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i Medical Microbiology Laboratory, Public Health Service (GGD), Amsterdam, The Netherlands
j Academic Medical Center, Amsterdam, The Netherlands
Evaluation of immune responses to combined hepatitis A and B vaccine in HIV-infected children and children on immunosuppressive medication
Abstract

Objective

A phase IV interventional study with a combined hepatitis A and B vaccine was conducted in HIV-infected children and children receiving immunosuppressive medication for treatment of rheumatic diseases to evaluate immune responses.

Methods

Both groups (1–16 years of age) received combined (inactivated) HAV and (rDNA) HBV vaccine Ambirix® at months 0 and 6. Serum samples were taken at four time points and tested for anti-HAV and anti-HBs antibodies. Anti-HAV concentrations ≥20 mIU/mL or anti-HBs concentrations ≥10 mIU/mL were considered protective. Seropositivity percentages were calculated and geometric mean concentrations (GMCs) were compared by nonparametric Mann–Whitney U-test or Kruskal–Wallis one-way-analysis-of-variance.

Results

Of 80 HIV-infected children who completed the study, 67 were HAV-susceptible and 68 HBV-susceptible at enrolment. Of 80 children with rheumatic diseases who completed the study, 65 were HAV-susceptible and 74 HBV-susceptible at enrolment. Immune responses to HAV after first dose of vaccine in both study groups were low: 71% and 55% respectively, whereas immune responses after the second dose were 99% and 100% respectively. Immune response to HBV after first dose of vaccine in both groups was also low: 27% and 17% respectively. Immune responses after the second dose were 97% and 93%, respectively.
A larger proportion of children on combination antiretroviral therapy (cART) and of children with viral load <50 copies/mL responded to HBV, and also showed a significantly higher GMC. Conclusions: Although immune response after full series of combined HAV and HBV vaccine in both groups was excellent and comparable to healthy children, a substantial proportion of both groups was not protected for HAV after first dose of vaccine. This protection gap is especially important for HAV in travel health and postexposure prophylactic treatment: both groups of children should be serologically tested for anti-HAV prior to travel to ensure protection if there is no time to await second dose of vaccine.

**Introduction**

The availability of better treatment options for HIV-infected children [1] and children with rheumatic diseases has resulted in their increased survival and improved quality of life [2,3]. An infection in HIV-infected persons [4–10] and those on immunosuppressive medication [11–15], however, may result in increased morbidity and mortality, partly because of changes in cellular immunity induced by the disease itself or because of treatment with immunomodulatory or immunosuppressive agents.

Even after the introduction of HBV vaccination as part of routine immunization programs in most countries, both hepatitis A (HAV) and B virus (HBV) infections are endemic in many countries worldwide and are therefore still an important health problem [16–18], especially for immunocompromised persons. Due to high numbers of international travelers [19], susceptible persons may be exposed to HAV and HBV infection. Whenever HAV is introduced to regions with intermediate or low endemicity, outbreaks of HAV infection can occur and contacts must be protected by postexposure vaccination [20]. In the Netherlands, HAV vaccine is advised to people at risk, such as all persons traveling to HAV-endemic countries and contacts of acute hepatitis A cases. Targeted vaccination programs for HBV risk groups exist and a universal HBV program for all newborns was introduced in 2011.

Studies on the antibody response to HAV vaccine or HBV vaccine in HIV-infected children [21–27] and in particular in children on immunosuppressive medication for rheumatic diseases [14–29] are limited, and thus far have only involved small groups of children.
When children need to be protected against HAV and HBV, this is preferably done through a combined vaccine to overcome the constraints of multiple injections. Since, to our knowledge, no data are available on the immunogenicity of combined vaccines in HIV-infected children and children with rheumatic diseases receiving immunosuppressive medication, we conducted a phase IV interventional study with a combined HAV and HBV vaccine in HIV-infected children and children with rheumatic diseases receiving immunosuppressive medication to evaluate their immune responses.

