Hepatitis B and pertussis antibodies in 4- to 5-year-old children previously vaccinated with different hexavalent vaccines

Timo Vesikari b, Jin Xu c, David R. Johnson c, Jessie Hall b, Tomáš Mrček d, Michelle G. Gouveia b, Camilo J. Acosta d, and Andrew Wen-Tseng Lee b

Department of Pediatrics, University of Tampere, Tampere, Finland; bMerck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc., Kenilworth, NJ, USA; cSanofi Pasteur, Swiftwater, PA, USA; dMCM Vaccine B. V., Leiden, The Netherlands

ABSTRACT
In randomized active-comparator controlled studies, DTaP5-HB-IPV-Hib showed comparable immunogenicity and safety to other licensed vaccines. This study assessed persistence of anti-hepatitis B surface antigen (HBs) and anti-pertussis antibodies, when children were 4 to 5 years of age, 3 to 4 years after initial infant/toddler hexavalent vaccination. This was an extension of 2 European studies in which infants/toddlers received either DTaP5-HB-IPV-Hib or DTaP3-HB-IPV/Hib on a 2 + 1 or 3 + 1 schedule. Primary endpoints included percentages with anti-HBs ≥10 mIU/mL, and anti-pertussis toxin (PT), anti-filamentous hemagglutinin (FHA), anti-pertactin (PRN), and anti-fimbriae types 2 & 3 (FIM) greater than or equal to the lower limit of quantitation (LLOQ). One month after 2 + 1 or 3 + 1 dosing, nearly all toddlers had anti-HBs ≥10 mIU/mL, and responded to the received pertussis antigens. Approximately 3 to 4 years later, 65.8%-70.2% in the DTaP5-HB-IPV-Hib and 82.0%-83.7% in the DTaP3-HB-IPV/Hib groups, respectively, had anti-HBs ≥10 mIU/mL. Percentages of children with pertussis antibodies above LLOQ after 2 + 1 dosing were 58.4% and 41.5% (anti-PT), 80.9% and 88.3% (anti-FHA), 66.1% and 72.6% (anti-PRN), and 94.4% and 3.3% (anti-FIM), in the DTaP5-HB-IPV-Hib and DTaP3-HB-IPV/Hib groups, respectively. This study demonstrated, as expected, waning of hepatitis B and pertussis antibodies during the 3 to 4 years after completion of a 3 + 1 or 2 + 1 hexavalent vaccination schedule. Nonetheless, anti-HBs levels ≥10 IU/mL and detectable antibodies against acellular pertussis antigens persisted in most study participants. The implications of these findings for the long-term prevention of hepatitis B and pertussis are further discussed.

Introduction
The complex recommended vaccination schedule for children younger than 2 years of age can be cumbersome for both healthcare professionals and parents, and may lead to missed opportunities for vaccination that increases the risk of epidemic outbreaks of otherwise preventable diseases.1–3 To help reduce the number of injections at office visits, 5- and 6-valent childhood vaccines have been long introduced in Europe, and more recently, in the United States. Numerous studies have shown that the use of combination vaccines increases coverage and on-time vaccination rates.4–7

