Hepatitis B immune memory in children primed with hexavalent vaccines and given monovalent booster vaccines: an open-label, randomised, controlled, multicentre study

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Summary
Background In 2000, hexavac and infanrix hexa were licensed in Europe for primary immunisation of children against diphtheria, tetanus, pertussis, poliomyelitis, hepatitis B, and invasive infections caused by Haemophilus influenzae b. In 2005, hexavac was suspended because of concerns about the long-term immunogenicity of its hepatitis B component. We aimed to assess the duration of immunity and need for booster injections in children primed with these vaccines.

Methods In an open-label, randomised, controlled, multicentre study in six local health units and at the Bambino Gesù Paediatric Research Hospital in Italy, antibody concentrations were measured 5 years after immunisation of infants with hexavac or infanrix hexa. Children with concentrations of antibodies to hepatitis B surface antigen (anti-HBs) lower than 10 mIU/L were randomly assigned by simple randomisation to receive a booster of HBVaxPro or engerix B monovalent hepatitis B vaccine and tested 2 weeks later. Primary endpoints were the proportion of children with anti-HBs concentrations of at least 10 mIU/mL, geometric mean concentrations (GMCs) of antibody 5 years after vaccination, and the proportion of children with anti-HBs concentrations lower than 10 mIU/mL who had anamnestic response to booster. The study is registered with Agenzia Italiana del Farmaco, code FARM67NFPN.

Findings 1543 children were enrolled, 833 had received hexavac and 710 infanrix hexa. 831 children who received hexavac and 709 who received infanrix hexa were included in the analysis. 319 children who received hexavac (38.4%, 95% CI 35.1–41.7) had anti-HBs concentrations of at least 10 mIU/mL compared with 500 who received infanrix hexa (83.2%, 80.5–86.0; p=0.0001). GMCs before booster were 4.5 mIU/mL in the hexavac group compared with 9.5 mIU/mL in the infanrix hexa group (p<0.0001). After booster 409 (92.1%, 89.6–94.6) of 444 children primed with hexavac and 99 (94.3%, 89.8–98.7) of 105 primed with infanrix hexa had anti-HBs concentrations of at least 10 mIU/mL (p=0.4). GMCs were 448.7 mIU/mL and 484.9 mIU/mL (p=0.6). The two booster vaccine groups did not differ in number of side-effects; no serious adverse events were reported.

Interpretation 5 years after immunisation with hexavalent vaccines, immunological memory seems to persist in children with anti-HBs concentrations lower than 10 mIU/mL, suggesting that booster doses are not needed. Additional follow-up is needed.

Funding Agenzia Italiana del Farmaco.

Introduction Safe and effective vaccines against hepatitis B have been available since the 1980s and have provided the opportunity worldwide to prevent and to control this disease and its consequences, such as cirrhosis and primary hepatocellular carcinoma. Strategies for hepatitis B vaccination initially targeted groups of patients and individuals who were at increased social or professional risk of exposure to hepatitis B virus. Failure of these strategies to reduce the burden of hepatitis B in the general population led WHO to recommend that all countries should introduce universal infant or adolescent hepatitis B vaccination into their national immunisation programmes by 1997. By the end of 2008, 177 countries had introduced hepatitis B vaccination into routine neonatal, infant, or adolescent immunisation programmes, or a combination thereof; Italy was one of the first countries to implement a universal strategy. Plasma-derived vaccines were first used for immunisation but were replaced by DNA recombinant vaccines in 1986, which are safe and provide long-lasting protection. Since the 1990s, a broad range of combined vaccines that include hepatitis B surface antigen (HBsAg) have been developed for vaccination of infants and young children. In the past decade, two hexavalent vaccines have been used for the prevention of infections caused by diphtheria, tetanus, pertussis, poliomyelitis, hepatitis B, and invasive infections caused by Haemophilus influenzae b—hexavac (Sanofi Pasteur MSD) and infanrix hexa (GlaxoSmithKline). These vaccines were licensed in the European Union in October, 2000, for primary and booster vaccination of children. In September, 2005, after reduced immunogenicity of the hepatitis B component in the hexavac vaccine was reported, the European Medicines Agency (EMA) recommended suspension of hexavac marketing authorisation. By contrast, no actions

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were taken with regards to infanrix hexa, which has comparable immunogenicity to that reported in the licensing documentations and to that after immunisation with monovalent hepatitis B vaccines.\textsuperscript{15–18}

