What Level of Hepatitis B Antibody Is Protective?

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This study assessed the level of vaccine-induced hepatitis B surface antibody that is protective against hepatitis B infection and carriage in The Gambia. Sera from 700 of a cohort of 1041 children vaccinated against hepatitis B in infancy were serially tested for markers of hepatitis B until age 7 years. No absolute level of protection against infection was found, but all children who attained a peak antibody response to vaccination of ≥10 IU/L were protected against carriage of hepatitis B surface antigen. Two-thirds of 45 infected children experienced brief infection (determined by loss of core antibody). This transient infection was likely related to surface antibody level. The data support the use of the peak antibody response as the best indicator of protection against carriage and suggest that most infections after vaccination are short-lived.

In countries with high prevalence of hepatitis B, the main intent of vaccination is to prevent the hepatitis B carrier state and associated chronic liver diseases. There has been debate over the level of antibody induced by vaccination that is protective against infection and against carriage [1, 2]. This is an important issue, since it may determine the need for booster vaccination and the need for testing of antibody level of vaccinees in more developed countries. Here we report the results of regular monitoring of serologic markers in a cohort of vaccinated Gambian children and estimate the risk of infection and the probability of loss of core antibody by surface antibody level.

Method

As part of The Gambia Hepatitis Intervention Study [3] initiated in 1986, which aims to evaluate the effect of hepatitis B vaccination on chronic liver disease, 1041 neonates were recruited to be geographically representative of the Gambian population. The method of recruitment has been described in detail elsewhere [4]. A sample of blood was obtained from the child’s mother at recruitment for assessment of her hepatitis B status. The child then received 4 doses of hepatitis B plasma-derived vaccine (Merck Sharpe & Dohme [West Point, PA]; 10 μg/dose). The target ages for these doses were as soon after birth as possible, 8 and 16 weeks, and 9 months. The vaccine was delivered through the Gambian routine immunization program. Thus, the actual age at vaccination varied within the cohort [5]. At ages 1, 2, 3, 4, 5, and 7 years, a fingerstick sample of blood was obtained, separated, and stored at −70°C until analyzed. The sera were analyzed at the MRC Laboratories in The Gambia for hepatitis B surface antigen by reverse passive hemagglutination (Wellcotest; Murex Diagnostics, Dartford, UK). For positive samples, the test was repeated after neutralization with rabbit anti-HBs. Hepatitis B core antibody, surface antibody, and e antigen were measured by RIA (Sorin Biochemica, Saluggia, Italy). Quantitation of anti-HBs concentrations was done using the World Health Organization reference standard.

In order to roughly quantitate the level of core antibody, the percentage inhibition was calculated using an assay cutoff value of 0%. Samples were considered positive if inhibition was ≥10%. Geometric mean surface antibody levels were calculated at each follow-up time to monitor antibody decay. Only children who showed no serologic evidence of infection were included in these calculations. Mean levels only included children with a surface antibody concentration of ≥10 IU/L. Antibody levels were adjusted by a regression model to allow for time between vaccination and sampling.

The risk of infection was estimated for each year by antibody stratum, and these risks were then aggregated over the years to give rates of infection by antibody stratum. Individuals were assigned to antibody strata in two ways: by peak level (antibody level at year 1 of follow-up) and by the most recent level preceding core antibody conversion. Thus, a child would always be classified to the same “peak” stratum but could move between “most recent” strata year to year.

Logistic regression was used to estimate risks of infection by antibody stratum adjusted for year of infection. The probability of loss of core antibody by stratum level was calculated as the number of those infected who lost core antibody in a particular surface antibody stratum divided by the total number infected in that stratum. The analysis by most recent antibody stratum used the level immediately before infection. All statistical analyses were performed using the Epilog (Epicenter Ware, Pasadena), Epi Info...
Results

Although 1041 children were recruited, by the first year of follow-up, only 764 (75%) could be traced and sera obtained due to both migration and mortality. Since then, loss to follow-up has been minor, with 704 children (68%) traced at age 7 years. The children traced were representative of the recruited cohort in terms of sex and maternal hepatitis B status.

