Incidence and Significance of Hepatitis B Core Antibody in a Healthy Blood Donor Population

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To determine the current incidence of hepatitis B core antibody (anti-HBc) in a healthy blood donor population, 1,893 donors were screened for anti-HBc. Forty-one (2.16%) were found to be initially positive and 35 (1.85%) repeatably positive. Sera from the repeatably positive donors were further screened for hepatitis B surface antibody (anti-HBs), and hepatitis B virus DNA (HBV DNA) by dot hybridisation. The repeatably positive donors were subsequently recalled for further investigation, and their peripheral blood lymphoyctes were also screened for HBV DNA by dot hybridisation. Eighteen (51.4%) of the anti-HBc-positive donors were also anti-HBs-positive. HBV DNA was not detected in the serum or the lymphocytes of any of the anti-HBc-positive donors.

Key words: HBV, anti-HBc, surrogate testing, NANB hepatitis

INTRODUCTION

It is now considered in the United States that approximately 95% of cases of posttransfusion hepatitis (PTH) are due to non-A non-B hepatitis (NANB) [Alter, 1985]. At present, there are no specific screening tests for NANB, and a number of workers have investigated the value of other markers in predicting increased risk of transmission of NANB by transfusion [Stevens et al., 1984; Koziol et al., 1986]. Antibody to hepatitis B

Accepted for publication October 26, 1987.

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core antigen (anti-HBc) and elevated serum alanine aminotransferase (ALT) activity are both thought by some workers to be possible markers for the prediction of occurrence of NANB, since some studies have shown an increase in cases of NANB in recipients of blood from donors with circulating anti-HBc [Cossart et al., 1982; Vyas and Perkins, 1982] or elevated ALT activity [Aach et al., 1981].

Anti-HBc in the serum of an individual indicates recent or active (IgM anti-HBc) or previous (IgG anti-HBc) infection with hepatitis B (HBV) and is usually produced during the acute phase of infection, until virus replication ceases, but may persist for life. The presence of anti-HBc may therefore define a potentially infectious stage of HBV infection. Nonetheless, Cossart et al. [1982] and Vyas and Perkins [1982] have associated the presence of anti-HBc in the serum of hepatitis B surface antigen (HBsAg)-negative donors with the transmission of NANB in the absence of HBV. Screening of donors for anti-HBc may therefore help to reduce the risk of PTH from NANB and should also reduce the already low incidence of PTH from HBV infection from donors whose HBsAg levels are below detectable limits. This study has been undertaken to determine the current incidence of anti-HBc in the donor population covered by the North East Thames Regional Transfusion Centre, to try to assess the significance of the anti-HBc in the serum of these donors, and to try to identify any HBsAg-negative but potentially infectious donors in this group.

MATERIALS AND METHODS

Donor Samples

Serum samples from 1,893 random donors were tested. All the donors were found to be negative for HBsAg (RIA, Blood Products Laboratory), antibody to HIV (Wellcozyme, Wellcome Diagnostics), and antibody to *Treponema pallidum* (Fujirebio), and their donations were considered suitable for tranfusion according to the current U.K. criteria for the microbiological screening of blood donors. Donors who were found to be repeatably anti-HBc-positive were recalled for further investigation. The peripheral blood lymphocytes from those donors who were rebled were separated using a standard ficol/triosil density gradient.

Assays

The donor samples were tested for anti-HBc and anti-HBs using the Corzyme EIA and Ausab EIA kits (Abbott Diagnostics), respectively, in accordance with the manufacturers' instructions. Samples that gave clearly positive or equivocal results on initial testing for anti-HBc were retested in duplicate. The mean of the absorbance/cut-off values for each test was used to determine final reactivity.

DNA Hybridisation

The sera of the anti-HBc-positive donors were tested for HBV DNA by DNA:DNA hybridisation using a dot-blot procedure as described by Harrison et al. [1985]. Lymphocyte DNA (approximately 10^6 cells) was prepared for hybridisation by phenol extraction following treatment of the cells with sodium dodecyl sulphate (SDS) and pronase.

TABLE I. Results of Anti-HBc Testing of 1893 Random Donor Samples

Serological state	Total	Percent
Initially positive*	41	2.16
Repeatably positive	32	1.69
Repeatably equivocal	3	0.16
Total samples tested	1,893	

*Initially positive group includes equivocal results.

figure dropped to 35 (1.85%) repeatably positive samples. Of these 35 samples, 32 (1.69%) were clearly positive and three (0.16%) were equivocal, i.e., within 10% of the cut-off value. When the comparative titre of anti-HBc is considered, two populations of anti-HBc-positive donors were apparently present. The distribution of the individual absorbance/cut-off ratios of the anti-HBc-positive samples, shown in Figure 1, clearly demonstrates the two populations of anti-HBc-positive donors, 20 (57.2%) having higher titre and 15 (42.8%) having lower titre anti-HBc.

