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Serological evidence of presence of HBsAg undetectable by conventional radioimmunoassay in anti-HBc positive blood donors

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SUMMARY Sera from two blood donors, one of whom was implicated in a case of post-transfusion hepatitis B, were found to be positive for anti-HBc and negative for HBsAg by conventional radioimmunoassay and were retested for HBsAg after concentration (pepsin digestion and polyethylene glycol precipitation). The presence of occult HBsAg was confirmed in both. These observations have implications for blood transfusion, and wider studies of anti-HBc in blood donors are recommended before the introduction of routine screening for anti-HBc and exclusion of the positive donors from blood donation.

Post-transfusion hepatitis B has been reported occasionally in recipients of blood negative for HBsAg by radioimmunoassay (Hollinger *et al.*, 1973; Koretz *et al.*, 1973). A possible explanation for such occurrences may be a relative insensitivity of the currently most sensitive techniques for HBsAg detection. Gerlich *et al.* (1976) have reported the sensitivity of radioimmunoassay to be between 1·2 and 2·4 ng HBsAg/ml, while the sensitivity of the enzyme-immunoassay varies between 2·4 and 10·5 ng HBsAg/ml (Wolters *et al.*, 1976). The description of a new antigen/antibody system by Almeida *et al.* (1971), comprising the cores of Dane particles and antibodies to them (HBcAg and anti-HBc), has provided an additional tool for detecting the presence of hepatitis B virus (HBV) markers. Hoofnagle *et al.* (1973, 1974) have proposed an hypothesis, according to which a positive anti-HBc as a sole marker of HBV infection may be an indicator of a continued multiplication of HBV in liver cells, with a possible intermittent viraemia, accounting thus for the occurrence of post-transfusion hepatitis B in some recipients of such blood. In none of such donors, however, was there evidence of HBsAg in circulation.

We report the results of an attempt to determine the presence of HBsAg in quantities undetectable by conventional radioimmunoassay screening in two blood donor sera in which the sole HBV marker was a positive test for anti-HBc. One of these donors was implicated as a probable cause of a post-transfusion hepatitis B; the other was tested for anti-HBc in addition to the routine screening for HBsAg because he had an episode of hepatitis in anamnesis.

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Material and methods

TESTS FOR HBV MARKERS

HBsAg and anti-HBs were detected by radioimmunoassay (Ausria II and Ausab, Abbott Laboratories, North Chicago, Ill., USA). The specificity of HBsAg positives was determined by neutralisation with human anti-HBs. HBeAg and anti-HBe were detected by double immunodiffusion in agarose. Anti-HBc was detected by indirect immunofluorescence, using post-mortem human liver rich in HBcAg as substrate, as described by Kater and Vogten (1976), with a modification using formalin-fixed tissue. The specificity of this technique was controlled by complete abolition of anti-HBc positivity of a known positive serum after absorption with a suspension of HBcAg positive liver tissue (other than the substrate), while the absorption with anti-HBc negative liver suspension had no effect.

DETERMINATION OF HBSAG IN QUANTITIES UNDETECTABLE BY CONVENTIONAL RADIOIMMUNOASSAY

(hereafter referred to as 'occult HBsAg').

The technique described by Harris *et al.* (1977) was

used, consisting briefly of sequential pepsin digestion of non-HBsAg proteins at low pH and polyethylene glycol precipitation of remaining HBsAg. This technique claims an increase of sensitivity in subsequently performed radioimmunoassay by at least a factor 100. The sera before the application of this procedure and the resulting material were tested by Ausria II. The specificity was confirmed by neutralisation with human anti-HBs.

PATIENT AND DONORS

Post-transfusion hepatitis B. A 41-year old woman, who underwent vaginal uterus extirpation, developed classic acute hepatitis B with jaundice and positive HBsAg about 130 days after a transfusion of four units of blood from donors negative for HBsAg by radioimmunoassay at routine screening. The original serum samples of the four implicated donors (Nos. W0869, W9365, V0472, and 13-2483) were retrieved from the serum collection kept at −20°C, retested for HBsAg and—in addition to this—tested for other HBV markers (anti-HBs, HBeAg, anti-HBe, and anti-HBc). The one positive for anti-HBc was also tested for occult HBsAg.

An occasional anti-HBc positive donor. This donor (No. 11055/77) was excluded from giving blood on the basis of his anti-HBc positivity and an episode of hepatitis in anamnesis. He was also tested for other HBV markers, along with testing for occult HBsAg.