At year 1 of follow-up, 94% of children were surface antibody-positive (anti-HBs >10 IU/L) but negative for hepatitis B core antibody, indicating they had been successfully vaccinated and were uninfected. A small proportion (1%) had no evidence of response to the vaccine. Four percent were positive for antibodies to both surface and core antigens. However, 31 of these 33 became negative for core antigens during the second year of life, indicating passive transfer of the antibody from the mother. A third of the mothers (11) were hepatitis B carriers, and 6 were e antigen-positive, suggesting they had high concentrations of circulating core antibody. Finally, 4 children were negative for surface antibody but positive for core antibody. Two of these children were also surface antigen-positive and have remained so throughout the follow-up period, indicating they are carriers. Since both of their mothers were e antigen-positive carriers, it seems likely they were infected perinatally.

Antibody decay: In subsequent years, there has been a decay in surface antibody from a geometric mean concentration of 2131 (95% confidence interval [CI], 1860–2443) in year 1 to 88 (76–101) in year 7. This decay is shown in figure 1 for children who have never been positive for core antibody. This has resulted in a decline in the proportion of children who are surface antibody-positive, which has been particularly marked after year 4. Thus, in year 5, 84% remained surface antibody-positive and uninfected, whereas by year 7, only 68% had this status. The proportion of children with anti-HBs<10 IU/L rose from 1% in year 1 to 4% in years 2–4, 11% in year 5, and 25% in year 7. In addition, the number of children who were core antibody-positive rose after year 2; the proportion of children who were surface antibody-negative but core antibody-positive, and therefore infected, rose to 2.7% in year 7. In total, 73 children became core antibody-positive during follow-up. The relative risk of seroconversion in children with carrier mothers (vs. noncarrier mothers) was 1.99 (95% CI, 1.01–3.89). The relative risk for those with e antigen-positive versus e antigen-negative carrier mothers was 6.92 (95% CI, 2.34–20.4).

Infections. Surface antigen was detected in 4 infected children. One child became positive for core antibody in year 2 and for core antibody and surface antigen in year 3, and he was not seen again until year 7, when he was surface antigen-negative but core antibody-positive. Two children who seroconverted became carriers. One was positive at year 3 and the other at year 7. Neither responded to the vaccine. Another child was surface antigen-positive. At first follow-up (year 3), she

![Figure 1. Geometric mean concentration (GMC) of hepatitis B surface antibody and % negative (<10 IU/L) by year of follow-up in 1041 vaccinated Gambian children. CI, confidence interval.](image-url)
was found to be a carrier and has so remained. Her vaccine response is not known nor is it known when she was infected, although her mother was an e antigen–negative carrier.

**Risk of infection.** Because exposure to infection and decay in antibody may change over time, the analysis of risk of infection by surface antibody was adjusted for year of seroconversion. Two comparisons were made: First, the relative risk of infection was assessed by peak antibody level and compared with children who did not respond to the vaccine, and second, the relative risk of infection was assessed by surface antibody level immediately preceding seroconversion and compared with children who had antibody levels of <10 IU/L immediately before conversion. The adjustment for year of seroconversion was valid, as there was no statistically significant variation in rate of seroconversion from one year to another after controlling for surface antibody level. The results of the analysis by antibody level after adjustment are shown in table 1. The trend in risk of seroconversion adjusted for year was highly significant whether peak \( (P < .001) \) or most recent \( (P < .001) \) antibody was used, although the fit of the model was considerably better using most recent antibody levels. There was no evidence of complete protection at any antibody level, although the protective efficacy in the highest antibody stratum \((>10,000)\) was 99% when the most recent antibody was considered.

**Core antibody reversion.** Many infected children were subsequently found to be negative for core antibody. The relationship of subsequent loss of core antibody or reversion to both peak and most recent surface antibody level is shown in table 1. In both of these calculations, children given vaccine after the first year were excluded because there was no measure of their peak response. Information was not available for children converting to core antibody in year 7, as further follow-up has not yet been done. A test of trend in seroconversion with peak antibody was highly significant \( (\chi^2 = 11.95, P < .001) \), as was the trend with most recent antibody level \( (\chi^2 = 12.66, P < .001) \); however, the results suggest that reversion of core antibody to negative is more closely associated with the most recent antibody level than with the peak level.