Table II shows the anti-HBs results in the anti-HBc-positive donors. Eighteen (51.4%) were found to be anti-HBs-positive. These donors were not, however, randomly distributed among the total number of anti-HBc-positive donors. As can be seen in Figure 1, 17 (94%) of the anti-HBs-positive donors were also in the group of higher titre anti-HBc-positive donors (Fig. 1). Twenty-one of 35 (60%) of the repeatably positive donors responded to our request to be bled for a further sample.

DNA Hybridisation

HBV DNA was detected neither in the serum nor in the lymphocytes of any of the anti-HBc-positive donors.

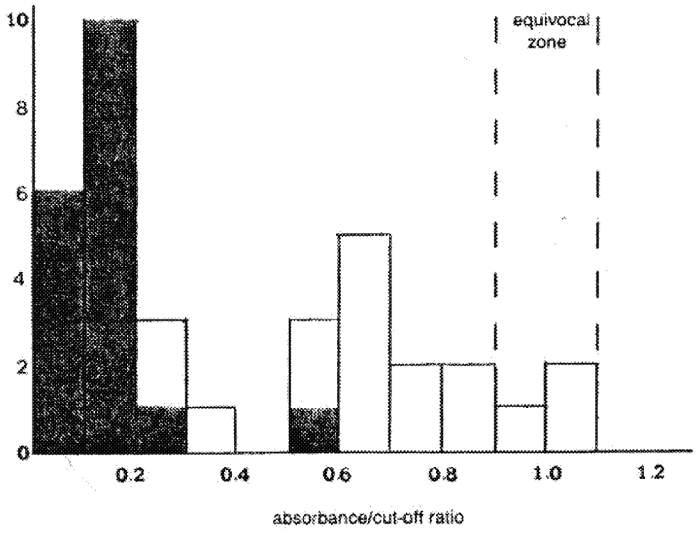

Fig. 1. Histogram showing distribution by comparative titre of anti-HBc-positive donors and corresponding anti-HBs status. Open bars, anti-HBc-positive; shaded bars, anti-HBc- + anti-HBs-positive. The equivocal zone includes sample absorbance values within 10% of the cut-off value and defines the borderline results.

TABLE II. Anti-HBs Status of Anti-HBc-Positive Donors

Serological state	Total	Percent anti-HBc-reactive donors	Percent original donor population
Anti-HBc-pos, anti-HBs-neg	14	40.0	0.74
Anti-HBc-pos, anti-HBs-pos	18	51.4	0.95
Anti-HBc-eq,[a] anti-HBs-neg	3	8.6	0.16
Anti-HBc-eq, anti-HBs-pos	0	0	0
Total	35		

[a]eq, within 10% of the cut-off value.

DISCUSSION

Anti-HBc was detected in 35 of 1,893 (1.69%) random blood donors negative for HBsAg. Anderson et al. [1987] have recently reported the results of two previously unpublished preliminary studies made in 1984 and 1985 to investigate the incidence of anti-HBc in donors bled by the North London Blood Transfusion Centre. In 1984, an incidence of 1.8% was found, and in a smaller study, in 1985, this figure had dropped 0.6%. A possible explanation put forward by the authors to explain this significant decrease was that the 1985 study, although carried out before testing for antibodies to human immunodeficiency virus (HIV) had begun, followed an education programme to promote the self-exclusion of donors at risk of transmitting HIV. Although our study was carried out at the end of 1986 on a population that was negative for antibody to HIV and that had also been educated in self-exclusion, the incidence of anti-HBc was higher than that found in the 1985 North London study and was similiar to the incidence found in the 1984 study. Although no other figures for the United Kingdom have been published recently, Donnellan et al. [1987] reported an incidence of 1% (42 of 4136) in blood donors in the Republic of Ireland, and Kline et al. [1987] and Hanson and Polesky [1987] reported incidences of 2.0% and 2.08%, respectively, in voluntary donors in the United States. The Abbott Corzyme assay used in this study is a second-generation assay using HBcAg produced by recombinant DNA techniques. Lee et al. [1987] have recently used immunoabsorption techniques to demonstrate the sensitivity and specificity of the assay, and Staller et al. [1987] have developed a confirmatory assay, which has shown that 92% borderline samples are true positives. It would therefore seem quite reasonable to accept that the repeatably positive samples do indeed contain anti-HBc, albeit at a low titre.