In an analysis of data concerning hexavac, over 95% of children vaccinated with hexavac had protective concentrations (≥10 mIU/mL) of antibodies against HBsAg (anti-HBs) when tested 1 month after primary immunisation;\textsuperscript{19} however, antibody concentrations were low (10–99 mIU/mL) in at least 5–20% of vaccinated children. In a small cohort of children aged 7–9 years who were vaccinated with hexavac and who received a booster dose of monovalent hepatitis B vaccine, those who had concentrations of anti-HBs of 10–100 mIU/mL after vaccination with hexavac responded less efficiently or not at all to the booster vaccine compared with children with antibody concentrations of 100–1000 mIU/mL.\textsuperscript{19} Moreover, children who were simultaneously given hexavac and a seven-valent pneumococcal conjugate vaccine (Prevenar, Wyeth) or hexavax and a meningococcal type C conjugated vaccine (NeisVac-C, Baxter) had lower than expected anti-HBs seroconversion rates and antibody geometric mean concentrations (GMCS).\textsuperscript{14,20}

Because peak antibody concentration achieved after primary immunisation is associated with the duration that concentrations remain within the protective range, the low antibody concentrations after first immunisation with hexavac might not provide protection against hepatitis B during adolescence and adulthood when risk of exposure to hepatitis B virus increases, possibly as a result of loss of immunological memory. This issue is crucial in Europe—especially in Germany, Austria, Italy, and to a lesser extent in France, Greece and Spain—where hexavac was given to several cohorts of infants from the end of 2000 until suspension in 2005.

We aimed to assess the duration of immunity from vaccination with hexavac or infanrix hexa and the need for a booster 5 years after primary vaccination.

**Methods**

**Patients**

The study included healthy children born to HBsAg-negative mothers in 2001–03 (ie, at least 5 years before the start of the study) and who had received three doses of the same hexavalent vaccine (hexavac or infanrix hexa) during the first year of life. Exclusion criteria were receipt of other hepatitis-B-containing vaccines either alone or in combination; present acute or chronic illness, or congenital or acquired immune disorder; history or symptoms of hepatitis B; and known sensitivity or allergy to any component of the study vaccines.

Children were invited to participate when they were offered the diphtheria–tetanus–pertussis vaccine booster dose that, according to the Italian programme of vaccination, is given at 5–6 years of age. Only one brand of hexavalent vaccine was used in every local health unit. Children had either received three 0.5 mL doses of hexavac (HBsAg content 5 μg) or infanrix hexa (HBsAg content 10 μg) at 3, 5, and 11 months, in accordance with the Italian vaccination schedule.

Before inclusion, children’s parents or legal guardians provided written informed consent, and verbal assent was given by each child. Approval was obtained from the ethics committee of the University of Milan before the start of the study.

**Procedures**

Demographic information (including age and gender, number of household members, and father’s standard of education) was collected with a standardised questionnaire. A blood sample (5 mL) was obtained from each child at enrolment to measure anti-HBs concentration and the presence of antibodies to hepatitis B core antigen (anti-HBc) as a marker of hepatitis B virus infection. Children who had anti-HBc were further tested for HBsAg and hepatitis B virus DNA. All assays were done at the University of Milan by personnel masked to the brand of vaccine used for the primary course of vaccination and for boosting.

Children with anti-HBs concentrations of at least 10 mIU/mL were considered immune. Those with concentrations less than 10 mIU/mL were offered a booster dose of a monovalent hepatitis B vaccine and assigned by simple randomisation to receive either 5 μg of HBVaxPro (Sanofi Pasteur MSD) or 10 μg of engerix B (GlaxoSmithKline). Parents or guardians of children who received booster injections were asked to keep a daily record of any adverse events for 7 days. A second blood sample was obtained from each child 2 weeks (give or take 3 days) after the booster dose to measure anti-HBs concentrations. An anamnestic response was defined as an increase in anti-HBs concentration of four times or more after the booster vaccine or providing an antibody concentration of at least 10 mIU/mL was achieved after the booster. Children who had antibody concentrations less than 10 mIU/mL after booster were offered two further doses of the same monovalent vaccine at 1 month and 6 months after the first booster vaccine. Children were tested again for anti-HBs 1 month after completion of vaccination.