**Methods**

**Study population**

Children between 1 and 16 years of age, known to be infected with HIV, and children receiving immunosuppressive medication for treatment of rheumatic diseases were recruited from August 2009 till June 2011 at the following outpatient clinics: Emma Children’s Hospital/Academic MC Amsterdam, Reade location Jan van Breemen Amsterdam, Erasmus MC Sophia Children’s Hospital, and Utrecht MC Wilhelmina Children’s Hospital. HIV-infected children were eligible for inclusion if the most recent CD4+ T cell percentage was 15% or higher. In case of rheumatic diseases, patients were eligible if they used immunosuppressive medication for at least 3 months including: prednis(ol)one ≥0.25 mg/kg body weight, methotrexate (Em-thexate® or Metoject®) or biological disease-modifying antirheumatic drugs (DMARDs) as anti-TNF- agents (etanercept [Enbrel®], infliximab [Remicade®], adalimumab [Humira®]) or anti-IL-1 agents (anakinra [Kineret®]), or other immunosuppressive medication (mycophenolate mofetil [CellCept®], azathioprine [Imuran®] or cyclosporine [Neoral®]). Exclusion criteria were: previous vaccination for both HAV and HBV, serologically confirmed prior infection with both HAV and HBV, and administration of immunoglobulin within 6 months prior to enrolment. Children who tested immune for either HAV or HBV could participate in the study as long as they were still susceptible for the other type of hepatitis. They received, as did all participants, the combined HAV and HBV vaccine.

The following data on all included children were collected from the medical records: sociodemographics and medication regimens (name of combination antiretroviral therapy [cART] or immunosuppressive medication, starting date, and dose). For HIV-infected children, the
following data were also collected: nadir CD4+ T-cell count, and both CD4+ T-cell count/percentage and HIV-RNA (viral load) at or close to enrolment as well as at the time of second dose of vaccine or endpoint of the study.

**Vaccine and laboratory testing**

Participants received the combined (inactivated) HAV and (rDNA) HBV vaccine Ambirix®, developed and manufactured by GlaxoSmithKline Biologicals (Rixensart, Belgium) twice: after inclusion in the study and again 26–30 weeks thereafter [30]. The vaccine contains 720 ELISA units of inactivated HAV, 20 mcg of hepatitis B surface antigen (HBs), and 0.45 mg of aluminium as salt, in a total volume of a 1 ml dose.

Blood for serological testing was drawn 4 times: prior to the first dose of vaccine; 4–6 weeks after the first dose of vaccine; prior to the second dose of vaccine; and 4–6 weeks after the second dose of vaccine. Serum samples were tested at the laboratory of the Public Health Service of Amsterdam. Immediately after arrival at the laboratory, blood samples were centrifuged for 10 min at 3000 rpm (210 g) and serum was tested for the presence of HAV and HBV antibodies. The first round serum samples from every subject were tested for qualitative detection of anti-HAV IgG (Abbott, Axsym, HAVAB 2.0) and for quantitative detection (Biomerieux, VIDAS, Anti-HAV Total HAVT). Also the first round samples were tested for qualitative detection of anti-HBc and, if positive, for qualitative detection of HBsAg (Abbott, Axsym, CORE), and quantitative detection of anti-HBs (Abbott, Axsym, AUSAB). All follow-up samples were tested for quantitative detection of anti-HAV IgG antibodies and quantitative detection of anti-HBs. An anti-HAV concentration ≥20 mIU/mL or an anti-HBs concentration ≥10 mIU/mL was considered protective for HAV or HBV infection respectively. Subjects who had a seronegative antibody concentration before the vaccination schedule and a protective concentration after second dose of vaccine were defined as “responders.”

**Medical Ethics Committee**

Study protocol was approved by the Central Committee on research involving human subjects (CCMO; file number NL23065.000.09). Informed consent was obtained from all parents/guardians and assent was obtained from all children ≥12 years of age.
Statistical analyses

Data analysis was performed with SPSS version 19.0.0.1 (2010, IBM, Somers, USA). Significant differences between proportions were assessed using Pearson’s chi-squared. Possible determinants for protective antibody concentration were assessed by means of univariable logistic regression modeling. The variables of sex, ethnicity, age at enrolment, CD4+ T-cell count, nadir CD4+ T-cell count, viral load, use of cART, and type of immunosuppressive medication were included in the univariable analysis.