Currently, 3 hexavalent vaccines are licensed in the European Union: Infanrix® hexa (combined diphtheria, tetanus toxoids, acellular pertussis, hepatitis B, inactivated poliomyelitis, and hepatitis b [Hib] conjugate vaccine adsorbed DTaP5-HB-IPV-Hib); 314 µg aluminum salts per 0.5-mL dose; GlaxoSmithKline Biologicals, Rixensart, Belgium), approved in 2000; Hexyon® (fully liquid diphtheria, tetanus, pertussis [acellular, component], hepatitis B [rDNA], poliomyelitis [inactivated] and Haemophilus influenzae type b [Hib] conjugate vaccine adsorbed, [DTaP2-HB-IPV-Hib]; 600 µg aluminum salts per 0.5-mL dose; Sanofi Pasteur Europe, Lyon, France), approved in 2013; and Vaxelis® (fully liquid diphtheria, tetanus, pertussis [acellular component], hepatitis B [rDNA], poliomyelitis [inactivated] and Haemophilus influenzae type b conjugate vaccine adsorbed DTaP5-HB-IPV-Hib); 314 µg aluminum salts per 0.5-mL dose; MCM Vaccine B. V., Leiden, The Netherlands), approved in 2016. These vaccines are indicated for the vaccination of infants and toddlers against the diseases caused by these pathogens.8–10 DTaP5-HB-IPV-Hib differs from DTaP3-HB-IPV/Hib and DTaP2-HB-IPV-Hib, as it contains 5 acellular pertussis antigens and utilizes a meningococcal outer membrane protein as the conjugate for the Hib antigen. DTaP5-HB-IPV-Hib was approved in Europe in February 2016 and in the United States in December 2018 based on its similar immunogenicity and safety compared with the other licensed comparator vaccines. To meet a request from the European Medicines Agency (EMA), this study was conducted to assess the long-term persistence of anti-hepatitis B surface antigen (HBs) and anti-pertussis antibodies 3 to 4 years after initial vaccination with the DTaP5-HB-IPV-Hib. The EMA had previously requested persistence studies for the other hexavalent vaccines, DTaP3-HB-IPV/Hib and DTaP2-HB-IPV-Hib.11,12

Methods
Study design
The clinical portion of this study was conducted in Finland from late April to early August 2016, as an extension of 2...
European pivotal studies: a study evaluating a 3 + 1 schedule conducted in Belgium, Finland, and Germany from late May 2011 to mid-March 2013 (NCT01341639) and a study evaluating a 2 + 1 schedule conducted in Finland, Italy, and Sweden from late November 2011 to early October 2013 (NCT01480258). In these randomized, double-blind trials, infants received either a 3-dose primary series of DTaP5-HB-IPV-Hib or DTaP3-HB-IPV/Hib at 2, 3, and 4 months of age and a toddler dose at 12 months of age or a 2 dose primary series of DTaP5-HB-IPV-Hib or DTaP3-HB-IPV/Hib at 2 and 4 months of age and a toddler dose at 11 to 12 months of age.

Four groups were defined according to previous vaccination schedule (3 + 1 or 2 + 1) and type of vaccine (DTaP5-HB-IPV-Hib or DTaP3-HB-IPV/Hib) received during each study

- **Group 1:** DTaP5-HB-IPV-Hib (3 + 1), those previously vaccinated with a 3-dose primary series and a toddler dose of DTaP5-HB-IPV-Hib
- **Group 2:** DTaP3-HB-IPV/Hib (3 + 1), those previously vaccinated with a 3-dose primary series and a toddler dose of DTaP3-HB-IPV/Hib
- **Group 3:** DTaP5-HB-IPV-Hib (2 + 1), those previously vaccinated with a 2-dose primary series and a toddler dose of DTaP5-HB-IPV-Hib
- **Group 4:** DTaP3-HB-IPV/Hib (2 + 1), those previously vaccinated with a 2-dose primary series and a toddler dose of DTaP3-HB-IPV/Hib

All participants in the 3 + 1 and 2 + 1 studies received concomitant conjugate pneumococcal vaccine (PCV13) and rotavirus vaccine. Participants in the 3 + 1 study also received concomitant measles-mumps-rubella-varicella vaccine.

No vaccine (eg, booster or challenge dose) was administered as part of this extension study. The long-term persistence of antibody to hepatitis B surface antigen (anti-HBs) was assessed approximately 3 to 4 years after completion of a 3 + 1 or 2 + 1 schedule when children were 4 to 5 years of age. The long-term persistence of pertussis antibodies was assessed in the 2 + 1 study only and not the 3 + 1 study. The 3 + 1 study started and finished earlier than the 2 + 1 study. Because the school-entry pertussis-containing booster vaccine is given at approximately 4 years of age in Finland, many of the children in the 3 + 1 study had already received their pertussis school-entry booster. They were therefore not eligible to undergo the anti-pertussis antibody measurements in this study.