HBsAg, anti-HBc, and anti-HBs were detected by commercially available kits (AxSYM HBsAg, CORE, and AUSAB, Abbott, IL, USA). The measurement range of AxSYM AUSAB is 2–1000 mIU/mL, defined by the limit of detection and the maximum of the calibration curve. Samples with anti-HBs greater than 1000 mIU/mL were diluted with the manual dilution protocol, according to the manufacturer’s instructions, to obtain...
the final sample concentration. Samples below the detection limit (2 mIU/mL) of the assay were recorded as undetectable. Hepatitis B virus DNA was detected by real-time PCR by the TaqMan HBV test (Roche, NJ, USA) with a 95% detection limit of 6·7 IU/mL (about 39 copies per mL).

Primary endpoints of this study were the proportion of children with anti-HBs of at least 10 mIU/mL at enrolment, GMCs of anti-HBs, and the proportion of children with anti-HBs less than 10 mIU/mL at enrolment who had an anamnestic response to monovalent booster vaccine. Secondary endpoints were the prevalence of serological markers of hepatitis B virus infection (anti-HBc, HBsAg, and viral DNA) in the two cohorts of children at enrolment and the proportion of children with antibody concentrations after booster lower than 10 mIU/mL who developed protective antibodies (≥10 mIU/mL) after two extra doses of monovalent vaccine.

The study protocol is registered with Agenzia Italiana del Farmaco, code FARM67NFPN.

**Statistical analysis**

Assuming that 5 years after the primary course of vaccination about 15% of children who received infanrix hexa and at least 30% of those vaccinated with hexavac would have anti-HBs concentrations below 10 mIU/mL, a sample size of 174 children for each cohort would be needed to detect a statistically significant difference between groups (α=0·05, β=0·10).

For samples with an undetectable antibody concentration, an arbitrarily value of 0·5 mIU/mL was assigned to allow calculation of GMCs. Percentages of children grouped by anti-HBs concentration and 95% CIs were calculated before and after the booster; the χ² test was used to compare the two groups primed with different hexavalent vaccines. GMCs and 95% CIs were calculated and the non-parametric Mann-Whitney U test was used to compare the two vaccine groups; p<0·05 was regarded as significant. After the univariate analysis we did a multivariate analysis in which logistic regression models were used to calculate the association between the type of
hexavalent vaccine and the absence of protective antibody concentrations, adjusted for potential confounding variables (age, time from the third vaccine dose, and father’s standard of education).

Statistical evaluations were done with Stata statistical software (version 8.2).

Role of the funding source

The study sponsor had no role in the study design, data collection, data analysis, data interpretation, writing of the report, or in the decision to submit the paper for publication. All authors had full access to all data reported in this study and had final responsibility for the decision to submit for publication.

Results

From February, 2008, to July, 2009, 1543 children were enrolled in the study (figure). Of these, 833 (54%) had previously been vaccinated with hexavac and 710 (46%) with infanrix hexa. Three children (two who were primed with hexavac and one with infanrix hexa) were anti-HBc positive but negative for both HBsAg and hepatitis B virus DNA and were excluded from further data analysis.

Demographics and baseline characteristics were comparable between groups (table 1). 909 (59·0%) of 1540 children had anti-HBs concentrations of at least 10 mIU/mL (figure, table 2). The proportion of children with protective antibody concentrations was lower in children primed with hexavac than in those primed with infanrix hexa (p<0·0001). Children primed with hexavac were more likely to have undetectable antibody than were those primed with infanrix hexa (p<0·0001). The proportion of children with protective antibody concentrations was lower in children primed with hexavac than among those primed with infanrix hexa (p<0·0001), as was the GMC (p<0·0001). In the multivariate analysis, the type of hexavalent vaccine given was the only determinant of anti-HBs concentration after adjustment (data not shown).

560 (88·8%) of the 631 children with anti-HBs below 10 mIU/mL at the start of the study were randomly assigned booster: 453 (88%) of children primed with hexavac and 107 (90%) of those primed with infanrix hexa to a booster vaccine of engerix B or HBVaxPro (figure). 71 children were not included because their parents or guardian declined participation. 11 children who had a booster vaccine did not give blood samples for testing. Blood samples for measurement of antibody concentrations after booster were obtained from 549 children. Of these, 508 (92·5%) showed an anamnestic response, and the remaining 41 (7·5%) still had antibody concentrations below 10 mIU/mL (table 3). The proportions of children