Anti-HAV IgG and anti-HBs antibody seropositivity percentages were calculated, and geometric mean concentration (GMC) calculations and 95% confidence interval (CI) were performed taking the anti-log of the mean of the log concentration transformations. Children with no detectable anti-HBs were assigned a value of 1 mIU/mL for calculation of geometric means, and subsequently 1 mIU/mL was also added to the immune response of all other children. As the highest value of the Axsym system is >1000 mIU/mL for anti-HBs concentration and >400 mIU/mL for anti-HAV, values of 1001 and 401 were assigned respectively for calculations. Anti-HBs and anti-HAV GMCs were compared using a nonparametric Mann–Whitney U-test or Kruskal–Wallis one-way variance analysis. CD4+ T-cell count and viral load at baseline and at the end of the study were compared using the Wilcoxon signed-rank test (WSRT). A p value <0.05 was considered statistically significant.
Results

HIV-infected children: study population and characteristics (Fig. 1 and Table 1)

A total of 100 HIV-infected children met the inclusion criteria, 81 of whom were willing to participate. Of these, 1 was lost to follow-up. The remaining 80 children completed the study. Thirteen children were HAV-immune, and 12 others were HBV-immune at baseline, resulting in 67 children susceptible for HAV at enrolment, and 68 for HBV.

Of the 80 HIV-infected children, 44 (55%) were male. The majority (65, 81%) had an African ethnicity. The median age was 10 years (IQR 7–12) with 37 (46%) between 6 and 10 years of age. At enrolment 55 (69%) had a viral load <50 copies/mL and 71 (89%) were on cART for a median duration of 86 months (IQR 41–115). A significantly larger proportion of children using cART had a viral load <50 copies/mL (55/71 [78%]; Pearson chi-square p < 0.001).

The median viral load at enrolment was 39 copies/mL (IQR 38–107); 55 (69%) had a viral load <50 copies/mL. The median time between first dose of vaccine and the viral load test was 0 days (IQR 0–91 days). Median CD4+ T-cell count was 858/mm3 (IQR 603–1134). All children had a CD4+ T-cell count >200/mm3 at enrolment, and most (72/80, 90%) ≥500/mm3.

The median time between the first dose of vaccine and determination of the CD4+ T-cell count was 0 days (IQR 0–75 days). There was no difference between the median CD4+ T-cell count and the viral load at baseline and at the end of the study (WSRT respectively p = 0.766 and p = 0.561).

Response to vaccination in HIV-infected children (Table 1)

The median time between the first and second dose of vaccine being given to the 80 HIV-infected children who completed the study was 28 weeks (IQR 27–30). The median interval between first dose of vaccine and second blood sample was 5 weeks (IQR 4–6) and between second dose of vaccine and fourth blood sample 6 weeks (IQR 4–7).
Fig. 1. HIV-infected children and children on immunosuppressive medication for rheumatic diseases treatment: study population and immune response to hepatitis A (HAV) and hepatitis B (HBV) vaccine.
**Table 1.** Characteristics and immune response to the combined hepatitis A and B vaccine in HIV-infected children, August 2009–June 2011.