### Immunogenicity measurements

The primary endpoints were the percentage of children with anti-HBs concentrations ≥10 mIU/mL, and anti-pertussis toxin (PT), anti-filamentous hemagglutinin (FHA), anti-pertactin (PRN), and anti-fimbriae types 2&3 (FIM) concentrations greater than or equal to the lower limit of quantitation (LLOQ), ≥2× LLOQ, and ≥4× LLOQ, with a LLOQ of 4 enzyme-linked immunosorbent assay (ELISA) units (EU)/mL for PT, PRN, FIM, and of 3 EU/mL for FHA. Secondary endpoints included anti-HBs, anti-PT, anti-FHA, anti-PRN and anti-FIM geometric mean concentrations (GMCs). Endpoint analysis was performed on the persistence analysis set approximately 4 years after the initial vaccination schedule. The persistence analysis set was defined as all children previously vaccinated with a complete 3 + 1 or 2 + 1 schedule who were not excluded per the previously noted criteria, and for whom immunogenicity results were available in the persistence study.

Anti-HBs was assessed using a hepatitis B-enhanced chemiluminescence (HepB ECiQ) assay at Focus Diagnostics Clinical Trials, San Juan Capistrano, California, USA. Anti-PT, FHA, PRN, and FIM antibodies were assessed using an ELISA at the Sanofi Pasteur Global Clinical Immunology platform, Swiftwater, Pennsylvania, USA.

### Safety assessments

No safety analyses were performed in this study, as no additional vaccinations were administered. Only serious adverse events (SAEs) related to study procedures (blood sampling) were collected.

### Statistics

It was estimated that with a sample size of approximately 180 evaluable children, the half width of the 2-sided 95% confidence interval (CI) was not to exceed 8% if the observed percentages of persistence were ≥60%. This was deemed acceptable to provide an appropriate estimate for the primary endpoints. The statistical analyses were performed using SAS® software version 9.2 (SAS Institute Inc., Cary, North Carolina, USA).

For categorical variables (except antibody concentrations), descriptive statistics of sample size, count by category, and proportion by category are presented. For continuous variables (except antibody concentrations), descriptive statistics of sample size, mean and standard deviation are presented. For
antibody concentrations, descriptive statistics of sample size, count greater than or equal to the threshold, proportion greater than or equal to the threshold, geometric mean, and 2-sided 95% CIs are presented.

Pertussis antibody concentrations reported by the laboratory as below the LLOQ were replaced by half of the LLOQ; those above the upper limit of quantitation were replaced by this limit. The 2-sided 95% CIs were provided for proportions and GMCs were calculated for immunogenicity endpoints. CIs for proportions were based on the exact method for binary variables. CIs for GMCs were calculated using the t distribution and sample variance of logarithms of individual concentrations.

**Results**

**Study population**

In the original 3 + 1 study, 1217 children were randomized, and in the original 2 + 1 study, 1315 children were randomized. Of these children (n = 2532), 760 were screened for possible inclusion into this study, with 754 children enrolled and 752 included in the persistence analysis set. No child screened was found to have had either hepatitis B or pertussis infection. All 752 children were evaluable for anti-HBs analysis; however, only those who had participated in the 2 + 1 study (n = 371) were included in pertussis follow-up (see Methods). Demographic data are presented in Table 1. The 3 + 1 and 2 + 1 persistence study cohorts were very similar to the original study cohorts in terms of female:male ratio and mean age at toddler dose.