<table>
<thead>
<tr>
<th>Total (n=549)</th>
<th>Primed with hexavac (n=444)</th>
<th>Primed with infanrix hexa (n=105)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;10 mIU/mL</td>
<td>41 (7·5%, 5·3−9·7)</td>
<td>35 (7·9%, 5·4−10·4)</td>
<td>0·6</td>
</tr>
<tr>
<td>≥10 mIU/mL</td>
<td>508 (92·5%, 90·3−94·7)</td>
<td>409 (92·1%, 89·6−94·6)</td>
<td>0·4</td>
</tr>
<tr>
<td>10-100 mIU/mL</td>
<td>69 (12·6%, 9·8−15·3)</td>
<td>62 (13·9%, 10·7−17·2)</td>
<td>0·06</td>
</tr>
<tr>
<td>100-1000 mIU/mL</td>
<td>220 (40·1%, 36·0−44·2)</td>
<td>162 (36·5%, 32·0−41·0)</td>
<td>0·0006</td>
</tr>
<tr>
<td>&gt;1000 mIU/mL</td>
<td>219 (39·3%, 35·8−44·0)</td>
<td>185 (41·7%, 37·1−46·3)</td>
<td>0·1</td>
</tr>
<tr>
<td>GMC (mIU/mL)</td>
<td>455·4 (374·0−554·6)</td>
<td>448·7 (359·6−559·9)</td>
<td>0·6</td>
</tr>
</tbody>
</table>

Data are number (%), 95% CI. GMC=geometric mean concentration.

Table 3: Post-booster anti-HBs concentration
who had an anamnestic response to booster did not differ between those who were primed with hexavac and those primed with infanrix hexa (p=0·4). Antibody GMCs after booster also did not differ significantly between the two groups (p=0·6). In both groups of children, anti-HBs concentrations after booster were higher in children with detectable antibody concentrations than in those with undetectable antibody concentrations at enrolment (table 4; group primed with hexavac p<0·0001; group primed with infanrix hexa p=0·002).

Of 549 children tested after the booster, 272 were given engerix-B, 270 were given HBVaxPro, and the type of booster vaccine was unknown in seven (figure). Anamnestic response rates and GMCs did not differ between children boosted with HBVaxPro and those boosted with engerix-B in either primed vaccine group (table 5).

The diary records of booster side-effects were returned by the parents or guardians of 535 of 560 children. 55 children (10·3%) had transient mild reactions mostly confined to the site of the injection (swelling and induration), with no differences between the two booster vaccine groups (31 [11%] of the 281 children primed with infanrix hexa vs 24 [9%] of the 272 children boosted with HBVaxPro; p=0·5); no serious adverse events were reported.

35 of 41 children (31 of 35 primed with hexavac and four of six primed with infanrix hexa) who had anti-HBs concentrations less than 10 mIU/mL after the booster dose of vaccine had two additional doses of vaccine. After 1 month, 32 (91%) of 35 children had anti-HBs concentrations greater than 100 mIU/mL. The remaining three children (9%), all of whom were primed with hexavac, had anti-HBs concentrations of 10–100 mIU/mL. Antibody GMC was lower in children primed with hexavac than in those primed with infanrix hexa (584·5 mIU/mL vs 2756·8 mIU/mL; p=0·008).

### Discussion

In our study, both the proportion of children with seroprotective concentrations of anti-HBs and their GMCs were significantly lower among children who received hexavac than among those immunised with infanrix hexa, which is consistent with previous findings. Additionally, most children who received a monovalent booster vaccine showed a rapid anamnestic response, and the proportion of responders and their GMCs were similar between groups.

Previous data has shown that, 5–13 years after primary vaccination with monovalent recombinant vaccines, nearly 90% of patients with anti-HBs concentrations lower than 10 mIU/mL before booster responded to booster vaccination. Likewise, over 95% of children aged 4–9 years who received four doses of infanrix hexa as infants and had anti-HBs below 10 mIU/mL have had an anamnestic response when boosted. In a preliminary study done in Italy, the proportion of children who had antibody concentrations below 10 mIU/mL 15 months after primary vaccination was significantly higher among those who received hexavac than among those who were given infanrix hexa (31% vs 4%; p=0·0001). However, a similar rapid anamnestic response was observed in both groups,