<table>
<thead>
<tr>
<th>Demographics</th>
<th>Hepatitis A-immune (a) after 1st dose of vaccine</th>
<th>Hepatitis A-immune after 2nd dose of vaccine</th>
<th>Hepatitis B-immune after 1st dose of vaccine</th>
<th>Hepatitis B-immune after 2nd dose of vaccine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(N)</td>
<td>% (95% CI)</td>
<td>(N)</td>
<td>% (95% CI)</td>
</tr>
<tr>
<td><strong>Total susceptible</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>62</td>
<td>44</td>
<td>71% (59-81)</td>
<td>67</td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>35</td>
<td>28</td>
<td>79%</td>
<td>39</td>
</tr>
<tr>
<td>Female</td>
<td>26</td>
<td>16</td>
<td>62%</td>
<td>29</td>
</tr>
<tr>
<td><strong>Ethnicity</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Western</td>
<td>6</td>
<td>6</td>
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<td>African</td>
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<td>39</td>
<td>78%</td>
<td>55</td>
</tr>
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<td>Asian</td>
<td>1</td>
<td>0</td>
<td>0%</td>
<td>2</td>
</tr>
<tr>
<td>Latin American</td>
<td>4</td>
<td>3</td>
<td>75%</td>
<td>4</td>
</tr>
<tr>
<td><strong>Age at enrolment</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>up to 5 years</td>
<td>13</td>
<td>8</td>
<td>62%</td>
<td>13</td>
</tr>
<tr>
<td>6 - 10 years</td>
<td>30</td>
<td>21</td>
<td>70%</td>
<td>32</td>
</tr>
<tr>
<td>11 - 16 years</td>
<td>19</td>
<td>15</td>
<td>79%</td>
<td>22</td>
</tr>
<tr>
<td><strong>HIV-specific factors</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD4 count at enrolment, median, cells/mm(^3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥200 to &lt;500</td>
<td>4</td>
<td>3</td>
<td>75%</td>
<td>5</td>
</tr>
<tr>
<td>≥500</td>
<td>58</td>
<td>41</td>
<td>71%</td>
<td>62</td>
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<tr>
<td>Nadir CD4 count, cells/mm(^3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;200</td>
<td>7</td>
<td>7</td>
<td>100%</td>
<td>10</td>
</tr>
<tr>
<td>≥200 to &lt;500</td>
<td>22</td>
<td>12</td>
<td>55%</td>
<td>25</td>
</tr>
<tr>
<td>≥500</td>
<td>33</td>
<td>25</td>
<td>76%</td>
<td>32</td>
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<tr>
<td>Viral load at enrolment, median copies/mL</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;50</td>
<td>43</td>
<td>33</td>
<td>77%</td>
<td>46</td>
</tr>
<tr>
<td>50 - 1000</td>
<td>12</td>
<td>8</td>
<td>67%</td>
<td>13</td>
</tr>
<tr>
<td>&gt;1000</td>
<td>7</td>
<td>3</td>
<td>43%</td>
<td>8</td>
</tr>
<tr>
<td>On cART at the time of vaccination</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>5</td>
<td>2</td>
<td>40%</td>
<td>6</td>
</tr>
<tr>
<td>Yes</td>
<td>57</td>
<td>42</td>
<td>74%</td>
<td>61</td>
</tr>
</tbody>
</table>

\(^a\) Immune: for hepatitis A, if anti-HAV IgG ≥20 mIU/mL; for hepatitis B, if anti-HBs ≥10 mIU/mL.

\(^b\) 95% confidence interval.
Response to hepatitis A component

Of 67 HAV-susceptible children, a protective antibody response developed in 44/62 (71%) who donated a blood sample after the first dose of vaccine. Univariable analysis showed no associations for developing a protective antibody concentration after the first dose of vaccine (all p > 0.135).

After the second dose of vaccine, a protective antibody response developed in 66/67 (99%). The GMC of the children was 40 mIU/mL after the first dose of vaccine and 327 mIU/ml after the second dose of vaccine. Only one child, a 12-year-old on cART with a mean CD4+ T-cell count >500/mm3 and a viral load <50 copies/ml during the vaccination schedule, did not seroconvert for HAV. She also did not seroconvert for HBV after the first dose of vaccine, but did seroconvert after the second dose of vaccine with an anti-HBs concentration >1000 mIU/ml.

Response to HBV component

Out of 68 HBV-susceptible HIV-infected children, 17/64 (27%) developed a protective antibody response after the first dose of vaccine and 66/68 (97%) after the second dose of vaccine. Univariable analysis showed no associations for developing a protective antibody concentration after the first or second dose of vaccine (all p > 0.130). The GMC of the children after the first dose of vaccine was 5 mIU/ml, and 483 mIU/ml after the complete vaccination schedule.

One 10-year-old male and one 9-year-old female who did not respond after the second dose of vaccine were not on cART. Both had a CD4+ T-cell count ≥500/mm3 and a viral load >10,000 copies/ml at enrolment. They both showed anti-HAV concentrations > 400 mIU/ml.

