Short reports

Routine antenatal screening for hepatitis B using pooled sera: validation and review of 10 years experience

R Cunningham, J L Northwood, C D Kelly, E H Boxall, N J Andrews

Abstract

Aims—To validate the sensitivity of universal antenatal screening for hepatitis B surface antigen (HBsAg) by testing pools of 10 sera, and to review 10 years' experience using this method.

Methods—66 945 antenatal patients were tested between 1986 and 1996 using the pooled method. All sera from 1996 (n = 6050) were retrieved and retrospectively tested individually. An *in vitro* determination of the effect of pooling on sensitivity was performed by checkerboard neutralisation assay.

Results—26 HBsAg positive women were detected by universal screening over 10 years; 12 had non-European surnames and five had known risk factors for hepatitis B infection. High titre anti-HBs sera in the pool reduced the sensitivity of the HBsAg assay, though the effect was only significant at low levels of HBsAg carriage.

Conclusions—The prevalence of hepatitis B is extremely low in the antenatal population served by Plymouth PHL. Pooling is unlikely to reduce sensitivity enough to lead to significant preventable vertical transmission, and is a cost-effective and valid strategy in areas of low seroprevalence.

(*J Clin Pathol* 1998;51:392-395)

Keywords: antenatal; hepatitis B; screening

Plymouth Public Health Laboratory,
Derriford Hospital,
Plymouth, UK
R Cunningham
J L Northwood
C D Kelly

Birmingham Public Health Laboratory,
Birmingham, UK
E H Boxall

PHLS Statistics Unit,
Colindale, London
NW9, UK
N J Andrews

Correspondence to:
Dr R Cunningham,
Plymouth Public Health Laboratory,
Derriford Rd,
Plymouth PL6 8DH, UK;
email: richard.cunningham@phnt.swest.nhs.uk.

Accepted for publication
30 January 1998

complete course of vaccine, in some cases with hepatitis B immunoglobulin.³ These guidelines also state that antenatal clinics should consider offering screening to all antenatal patients, though at present only 27% of pregnancies in England and Wales are screened universally.⁴ Most laboratories test for hepatitis B where the mother is identified as falling into an at risk group. This relies on antenatal clinic staff identifying parenteral drug misuse, family history, and sexual contact with perceived at risk partners. A foreign surname is frequently used within laboratories as a marker of ethnic origin.

It is recognised that selective screening using the above criteria is a very insensitive way of identifying HBV carriers. Chrystie *et al* found that 40% of HBV positive antenatal patients in West Lambeth Health Authority were not screened using conventional selective criteria, despite publicising early results of the survey among the antenatal staff concerned.² Universal testing of antenatal sera is the most sensitive way of detecting carriers though its cost-effectiveness is proportional to maternal HBsAg prevalence.⁵

As resources have not permitted individual testing of all antenatal sera, Plymouth Public Health Laboratory (PHL) has for 10 years tested antenatal patients with identified risk factors or foreign surnames individually, then tested the remainder in pools of 10 sera. When a positive result is found the constituent sera in the pool are tested individually.

This policy began in 1986 following transmission of hepatitis B to an infant whose mother had no identified risk factors. The 10-fold reduction in consumables costs has allowed universal testing to be done, though there is inevitably some loss of sensitivity. The high uptake of HBV immunisation in female healthcare workers in the Plymouth area led to concerns that anti-HBs from vaccinated women might neutralise HBsAg within a pool, giving a false negative result. To investigate this possibility we reviewed the results of our screening method and evaluated its sensitivity *in vitro* and by individually testing one year's antenatal sera retrospectively.

Hepatitis B is a serious condition affecting over 200 million people worldwide.¹ The United Kingdom is an area of low endemicity, though carriage rates over 1% have been found in antenatal women from clinics serving a high proportion of immigrants.² Chronic hepatitis B (HBV) infection reduces life expectancy by causing cirrhosis and hepatocellular carcinoma, and is most common when infection occurs at birth. A vital aspect of controlling HBV infection is the interruption of vertical transmission. Current Department of Health guidance is that babies born to mothers who are chronic carriers of hepatitis B or have acute hepatitis B during pregnancy should receive a

Figure 1 HBsAg concentrations in 153 antenatal carriers.

Methods

ROUTINE TESTING

Since 1986 all sera submitted to Plymouth PHL for antenatal rubella and syphilis serology have also been tested for HBsAg. Sera from patients with non-European surnames or clinical information indicating a risk of hepatitis B are tested individually for HBsAg by enzyme linked immunosorbent assay (ELISA). The remainder are mixed in pools of 10 using an automated sample handling robot (Biomek 2000, Beckman Instruments UK, High Wycombe, Buckinghamshire, UK) and the pool tested within one hour using a highly sensitive sandwich ELISA, currently the Bioelisa HBsAg (Launch Biokit, Longfield, Kent, UK). The assay used has varied over the years but has always been chosen primarily on the basis of high sensitivity. Apart from the initial pooling of sera, manufacturer's methods are used throughout, with appropriate controls and internal quality control sera. Positive and indeterminate pools are identified and the constituent sera tested individually for HBsAg. They are referred for e antigen and antibody testing if positive, and appropriate prophylaxis and follow up of the infant arranged.