**Hepatitis B immunogenicity**

All of the children (100%) in the persistence study cohort had seroconverted (ie, achieved anti-HBs ≥10 mIU/mL) 1 month after the 3 + 1 vaccination and 2 + 1 schedules with DTaP5-HB-IPV-Hib or DTaP3-HB-IPV/Hib, reflecting robust hepatitis B responses for both vaccine groups. These high seroconversion rates in the persistence study cohort were consistent with the anti-HBs seroconversion rates (>99% in both vaccine groups following the 3 + 1 schedule and >98% in both vaccine groups following the 2 + 1 schedule) in the overall study populations (Supplemental Tables 1 and 2). The percentages of toddlers with anti-HBs ≥100 mIU/mL were also similar between DTaP5-HB-IPV-Hib and DTaP3-HB-IPV/Hib in the 3 + 1 schedule (96.8% and 98.4%, respectively, Table 2) and in the 2 + 1 schedule (97.6% and 97.7%, respectively, Table 2), with overlapping 95% CIs. The anti-HBs GMCs after the toddler dose were lower for DTaP5-HB-IPV-Hib compared with DTaP3-HB-IPV/Hib, with overlapping 95% CIs for the 3 + 1 schedule, but non-overlapping 95% CIs for the 2 + 1 schedule (Table 2).

The persistence of anti-HBs was lower after DTaP5-HB-IPV-Hib than DTaP3-HB-IPV/Hib. Approximately 4 years after completion of a 3 + 1 schedule, the percentage of children with anti-HBs ≥10 mIU/mL was 70.2% (95% CI, 63.1%–76.5%) in Group 1 and 82.0% (95% CI, 75.8%–87.2%) in Group 2. Of those who received the 2 + 1 schedule, the percentage of children with anti-HBs ≥10 mIU/mL was 65.7% (95% CI, 58.3%–72.6%) in Group 3 and 83.7% (95% CI, 77.6%–88.6%) in Group 4. The persistence of anti-HBs GMCs across study groups were consistent with the initial GMC results, with lower levels at 4 years after completion of the original schedules of DTaP5-HB-IPV-Hib (Table 2).

**Pertussis immunogenicity**

Approximately 4 years after completion of the 2 + 1 schedule, the proportions of children with antibodies above LLOQ were 58.4% and 41.5% for anti-PT, 80.9% and 88.3% for anti-FHA, 66.1% and 72.6% for anti-PRN, and 94.4% and 3.3% for anti-FIM in the DTaP5-HB-IPV-Hib and the DTaP3-HB-IPV/Hib groups, respectively (Table 3). The 95% CIs overlapped for DTaP5-HB-IPV-Hib and DTaP3-HB-IPV/Hib for FHA and PRN but were non-overlapping for PT and FIM. In the original 2 + 1 study, all of these percentages were 100% 1 month after the 2 + 1 vaccination schedule with DTaP5-HB-IPV-Hib or DTaP3-HB-IPV/Hib, except for FIM, of which 100% of children in the DTaP5-HB-IPV-Hib group had anti-FIM antibodies above LLOQ versus 8.0% in the DTaP3-HB-IPV/Hib group.

For the secondary endpoint of GMCs assessed at 4 years after completion of the original 2 + 1 vaccination schedule, persistence of pertussis-related antibodies was generally consistent with post-primary immunization levels. Anti-PT and anti-FIM GMCs were higher, anti-FHA was lower, and anti-PRN GMCs were similar in the DTaP5-HB-IPV-Hib group compared with the DTaP3-HB-IPV/Hib group (Table 4).

**Safety**

No SAEs related to study procedures were reported during the study period.