Geometric mean concentration of all children by category (Fig. 2)

For HAV, no statistical differences in GMC were found regarding CD4+ T-cell count, viral load, or use of cART (all p > 0.266). For HBV, a significantly higher GMC was found in children with a lower viral load and in children on cART compared to children not on cART at the time of the second dose of vaccine and after the second dose of vaccine (all p < 0.002).
Fig. 2. Geometric mean concentration (GMC) anti-HAV and anti-HBs of all HIV-infected children, categorized by CD4+ T-cell count at enrolment, viral load, and use of cART, August 2009–June 2011.

GMC anti-HAV of all HIV-infected children, categorized by CD4+ T-cell count

GMC anti-HAV of all HIV-infected children, categorized by viral load (VL)

GMC anti-HAV of all HIV-infected children, categorized by use of cART

GMC anti-HBs of all HIV-infected children, categorized by CD4+ T-cell count

GMC anti-HBs of all HIV-infected children, categorized by viral load (VL)

GMC anti-HBs of all HIV-infected children, categorized by use of cART

\* cART: combination antiretroviral therapy. \= Time points: 1 (time prior to first dose of vaccine), 2 (after first dose of vaccine), 3 (prior to second dose of vaccine), 4 (after second dose of vaccine). \+ Significant difference in GMC at time points 3 and 4 (both p < 0.0001).

\* Significant difference in GMC at time points 3 and 4 (respectively p < 0.0001 and p = 0.002)
Children on immunosuppressives: study population and characteristics (Fig. 1 and Table 2)

A total of 140 children using immunosuppressive medication for rheumatic diseases met the inclusion criteria, 85 of whom were included in the study. Of these, 5 children were lost to follow-up during the study, 4 of whom were susceptible for HAV and 4 for HBV. The remaining 80 children completed the study. Of these 80 children, 15 were HAV-immune at baseline, and 6 other children were HBV-immune by previous vaccination, resulting in 65 children susceptible for HAV and 74 for HBV at enrolment.

Of the 80 children, 51 (64%) were female. The majority (62, 78%) had a Western ethnicity, and the median age was 12 years of age (IQR 9–14) with 50 children (63%) between 11 and 16 years of age. Most children (71, 89%) were diagnosed juvenile idiopathic arthritis (JIA); 3 (4%) uveitis; 2 (3%) systemic lupus erythematosus; 1 (1%) panuveitis; 1 (1%) autoimmune syndrome; and 1 (1%) juvenile dermatomyositis. Most children (42, 53%) were using only methotrexate, 28 (35%) methotrexate in combination with an anti-TNF- agent (n = 24), both an anti-TNF- and prednisone (n = 2), anakinra (n = 1), or prednisone (n = 1), and 10 (13%) used another immunosuppressive regimen (including only anti-TNF- (n = 4); anti-TNF- in combination with cyclosporine (n = 1); anakinra (n = 1); azathioprine (n = 1); cyclosporine (n = 1); mycophenolate mofetil (n = 1), or mycophenolate mofetil in combination with prednisone (n = 1)). Of the children who used methotrexate, the median dose used was 0.40 mg/kg (IQR 0.32–0.49). The median duration of immunosuppressive treatment of all children before first dose of vaccine was 32 months (IQR 16–54).

Response to vaccination in children on immunosuppressives (Table 2)

The median time between the first and second dose of vaccine in those who completed the study was 28 weeks (IQR 26–29). The median interval between first dose of vaccine and second blood sample and between second dose of vaccine and fourth blood sample was 5 weeks in both situations (IQR 4–6).

Response to hepatitis A component

After the first dose of vaccine 37/67 (55%) children developed a protective antibody response. Univariable analysis showed no associations for developing a protective antibody concen-
tration for immunity after the first dose of vaccine (all $p > 0.405$). After the second dose of vaccine, all 65/65 (100%) children developed a protective antibody response. The GMC of the children was 31 mIU/ml after the first dose of vaccine and 288 mIU/ml after the second dose of vaccine.

Response to HBV component

Of 76 HBV-susceptible children, 13 (17%) developed a protective antibody response after the first dose of vaccine. After the second dose of vaccine 69/74 (93%) developed a protective antibody response. The GMC of the children after the first dose of vaccine was 3 mIU/ml, and 321 mIU/ml after the second dose of vaccine. The 5 children, 4 girls and a boy, who did not seroconvert for HBV after the second dose of vaccine, were between 9 and 15 years of age at enrolment (median 12 [IQR 10–15]). No significant association was found between sex, age, ethnicity, or type of medication and no response after the full vaccination series (all $p > 0.441$).