A donor false-positive for HBsAg. The serum of this donor (No. 22076/78), false positive for HBsAg by radioimmunoassay, was used as a negative control in testing for occult HBsAg.

Results

The results of tracing the donor presumably responsible for the post-transfusion hepatitis B are shown in Table 1.

One of the four blood donors implicated in the case of post-transfusion hepatitis B (donor No. 13-2483) was found, of all the HBV markers tested, positive only for anti-HBc. This donor was bled again, more than six months after the original donation, and again found positive only for anti-HBc. This donor had no hepatitis episode in anamnesis. Her blood had previously (more than six months before the donation in question) been given to another recipient. This recipient was traced and his serum found to be negative for all the HBV markers tested.

The results of testing for occult HBsAg are shown in Table 2. In the sera of both anti-HBc positive blood donors a reactivity for HBsAg was detected after concentration. The neutralisation with human anti-HBs reduced this reactivity by 72-85%. The addition of normal human serum devoid of anti-HBs activity had no effect. In contrast, occult HBsAg could not be detected in the control serum (No. 22076/78) which was false-positive in HBsAg screening.

Table 1 *Tests for HBV markers in four donors implicated in a case of post-transfusion hepatitis B*

Serum	*Date*	*Routine screening*	*Additional tests*			
		HBsAg	*anti-HBs*	*HBeAg*	*anti-HBe*	*anti-HBc (recipr. titre)*
Donors						
W 0869	25 July '77	Negative	Negative	Negative	Negative	Negative
W 9365	25 July '77	Negative	Negative	Negative	Negative	Negative
V 0472	25 July '77	Negative	Negative	Negative	Negative	Negative
13-2483	25 July '77	Negative	Negative	Negative	Negative	320
	2 Feb. '78	Negative	Negative	Negative	Negative	160
Recipient transfusion 26 July '77	7 Jan. '78	++	Negative	Negative	Negative	5120

Table 2 *Results of testing for occult HBsAg in two anti-HBc positive blood donors*

Serum No.	*Tests for usual HBV-markers*			*Tests for occult HBsAg*	
	anti-HBc	*anti-HBs*	*HBsAg ratio Ausria II‡*	*ratio Austria II after concentration*	*Specificity (% cpm decrease)**
13-2483	320	Negative	0·7	1·8	72
11055/77	5120	Negative	1·1	4·7	85
22076/78	Negative	Negative	11·0†	1·2	36

*Regarded specific if ⩾ 50%; †False positive in confirmation test; ‡Regarded positive if ⩾ 2·1

Discussion

Although a pre-transfusion serum sample from the recipient of transfusion who subsequently developed hepatitis B was not available, it seems acceptable to assume that donor 13-2483 (Table 1) was the cause of this infection. Anti-HBc persisted in the donor for at least 6 months after the blood donation and the result of testing for occult HBsAg, however low the ratio (see Table 2), seems to be specifically confirmed. Aside from the results of Hoofnagle *et al.* (1973, 1974), and more recently of Lander *et al.* (1978), who reported post-transfusion hepatitis B after transfusions of anti-HBc positive, HBsAg negative blood, it has been shown that persons with positive anti-HBc (HBsAg negative in blood) may have both HBsAg and HBcAg in liver cells (Ray *et al.*, 1976; Kojima *et al.*, 1977). Depending on some extraneous factors, such as immunosuppression, reactivation of hepatitis B has been observed, eg, in renal transplant patients whose sole marker of HBV infection was a positive anti-HBc (Nagington *et al.*, 1977).

Our results on the determination of occult HBsAg in blood donors, negative for HBsAg by conventional radioimmunoassay, whose sole marker of HBV infection is a positive anti-HBc, seem to support the hypothesis of Hoofnagle *et al.* (1973, 1974). After concentration the occult HBsAg had apparently been detected in at least one of the two sera (No. 11055/77, Table 2). The specificity for HBsAg, as far as the serological criteria are concerned, has been confirmed in both sera. It is desirable to obtain more such data before any definite conclusion is reached. Testing for the presence of HBsAg and Dane particles by electron immune microscopy in such concentrates is warranted.

The reported association of anti-HBc positive blood with infectivity for HBV (Hoofnagle *et al.*, 1973, 1974; Lander *et al.*, 1978) is highly suggestive. However, in view of the practical consequences for blood banking, extensive retrospective studies of anti-HBc in blood donor populations, possibly combined with attempts to detect occult HBsAg in anti-HBc positive samples, and the consequences in recipients of such samples, are needed before a definite conclusion is reached on the introduction of routine screening of blood donors for anti-HBc and exclusion of the positive ones from donation.

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