Table 1. Demographic characteristics of the persistence analysis set.

| Parameter                        | 3 + 1                | 2 + 1                |
|----------------------------------|----------------------|----------------------|
|                                 | Group 1 (N = 191)    | Group 2 (N = 189)    | Group 3 (N = 181)    | Group 4 (N = 191)    |
|                                 | All (N = 380)        | All (N = 372)        | All (N = 752)        |
| Gender, n (%)                    |                      |                      |                      |
| Female                           | 98 (51.3)            | 86 (45.5)            | 184 (48.4)           | 84 (46.4)            |
| Male                             | 93 (48.7)            | 103 (54.5)           | 196 (51.6)           | 97 (53.6)            |
| Mean age at toddler dose in months (SD) | 12.2 (0.4)           | 12.2 (0.4)           | 12.2 (0.4)           | 11.2 (0.4)           |
| Mean age at the persistence time point, months (SD) | 4.8 (0.2)            | 4.8 (0.2)            | 4.8 (0.2)            | 3.9 (0.1)            |
| Mean weight at the persistence time point, kg (SD) | 19.2 (2.5)           | 19.3 (2.6)           | 19.2 (2.6)           | 16.9 (2.1)           |

SD, standard deviation.

Group 1: DTaP5-HB-IPV-Hib (3 + 1): those previously vaccinated with a 3-dose primary series and a toddler dose of DTaP5-HB-IPV-Hib.

Group 2: DTaP3-HB-IPV/Hib (3 + 1): those previously vaccinated with a 3-dose primary series and a toddler dose of DTaP3-HB-IPV/Hib.

Group 3: DTaP5-HB-IPV-Hib (2 + 1): those previously vaccinated with a 2-dose primary series and a toddler dose of DTaP5-HB-IPV-Hib.

Group 4: DTaP3-HB-IPV/Hib (2 + 1): those previously vaccinated with a 2-dose primary series and a toddler dose of DTaP3-HB-IPV/Hib.
Table 2. Summary of anti-HBs responses: persistence analysis set.

| Schedule Parameter | DTaP5-HB-IPV-Hib | DTaP3-HB-IPV/Hib | DTaP5-HB-IPV-Hib | DTaP3-HB-IPV/Hib | DTaP5-HB-IPV-Hib | DTaP3-HB-IPV/Hib |
|--------------------|------------------|------------------|------------------|------------------|------------------|------------------|
| 3 + 1              |                  |                  |                  |                  |                  |                  |
| Anti-PT            |                  |                  |                  |                  |                  |                  |
| ≥LLOQa             | 178              | 104 (58.4)       | 50.8, 65.8       | 188              | 78               | 41.5, 48.9       |
| ≥2xLLOQ            | 72 (40.5)        | 33.2, 48.1       | 41                | 161              | 16.1, 28.4       |
| ≥4xLLOQ            | 26 (14.6)        | 9.8, 20.7        | 7                 | 3.7              | 1.5, 7.5         |
| Anti-FHA           |                  |                  |                  |                  |                  |                  |
| ≥LLOQ              | 173              | 140 (80.9)       | 74.3, 86.5       | 188              | 166              | 88.3, 92.9       |
| ≥2xLLOQ            | 81 (46.8)        | 39.2, 54.5       | 133              | 70.7             | 63.7, 77.1       |
| ≥4xLLOQ            | 45 (26.0)        | 19.7, 33.2       | 85                | 45.2             | 38.0, 52.6       |
| Anti-PRN           |                  |                  |                  |                  |                  |                  |
| ≥LLOQ              | 180              | 119 (66.1)       | 58.7, 73.0       | 190              | 138              | 72.6, 78.8       |
| ≥2xLLOQ            | 79 (43.9)        | 36.5, 51.5       | 97               | 51.1             | 43.7, 58.4       |
| ≥4xLLOQ            | 28 (15.6)        | 10.6, 21.7       | 35                | 18.4             | 13.2, 24.7       |
| Anti-FIM           |                  |                  |                  |                  |                  |                  |
| ≥LLOQ              | 177              | 167 (94.4)       | 89.9, 97.3       | 183              | 6                | 1.2, 7.0         |
| ≥2xLLOQ            | 156 (88.1)       | 82.4, 92.5       | 4                 | 2.2              | 0.6, 5.5         |
| ≥4xLLOQ            | 123 (69.5)       | 62.1, 76.2       | 2                 | 1.1              | 0.1, 3.9         |

aConfidence intervals were calculated based on the exact binomial method of D. Collett.15
bLower limit of quantification (LLOQ) = 4 EU/mL for PT, PRN, and FIM; LLOQ = 3 EU/mL for FHA.