Geometric mean concentration of all children by category (Fig. 3)

No statistical differences were found in GMC for HAV or HBV regarding type of immunosuppressive medication (all $p > 0.118$).
Table 2. Characteristics and immune response to the combined hepatitis A and B vaccine in children on immunosuppressive treatment for rheumatic diseases, August 2009–June 2011.

<table>
<thead>
<tr>
<th>Demographics</th>
<th>Hepatitis A-Immune* after 1st dose of vaccine</th>
<th>Hepatitis A-Immune after 2nd dose of vaccine</th>
<th>Hepatitis B-Immune after 1st dose of vaccine</th>
<th>Hepatitis B-Immune after 2nd dose of vaccine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(N)</td>
<td>No.</td>
<td>% (95%CI)</td>
<td>(N)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Male</td>
<td>25</td>
<td>14</td>
<td>56%</td>
<td>23</td>
</tr>
<tr>
<td>Female</td>
<td>42</td>
<td>23</td>
<td>55%</td>
<td>42</td>
</tr>
<tr>
<td>Ethnicity</td>
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<tr>
<td>Western</td>
<td>54</td>
<td>27</td>
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<td>African</td>
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<td>50%</td>
<td>4</td>
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<tr>
<td>Asian</td>
<td>1</td>
<td>1</td>
<td>100%</td>
<td>1</td>
</tr>
<tr>
<td>Latin American</td>
<td>4</td>
<td>4</td>
<td>100%</td>
<td>4</td>
</tr>
<tr>
<td>Other*</td>
<td>4</td>
<td>3</td>
<td>75%</td>
<td>4</td>
</tr>
<tr>
<td>Age at enrolment</td>
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</tr>
<tr>
<td>Up to 5 years</td>
<td>7</td>
<td>4</td>
<td>57%</td>
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<td>6 - 10 years</td>
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<td>41%</td>
<td>17</td>
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<tr>
<td>11 - 16 years</td>
<td>43</td>
<td>26</td>
<td>60%</td>
<td>41</td>
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<tr>
<td>Immunosuppressive-specific factors</td>
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<tr>
<td>Medication, type</td>
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<tr>
<td>Methotrexate</td>
<td>40</td>
<td>23</td>
<td>58%</td>
<td>37</td>
</tr>
<tr>
<td>Methotrexate combined†</td>
<td>20</td>
<td>9</td>
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</tr>
<tr>
<td>Other treatment†</td>
<td>7</td>
<td>5</td>
<td>71%</td>
<td>7</td>
</tr>
</tbody>
</table>

*Immune: for hepatitis A if anti-HAV IgG ≥20mIU/mL, for hepatitis B if anti-HBs ≥10mIU/mL.
†95% Confidence Interval
‡Other: mother born in Turkey (n=5), Lebanon (n=1) or Iraq (n=1)
§Methotrexate combined: treatment with methotrexate in combination with an anti-TNF-α agent, anakinra and/or prednisone
¶Other treatment: adalimumab, anakinra, azathioprine, ciclosporine, etanercept, mycophenolate mofetil, or prednisone
Fig. 3. Geometric mean concentration (GMC) anti-HAV and anti-HBs of all children on immunosuppressive medication for rheumatic diseases, categorized by type of medication, August 2009–June 2011.

*S Time points: 1 (time prior to first dose of vaccine), 2 (after first dose of vaccine), 3 (prior to second dose of vaccine), 4 (after second dose of vaccine).
Discussion

In this interventional study with combined HAV and HBV vaccine, the initial immune response to HAV in both HIV-infected children and children on immunosuppressive medication for rheumatic diseases treatment was low: only 71% and 55% respectively developed a protective antibody response after the first dose of vaccine. This was much lower than the immune response found in healthy children (90–100% immunity) [31,32]. Two other studies evaluating protective antibody response after a first hepatitis A dose of vaccine in HIV-infected children found responses of 86% and 69% [21,26]. However, these study results are difficult to compare with ours because of differences in vaccines, characteristics of study populations, and setting. For example, Sudjaritruk et al. [26] studied HIV-infected children living in Thailand, an endemic country for HAV, where immune responses could have been influenced by community exposure to HAV during the study period. We are not aware of studies that show data on anti-HAV immune response after the first dose of vaccine in children on immunosuppressive medication for treatment of rheumatic diseases.