### Table 1. The risk of infection and probability of core antibody reversion by peak and most recent surface antibody stratum.

<table>
<thead>
<tr>
<th>Antibody stratum IU/L</th>
<th>No. of infections/person years at risk</th>
<th>Adjusted RR (95% confidence interval)</th>
<th>P of core antibody reversion (children reverting/total at risk)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1000–</td>
<td>5/538</td>
<td>0.03 (0.005–0.15)</td>
<td>1 (4/4)</td>
</tr>
<tr>
<td>100–</td>
<td>24/1321</td>
<td>0.07 (0.02–0.30)</td>
<td>.94 (15/16)</td>
</tr>
<tr>
<td>10–</td>
<td>6/142</td>
<td>0.36 (0.07–1.81)</td>
<td>0 (0/4)</td>
</tr>
<tr>
<td>&lt;10</td>
<td>2/44</td>
<td>1</td>
<td>.50 (1/2)</td>
</tr>
<tr>
<td>Antibody level at 1 year</td>
<td>Antibody level in year preceding conversion</td>
<td>Adjusted RR (95% confidence interval)</td>
<td>P of core antibody reversion (children reverting/total at risk)</td>
</tr>
<tr>
<td>No. of infections/person years at risk</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adjusted RR</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>12156</td>
<td>20/1094</td>
<td>0.06 (0.03–0.13)</td>
<td>.88 (14/16)</td>
</tr>
<tr>
<td>12856</td>
<td>18/1094</td>
<td>0.04 (0.02–0.09)</td>
<td>1 (9/9)</td>
</tr>
<tr>
<td>12570</td>
<td>25/1770</td>
<td>0.18 (0.09–0.36)</td>
<td>.36 (5/14)</td>
</tr>
<tr>
<td>14/16</td>
<td>1 /209</td>
<td>0.01 (0.001–0.09)</td>
<td>1 (1/1)</td>
</tr>
</tbody>
</table>

**Discussion**

This cohort study clearly demonstrates the decay in antibody level over time. The decay curve has the characteristic logarithmic form that has been described in both children and adults [6, 7] in both Africa and Europe. This decay is associated with increasing rates of infection.

The risk of infection among children vaccinated against hepatitis B is not an all-or-nothing phenomenon but is related to the antibody level attained after vaccination. However, among the seroconverters, the 2 children who became persistently surface antigen–positive (carriers) never mounted antibody responses >10 IU/L. This justifies the use of this cutoff as a measure of vaccine response. Because there was no control group, it was not possible to estimate whether there was protection from infection at antibody levels ≤10 IU/L.

The probability that children will subsequently revert to negative for core antibody was also related to surface antibody level, as was found in a separate study of vaccinees in the Gambian villages of Keneba and Manduar [8]. All children whose most recent antibody level was >1000 IU/L lost the marker of infection. It is possible that this core antibody positivity is false-positive in some cases; however, the clear relationship to surface antibody argues against this and suggests a transient infection without establishment of a source of core antigen in the liver. These transient infections and the infections manifested by long-term core antibody conversion are unlikely to be important in terms of transmission of infection or subsequent morbidity. The implications for vaccine policy in terms of booster vaccinations are clear in that persistent infection manifested by surface antigen carriage was prevented up to 7 years after vaccination, despite decay in antibody levels.

One aspect of this relationship between conversion, reversion, and surface antibody is that the phenomena noted here are transitory. Once the children have antibody levels ≤100 IU/L, they will be at similar risk of infection and reversion. Whether the risk of becoming a carrier remains zero at that point requires continuing follow-up. A problem is that the peak transmission period for these children is now past (~4 years of age), and further high levels of challenge to their immunity may be at the age at which they begin sexual exposure to the virus.

Finally, the recognition of mutant hepatitis B virus in the vaccinated populations of Keneba and Manduar villages [9] raises the possibility that some of these phenomena may be related to mutations in the S gene of hepatitis B. This will be examined in detail in year 9 of follow-up.
References