REVIEW OF HBSAg CARRIERS DETECTED

All antenatal cases detected between 1986 and 1996 were identified from laboratory records. The surnames were arbitrarily identified as oriental, African, Asian, or other. The original laboratory request forms were reviewed to determine whether HBV testing had been requested by the clinician, and whether information on risk factors was supplied.

Table 1 Anti-HBs concentrations in pools of 10 antenatal sera

Anti-HBs (IU/ml)	No of pools	Percentage
<10	39	44.3
10-99	21	23.9
100-900	11	12.5
901-1900	5	5.7
1901-7500	8	9.1
>7500	4	4.5

HBSAg CONCENTRATIONS IN ANTENATAL CARRIERS

HBsAg concentrations in 153 antenatal carriers identified by Birmingham PHL were quantified using "Amerlite" enhanced luminescent immunoassay (Johnson and Johnson, Amer-sham, UK) for HBsAg against a standard curve ranging from 1 to 10 000 IU/ml HBsAg (Standard 80/549, National Institute for Biological Standards and Control, London). HBeAg and anti-HBe were also determined using the "Amerlite" semiquantitative enhanced luminescent immunoassay (Johnson and Johnson).⁶

ANTI-HBS CONCENTRATIONS IN POOLED ANTENATAL SERA

Anti-HBs concentrations in randomly selected, routine antenatal pools from Plymouth patients were assayed using the Biokit Bioelisa anti-HBs (Launch Biokit, Longfield, Kent, UK) in two trials. In the first trial, anti-HBs concentrations in 120 pools were quantified within the following ranges: negative (anti-HBs < 10 IU/ml), positive (anti-HBs 10-100 IU/ml), and strongly positive (anti-HBs > 100 IU/ml). In the second trial, 88 pools were assayed then serially diluted to give a semi-quantitative measure of anti-HBs with a range of values from < 10 IU/ml to > 10 000 IU/ml.

RETROSPECTIVE TESTING OF STORED SERA

All antenatal sera are stored individually at -20°C for at least three years. In order to determine whether any false negatives had occurred as a result of testing pools, all sera received in the year to December 1996 were retrieved and tested individually. This comprised a total of 6050 sera. Staff performing these tests were not aware of the results obtained when they had been tested as part of a pool.

EVALUATION OF HBSAg ASSAY SENSITIVITY WHEN TESTING POOLED SERA

In order to determine the effect of dilution in pools on HBsAg detection, and the potential neutralising effect of sera containing high

Table 2 Detection limits of HBsAg pooled with various concentrations of anti-HBs

<i>HbsAG</i> (<i>IU/ml</i>)	<i>Anti-HBs (IU/ml)</i>											
	<i>10</i>	<i>20</i>	<i>40</i>	<i>80</i>	<i>160</i>	<i>300</i>	<i>600</i>	<i>1250</i>	<i>2500</i>	<i>5000</i>	<i>10 000</i>	<i>20 000</i>
0,5	++*	+	+	+	+	+	+	—**	—	—	—	—
1	+	+	+	+	+	+	+	—	—	—	—	—
2	+	+	+	+	+	+	+	+	—	—	—	—
20	+	+	+	+	+	+	+	+	+	+	—	—
200	+	+	+	+	+	+	+	+	+	+	+	+
2000	+	+	+	+	+	+	+	+	+	+	+	+
20 000	+	+	+	+	+	+	+	+	+	+	+	+
40 000	+	+	+	+	+	+	+	+	+	+	+	+

*Optical density (OD) > mean OD of negative controls +0.040 OD units (positive).

**OD < mean OD of negative controls +0.040 OD units (negative).

concentrations of anti-HBs, a checkerboard neutralisation assay was performed. Serial dilutions of sera containing HBsAg ranging from 0 to 20 000 IU/ml were mixed with anti-HBs sera ranging from 0 to 5000 IU/ml. The mixtures were held at room temperature for one hour to simulate routine laboratory practice, and then tested in the usual way. National Institute for Biological Standards and Control sera containing 0.125 (monitor serum) and 0.5 IU/ml (British working standard) HBsAg were included in each run. Mixtures were reported as positive or negative using standard manufacturer's criteria of the mean optical density (OD) of negative controls +0.040 OD units.

Results

In all, 66 945 antenatal sera from Plymouth patients were tested for HBsAg between 1986 and 1996. In that time 26 positive patients were detected. Twelve would have been identified by non-European surname; nine patients would have remained undetected if selective testing had been done on the basis of a foreign surname or risk factor information supplied by the requesting clinician.

All 6050 antenatal sera submitted in 1996 were available for retrieval and individual testing. Two HBsAg positive patients had been detected using the routine method over that period. Both of these were again found to be positive on retesting; no extra HBsAg positives were detected.