After the second dose of vaccine, the immune response to hepatitis A in both HIV-infected children and children on immunosuppressive medication for rheumatic diseases was much better: 99% (95% CI 93–100) and 100% (95% CI 96–100) responded, respectively. These percentages are comparable to other studies in both healthy and HIV-infected children [21–31], but higher than the 85% (95% CI 75–92) found by Siberry et al. [25], and the 92% (95% CI 81–97) found by Erguven et al. in children with JIA with or without immunosuppressive medication [28]. The latter study found lower immune response rates in children on anti-TNF- treatment, a finding that was not confirmed in our study. In the study of Siberry et al. on HIV-infected children, higher CD4+ T-cell count and younger age (<12 years) were found to be associated with anti-body response to HAV. This finding was also not confirmed in our study. The immune response to HBV after the first dose of vaccine in both HIV-infected children and children on immunosuppressive medication for rheumatic diseases was low: 27% (95% CI 17–38) and 17% (95% CI 10–27) responded, respectively. Low to slightly higher percentages after the first dose of vaccine, varying from 34% to 51% (95% CI 23–47 and 40–61 respectively), are known to occur among healthy children and adolescents [33].

As opposed to the response following full vaccination series for HAV, the low HBV response
after the first dose of vaccine has little clinical significance, since exposure to HBV virus is less likely and can easily be prevented until the vaccination schedule has been completed. After the second dose of vaccine in HIV-infected children and children on immunosuppressive medication for rheumatic diseases, we found an immune response for hepatitis B of 97% and 93% respectively. Similar to hepatitis A, after completion of the full hepatitis B vaccination series, seroconversion percentages in our study were comparable to those in healthy children and adolescents [30–32]. One comparable study among children on immunosuppressive medication for JIA showed immunity results for hepatitis B comparable to our study [29]. However, two other studies among HIV-infected children found lower seroconversion percentages (60–71%) [23,24]. In these studies and also in studies among HIV-infected adults, viral load, CD4+ T-cell count, and cART were found to be associated with seroconversion [7,23,24]. We could not confirm these associations, likely due to the high median CD4+ T-cell count (858 cells/mm3), low median viral load (39 copies/mL), and almost all children (89%) using cART in our study. We did find that a larger proportion of children on cART and children with a viral load <50 copies/mL were responders, whereby, not surprisingly, all children not on cART had a viral load >50 copies/mL. Furthermore, a significantly higher GMC was found in the same two groups.

The GMCs we found in both HIV-infected children and children on immunosuppressives for rheumatic diseases were lower compared to other studies [7,21,22,26,29,34]; this may be due to the cut-off values of 1000 mIU/mL for anti-HBs and of 400 mIU/mL for anti-HAV that were used in our study. Having had precise antibody concentrations could have contributed to a better comparison with other studies. However, data on seroconversions and associations are more important in clinical practice than GMCs.

In conclusion, although the immune response after a full series of combined HAV and HBV vaccine in the two groups of children was excellent and compared favorably to healthy children, a substantial proportion of HIV-infected children and children on immunosuppressive medication for rheumatic diseases were not protected for HAV after the first dose of combined HAV and HBV vaccine. Especially in travel health and postexposure prophylactic treatment for HAV, the following measures are suggested: HIV-infected children and children on immunosuppressive medication should be serologically tested for anti-HAV prior to travel to ensure they are protected if there is no time to await the second dose of vaccine; and, children
who cannot be protected before travel and those who need postexposure prophylactic treatment for HAV should receive immunoglobulines.

Testing anti-HBs after a full series of vaccine could be considered to ensure protection, in particular in HIV-infected children who are not receiving cART and in children with a viral load >1000 copies/mL.

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Conflict of interest
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