiologic background, there is a high incidence of transition from acute hepatitis to chronic forms of this disease, including the development of liver cirrhosis.

(ii) NANB hepatitis is predominantly transmitted by overt or covert percutaneous exposure (transfusion of blood or blood components, drug addicts, hemodialysis patients, renal transplant recipients, patients undergoing plasmapheresis). However, a person-to-person transmission including maternal-fetal and mother-infant is also suggested.

(iii) NANB hepatitis is caused by one or more virus-like agents based on experimental transmission and serial passage in human volunteers and chimpanzees.

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