Further analysis of the comparative titres of the anti-HBc-positive donors together with their anti-HBs status clearly demonstrates two populations of donors. Seventeen of 18 (94%) anti-HBs-positive donors also had high titres anti-HBc, only one of the anti-HBs positive donors having a low titre of anti-HBc. The implication is that each of the donors with high titre anti-HBc and anti-HBs are immune following presumed natural infection. None of the donors in the study has admitted to any previous diagnosis of hepatitis, although this is perhaps not too surprising in that it is estimated that up to 65% of HBV infections are transient and subclinical, marked solely by the development of anti-HBs and anti-HBc and subsequent immunity [Hoofnagle and Schafer, 1986]. Further examination of the donor histories revealed that, of the group of 20 high titre anti-HBc donors, two were previously known to possess anti-HBs. One of them was born in India and the other had

received hepatitis B vaccination. The response of this last donor is unusual; high titre anti-HBc is present in the serum as well as anti-HBs. This is not the expected response to vaccination; the vaccine contains HBsAg but not HBcAg, and it is possible that this donor had actually been infected prior to vaccination and now has natural immunity with his anti-HBs level boosted by the vaccination.

The significance of the sole presence of low titre anti-HBc in the serum of the second group of anti-HBc-reactive donors is not so clear, although the results imply previous exposure to HBV. It is possible that this group of donors has had previous low-level infection, where anti-HBs either has not been produced or has declined more rapidly than anti-HBc, leaving anti-HBc as the sole marker. The three donors found to have high titre anti-HBc and no anti-HBs may fall into one of two classes, either the window period when HBsAg has disappeared and anti-HBs has not yet been produced, indicating recent infection, or a chronic carrier state where HBsAg is at a level too low to detect. Both of these situations have been considered to pose a risk of HBV infection [Hoofnagle et al., 1978], although in this case, using DNA hybridisation, we have shown that these donors are undoubtedly noninfectious. None of the donors involved in this study was subsequently reported to us as having been implicated in any case of PTH. DNA hybridisation analysis has not detected HBV DNA in the sera of the anti-HBc-positive donors, nor in the lymphocytes of those recalled donors from whom further blood samples were taken. On the basis of the results of this study, it appears that these donors present no increased risk of transmission of HBV, though a response to past infection is clearly demonstrated.

Although anti-HBc screening together with ALT screening was originally introduced by the American Assoication of Blood Banks (AABB) at the end of November, 1986, in an attempt to reduce the risk of transmission of NANB by transfusion, not all centres adopted this policy and, in fact, the requirement for anti-HBc screening has now been deferred until problems with false-positive reactions have been resolved. Some centres in the United States have, however, implemented full screening programmes for anti-HBc and defer donors with detectable circulating antibody [Lee et al., 1987]. At present, this proceedure has not been adopted in the United Kingdom and doubt has been expressed regarding the validity of any such surrogate testing [Anderson et al., 1987; Gunson, 1986]. Another group however, [McClelland et al., 1987] has recently called for the introduction of surrogate screening tests for NANB hepatitis in the United Kingdom. Justification for the doubts raised regarding the validity of surrogate testing is given by the results of a recent prospective study in France in which no evidence was found of any link between the presence of anti-HBc in donor blood and transmission of NANB in a group of cardiac surgery patients [Aymard et al., 1986].

It is generally accepted that the incidence of viral hepatitis is greater in the United States than in the United Kingdom and that in the United States PTH remains a serious and well documented problem accounting for an estimated 200,000–300,000 cases per annum [Alter, 1985]. In the United Kingdom, however, the incidence of PTH appears to be much lower and does not present such a serious problem. The reasons for this are unclear but may be due to the fact that many cases of PTH are essentially asymptomatic and, unless there is an obvious clinical response, they may not be diagnosed. Even when viral hepatitis is suspected and diagnosed, transfusion is not the only possible source of infection. There is also a significant difference between the reporting of PTH in the United Kingdom and in the United States. Most cases of PTH in the United Kingdom are reported primarily on the basis of clinical jaundice [Barbara and Briggs, 1981]; in the United States, 75% of cases are reported from prospective studies based on raised serum

liver enzyme activity, primarily ALT [Aach, 1978]. The apparent low incidence of reported cases of PTH in the United Kingdom is demonstrated by the fact that during the last 12 months in the area served by the North East Thames Regional Transfusion Centre, approximately 120,000 units of blood were transfused and only three cases of PTH were reported for follow-up of the implicated donors. In the light of the findings of this study, and the very small number of cases of PTH reported in the United Kingdom, we believe, as do other workers [Anderson et al., 1987; Aymard et al., 1986], that at the present time there is likely to be very little benefit in the introduction of anti-HBc screening of blood donors. The loss of approximately 2% of available donors because of deferment would cause problems for those transfusion centres facing shortages of donors, especially those serving the Greater London area. The cost of testing donations for the presence of anti-HBc is high and in the current financial climate would be hard to justify. A further consideration is the need to counsel those donors found to be anti-HBc-positive. Although the introduction of surrogate testing may eventually be unavoidable, we believe that only a controlled prospective study would provide the necessary information to determine the significance of donor anti-HBc levels in relation to PTH, especially NANB, in the United Kingdom.

ACKNOWLEDGMENTS

We are indebted to Abbott Diagnostics for providing the Corzyme assay kits and to Ms. Simone Deluce for excellent technical assistance. This study was partly funded by a generous award from the Wolfson Foundation.

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