Discussion

This immunogenicity study demonstrated the long-term persistence of hepatitis B antibodies following the administration of DTaP5-HB-IPV-Hib as a 3-dose primary series given at 2, 3, and 4 months of age and a toddler dose at 12 months of age, or a 2-dose primary series given at 2 and 4 months of age and a toddler dose at 11 to 12 months, and the persistence of pertussis antibodies after the latter vaccination schedule. In this study, the threshold for seroprotection was anti-HBs concentrations ≥10 mIU/mL, which is a widely accepted immune correlate of protection against hepatitis B virus infection.16 In contrast, pertussis lacks a simple correlate of protection, although logistic regression analysis of immune responses from household-exposed cohorts within acellular pertussis vaccine efficacy studies demonstrated statistically significant associations between the presence of antibodies against pertactin, fimbriae 2/3, and pertussis toxin, and clinical protection from pertussis.17,18

Anti-HBs results in this persistence study were consistent with those from the original studies, which showed similarly high rates of children with anti-HBs ≥10 and ≥100 mIU/mL for both DTaP5-HB-IPV-Hib and DTaP3-HB-IPV/Hib, with higher GMCs for the DTaP3-HB-IPV/Hib vaccine. All anti-HBs levels are expected to wane over time, regardless if from infection or vaccination, with anti-HBs protective level concentrations declining rapidly within the first year (down to 15%–50%) and more slowly thereafter (low or undetectable after 5–15 years). However, lower anti-HBs concentrations years after vaccination do not indicate the absence of protection against clinically important hepatitis B disease. It is well documented and accepted by international public health authorities that initial seroprotection following a complete series of hepatitis B vaccination determines long-term protection, and robust seroprotection rates following vaccination were observed in the original studies. After disappearance of vaccine-induced circulating antibodies, protection against hepatitis B results from an immune memory response.19–29 This immune memory or anamnestic response years after vaccination with hepatitis B-containing vaccine can be demonstrated with a challenge dose of hepatitis B vaccine.
which is composed of hepatitis B surface antigen and serves as a model of exposure to hepatitis B virus itself. Years after primary hepatitis B vaccination, children and adults demonstrate high rates of seroprotective antibody responses following a challenge dose of hepatitis B vaccine, including individuals with anti-HBs <10 mIU/mL prior to the challenge.\textsuperscript{30} Such anamnestic responses have been demonstrated as long as 22 years after infant and childhood vaccination with recombinant or plasma-derived hepatitis B vaccine in Taiwan,\textsuperscript{19} and 30 years after vaccination with plasma-derived hepatitis B vaccine in Alaska.\textsuperscript{31} Immunologic characterization of the 30-year Alaskan cohort showed that immune memory against hepatitis B correlated with the presence of natural killer T-cell responses.\textsuperscript{32} Similarly high rates of seroprotection have been demonstrated in response to hepatitis B challenge dosing years after primary vaccination with hepatitis B-containing hexavalent vaccines.\textsuperscript{33–36} These immune memory mechanisms are posited to include responses mediated by natural killer T cells. Given that there are no approved clinical tests for cell-mediated immune memory markers against hepatitis B, it is reasonable to monitor for anti-HBs ≥10 mIU/mL after vaccination in the research setting, while understanding that it does not provide the complete view of immune protection against hepatitis B resulting from vaccination. Accordingly, there are no public health recommendations for long-term monitoring of anti-HBs, nor are there routine hepatitis B booster dose recommendations based on anti-HBs levels years after vaccination.