### Table 5: Anti-HBs concentrations after booster

<table>
<thead>
<tr>
<th></th>
<th>&lt;10 mIU/mL</th>
<th>10–100 mIU/mL</th>
<th>100–1000 mIU/mL</th>
<th>&gt;1000 mIU/mL</th>
<th>GMC (mIU/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primed with hexavac*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Undetectable</td>
<td>270</td>
<td>35 (13·0%, 8·9–17·0)</td>
<td>53 (19·6%, 14·9–24·4)</td>
<td>107 (39·6%, 33·8–45·5)</td>
<td>75 (27·9%, 22·4–33·1)</td>
</tr>
<tr>
<td>2–10 mIU/mL</td>
<td>174</td>
<td>0</td>
<td>9 (5·2%, 1·9–8·5)</td>
<td>55 (31·6%, 24·7–38·5)</td>
<td>110 (63·2%, 56·1–70·4)</td>
</tr>
<tr>
<td>Primed with infanrix hexa*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Undetectable</td>
<td>47</td>
<td>6 (12·8%, 3·2–22·3)</td>
<td>6 (12·8%, 3·2–22·3)</td>
<td>24 (51·1%, 36·8–65·4)</td>
<td>11 (23·4%, 11·3–35·5)</td>
</tr>
<tr>
<td>2–10 mIU/mL</td>
<td>58</td>
<td>0</td>
<td>1 (1·7%, 1·6–5·1)</td>
<td>34 (58·6%, 45·9–71·3)</td>
<td>23 (39·7%, 27·1–52·2)</td>
</tr>
</tbody>
</table>

*Concentrations after booster in detectable compared with undetectable at enrolment in those primed with hexavac p<0·0001 and those primed with infanrix hexa p=0·002.

Data are number (%; 95% CI) or concentration (95% CI). GMC = geometric mean concentration.
groups of children when they were given a booster dose of monovalent hepatitis B vaccine in their third year of life.

Together, our data and previous findings suggest that in healthy vaccinated children the immunological memory for HBsAg might outlast the presence of antibody, providing effective protection even in those showing waning or undetectable concentrations of anti-HBs after primary vaccination. Thus, children who have lost preventive antibody concentrations might still maintain T-cell memory that is able to trigger anti-HBs production by B cells when activated by revaccination.30 Loss of antibody might not necessarily mean loss of protection because the long incubation period of hepatitis B could allow time for the immunological memory to protect children against acute disease and the development of a chronic carrier state. Data from the Italian surveillance system showed no cases of hepatitis B could allow time for the immunological memory to protect children against acute disease and the development of a chronic carrier state. Data from the Italian surveillance system showed no cases of hepatitis B were already positive at the time of vaccination or whether they were subsequently infected with hepatitis B virus is unknown. However, because these children were born to HBsAg-negative mothers, they probably acquired infection after vaccination. Most children who acquire infection tend to develop a chronic carrier state, thus vaccination might have helped to partly protect these children whose exposure to hepatitis B virus resulted in asymptomatic infections. All 35 children in our study who did not respond to the booster dose achieved protective antibody titres 1 month after a second course of vaccination.

A limitation of our study is that we had no data on antibody concentrations immediately after primary vaccination. Therefore, we do not know whether children with antibody concentrations below the protective concentration before booster were poor responders or non-responders to the primary vaccination or whether their antibody concentrations had decreased over time. However, this limitation does not affect the findings of this study, which was aimed at comparing the different seroprotection rates and GMCs in children primed with hexavalent vaccines, especially specific to the 5-year checkpoint. Additional follow-up of children immunised with hexavalent vaccines, especially those injected with hexavax, is required to identify whether immunological memory persists during adolescence and adulthood or whether a booster might be needed later in life to maintain lifelong protection.

Contributors
ARZ, LR, AET, and FD’A designed the study, collected, analysed, and interpreted data, and wrote the draft of the paper. LR did the laboratory serological assays. CG, AP, and AB coordinated the work of the local health units, collected and interpreted data, and critically revised the paper. VC, GB, GM, EV, MAM, and DM enrolled and followed up vaccinated individuals, collected and interpreted data, and critically revised the paper. Study group investigators were involved in the follow-up of children, and collected and interpreted the data. The final draft was written by ARZ and was seen and approved by all authors.

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Conflicts of interest
ARZ has received honoraria for participation in two scientific advisory boards meetings on seasonal influenza and for lectures from GlaxoSmithKline; and has served as scientific expert in a clinical trial on hepatitis B vaccination sponsored by Sanofi Pasteur MSD, for which his department received a research grant. EV has received travel or accommodation expenses from GlaxoSmithKline for participation in a national meeting. AET has received research grants for a study on rotavirus from GlaxoSmithKline and for a study on conjugated pneumococcal vaccine from Wyeth, and travel or accommodation expenses for participation in scientific meetings from GlaxoSmithKline and Wyeth. All other authors declare that they have no conflict of interest.

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References
1 World Health Organization Expanded Programme on Immunization