The distribution of HBsAg concentrations in Birmingham antenatal carriers is given in fig 1. The results are skewed because the assay can only measure up to 10 000 IU/ml. Thirty one cases exceeded this level; 122 (79.7%) were anti-HBe positive, with a median HBsAg of 2400 IU/ml; 20 (13.1%) were e marker indeterminate, with a median HBsAg of 4485 IU/ml; and 10 (6.5%) were HBeAg positive, with a median HBsAg of > 10 000 IU/ml.

Of the first 120 pools of Plymouth sera examined for anti-HBs, 50 (42%) contained no detectable anti-HBs, 31 (26%) contained 10–100 IU/ml, and 38 (32%) contained > 100 IU/ml. The distribution of anti HBs concentrations in the second trial is given in table 1, which shows that relatively few pools contain high concentrations of antibody.

The potential neutralising effect of anti-HBs on various concentrations of HBsAg is illustrated in table 2. The detection limit is 0.1 IU/ml HBsAg with no anti-HBs added; this rises progressively to 200 IU/ml HBsAg when

20 000 IU/ml anti-HBs are added. With this information the probability of a false negative result at any level of HBsAg can be calculated. As an example, 6.0% of the 150 antenatal carriers had HBsAg concentrations in the region of 20 to 199 IU/ml, giving a pooled concentration of 2.0 to 19.9 IU/ml. From table 2 we see that an anti-HBs level above approximately 1900 IU/ml may cause a false negative result when mixed with HBsAg concentrations in this range. From table 1 we can see that 12 of 88 pools (13.6%) contain such concentrations of anti-HBs, so this group of cases contributes 0.82% (6% × 13.6%) to the total missed. The overall percentage of positives which may be missed by pooling was estimated by summing the percentages potentially missed in each of the following HBsAg ranges: 1–4, 5–19, 20–199, 200–1999, > 2000 IU/ml. The overall reduction in sensitivity is estimated at 4.2% over the whole range of HBsAg concentrations encountered in the antenatal population, that is, less than 1 in 20 HBsAg positive specimens might be missed, those being ones with low HBsAg concentrations.

Discussion

Plymouth PHL clearly serves a very low prevalence area for hepatitis B, with only two to five acute cases a year in total detected over the last five years. Despite this low prevalence, our finding that nine of 26 antenatal cases detected had European surnames and no previously identified risk factors supports the case for universal screening. This service is valued by our obstetricians and general practitioners and also provides reassurance to healthcare workers who have not responded to hepatitis B immunisation.

While universal screening is widely regarded as desirable, the fact that only 27% of centres in England and Wales currently perform it suggests that many purchasers remain unconvinced about its cost-effectiveness in low prevalence areas. Our pooling method dramatically reduces the cost of universal testing but inevitably results in some loss of sensitivity in the low risk group screened in this way.

The loss in sensitivity associated with pooling samples must be assessed in the light of the aim of the test, that is, to identify women who are likely to transmit HBV infection to their infants. Modern HBsAg assays are so sensitive, and HBV carriers have such high circulating concentrations of HBsAg, that a false negative will be an extremely rare event, at

worst occurring every nine to 10 years in our antenatal population. To screen all sera individually would add about £11 000 a year to laboratory costs, giving a cost of approximately £99 000 for each extra antenatal carrier detected. Since these cases would not be HBeAg positive, the chance of vertical transmission resulting from a false negative screen would be substantially less than 10%. The bottom line is that individual screening of our antenatal population would at best prevent a single extra case of neonatal HBV infection every 90 years at a cost in excess of £990 000. We estimate a cost of approximately £13 300 per case of neonatal HBV infection prevented by the pooled screening method at current values.

Ultimately, decisions on the most appropriate screening method for hepatitis B can only be made by purchasing authorities taking into account the ethnic mix and prevalence of

hepatitis B in their area, and the opportunity costs of a comprehensive screening programme. National guidelines would be welcome but it is hard to see how a single strategy can be applied cost-effectively in both high and low prevalence areas.

We acknowledge the contribution of Dr Sheena Reilly and Dr Peter Wilkinson who were involved in developing the pooled screening method and Mr Mark Wallis for technical assistance.

- 1 Fields BN, Knipe DM, Howley PM. *Virology*. Philadelphia: Lippincott-Raven, 1996.
- 2 Chrystie IL, Sumner D, Palmer S, *et al*. Screening of pregnant women for evidence of current hepatitis B infection: selective or universal? *Health Trends* 1992;24:13–15.
- 3 Department of Health. *Immunisation against infectious disease*. London: HMSO, 1996.
- 4 Boxall EH. Antenatal screening for carriers of hepatitis B virus. *BMJ* 1995;311:1178–9.
- 5 Dwyer MJ, McIntyre PG. Antenatal screening for hepatitis B surface antigen: an appraisal of its value in a low prevalence area. *Epidemiol Infect* 1996;117:121–31.
- 6 Boxall EH, Peterson S, Diment J, *et al*. A novel assay for hepatitis B e markers. *J Med Virol* 1997;52:280–5.