In addition to long-term immune memory, the long-term efficacy and effectiveness of hepatitis B vaccination has been confirmed in cohort studies, and by real-world evidence, respectively. Multiple long-term follow-up cohort studies have demonstrated high rates of protection from chronic carrier state (HBs positivity) and infection (hepatitis B core antigen positivity), following vaccination for 15 to 22 years.\textsuperscript{19–27,29} Countries that have adopted universal vaccination against hepatitis B with recombinant HBV have seen dramatic decreases in hepatitis B disease rates,	extsuperscript{21,37} consistent with the high effectiveness of hepatitis B vaccination during infancy. Given the high long-term efficacy and effectiveness of infant hepatitis B vaccination, routine booster vaccination against hepatitis B is not recommended by a number of global organizations, including the World Health Organization, US Centers for Disease Control and Prevention, the Standing Committee on Vaccination at the Robert Koch Institute (STIKO), and the Viral Hepatitis Prevention Board.\textsuperscript{38,39}

In a prior study of concomitant vaccination between a hepatitis B-containing hexavalent vaccine (DTaP-IPV-HBV-Hib [Hexaxac], Aventis Pasteur MSD, Lyon, France) and a 7-valent pneumococcal conjugate vaccine (Prevnar 7, Wyeth), lower hepatitis B GMCS were noted with concomitant administration as opposed to separate administration.\textsuperscript{40} In order to overcome potential immune interference among hexavalent vaccine components, or with concomitant vaccine antigens, DTaP5-HB-IPV-Hib has been designed to optimize hepatitis B immunogenicity, with an increase from 5 to 10 µg of the HBs component, and a modified process aluminum phosphate adjuvant, which allows for greater accessibility of the adsorbed hepatitis B antigen to antigen-presenting cells.\textsuperscript{41} Multiple randomized, double-blind, comparator-controlled studies of hepatitis B vaccine with modified process adjuvant, demonstrated similar safety and higher hepatitis B GMCS compared with hepatitis B vaccine with original process adjuvant in a wide range of populations from infants to older adults.\textsuperscript{42–46}

Accordingly, the hepatitis B responses in the Phase 3 studies of DTaP5-HB-IPV-Hib have been robust, meeting all acceptability and non-inferiority criteria compared with the licensed vaccine regimens in the United States and Europe (pentavalent with separate monovalent hepatitis B, or DTaP3-HB-IPV/Hib, respectively). Indeed, as highlighted in the current manuscript, DTaP5-HB-IPV-Hib resulted in high and similar percentages of children with anti-HBs ≥10 and ≥100 mIU/mL compared with DTaP3-HB-IPV/Hib in the rigorous settings of the European Union (ie, without a hepatitis B birth dose and using a compressed 3-dose [2, 3, 4 months] or 2-dose [2, 4 months] regimen). Note that the increase in the ratio of aluminum phosphate to aluminum hydroxide in the modified process adjuvant does not result in higher total amounts of aluminum salt in DTaP5-HB-IPV-Hib (314 µg) as compared to DTaP3-HB-IPV/Hib (950 µg).

This study also provides information about the long-term persistence of pertussis antibody responses following the administration of DTaP5-HB-IPV-Hib as a 2-dose primary series given at 2 and 4 months of age, with a toddler dose at 11 to 12 months of age. Pertussis antibodies 3 to 4 years following a 2 + 1 hexavalent dosing schedule were higher in DTaP5-HB-IPV-Hib recipients for anti-PT and anti-FIM antibodies compared with those who received the DTaP3-HB-IPV/Hib vaccine. Levels of anti-FHA antibodies were higher in DTaP3-HB-IPV/Hib recipients compared with DTaP5-HB-IPV-Hib recipients, while anti-PRN antibodies were comparable across both vaccine groups. There are differing quantities of pertussis antigens in each 0.5-mL dose of the study vaccines. DTaP5-HB-IPV-Hib contains 20 µg PT, 20 µg FHA, 3 µg PRN and 5 µg FIM. In contrast, DTaP3-HB-IPV/Hib contains 25 µg PT, 25 µg FHA, 8 µg PRN and no FIM. The anti-pertussis antibody levels found in this study generally correlated with the pertussis antigen quantities in the respective vaccines, with the exception of PT antibody. Factors in addition to antigen quantity, such as source and formulation, may explain this somewhat counterintuitive finding.\textsuperscript{47}

In this study, anti-PT, anti-FHA, and anti-PRN GMCS had fallen for both vaccine groups back to the pre-vaccination levels in the original 2 + 1 study, while the anti-FIM GMC in the DTaP5-HB-IPV-Hib group persisted well above baseline levels. Although some have suggested that there are no serological correlates of protection, the opposite is actually the case. However, these correlates are multiple and complex.\textsuperscript{48} A validated model of pertussis protection,\textsuperscript{17,48} as well as other investigations,\textsuperscript{49,50} suggest that higher levels of anti-FIM antibodies, as seen in the DTaP5-HB-IPV-Hib group of the present study, may be associated with enhanced pertussis immunity.\textsuperscript{17,18,50} In spite of the persistence of anti-FIM antibodies in the DTaP5-HB-IPV-Hib recipients, the overall pertussis antibody findings of this study reinforce the importance of following recommendations for school-entry pertussis booster vaccination at around 5 years of age, as is done in the United States and the majority of countries in the European Union.\textsuperscript{51,52}
Limitations of this study include that it was descriptive and not powered for formal between-groups comparisons, and the monitoring of immune memory against hepatitis B was limited to tests approved for clinical use (anti-HBs levels). In addition, the study design only evaluated the persistence of anti-HBs and did not measure the response to a challenge dose of hepatitis B vaccine, which models exposure to hepatitis B.

In conclusion, this study demonstrated that although anti-HBs levels ≥10 IU/mL and detectable antibodies against acellular pertussis antigens persisted in a majority of study participants, there was waning of hepatitis B and pertussis antibodies during the 3 to 4 years after completion of the infant-toddler hexavalent vaccination schedule. These findings have different clinical and public health implications for the long-term prevention of these diseases. Regarding hepatitis B vaccination, responders to a complete series continue to be protected against hepatitis B disease and chronic infection, regardless of the level of circulating anti-HBs at the time of their exposure to hepatitis B virus. For pertussis, the waning of most anti-pertussis antibodies to prevaccination baseline levels support pertussis booster vaccination at school-entry and beyond.

Acknowledgments

Deepest appreciation and thanks go to all of the children, parents, and clinical trial staff who were involved in the trials. The authors also wish to thank Pierre Van Damme, MD, PhD, University of Antwerp, Antwerp, Belgium, for critically reviewing the manuscript. Writing assistance was provided by Meredith Rogers, MS, CMPP, The Lockwood Group, Stamford, CT, USA. This assistance was funded by MCM Vaccine B.V., Leiden, The Netherlands.

Disclosure of potential conflicts of interest

Timo Vesikari was an investigator for the sponsor, which was supported by research grants.

Andrew Wen-Tseng Lee, Jin Xu, Michelle G. Goveia, and Jessie Hall are employees of Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc., Kenilworth, NJ, USA. Camilo J. Acosta was employed by Merck Sharp & Dohme Corp. at the time the study was conducted.

Tomáš Marček is an employee of MCM Vaccine B.V., Leiden, The Netherlands.

David R. Johnson is an employee of Sanofi Pasteur, Swiftwater, PA, USA.

Funding

This study was sponsored by MCM Vaccine B.V., a partnership between Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc., Kenilworth, NJ, USA, and Sanofi Pasteur, Inc., Swiftwater, PA, USA; MCM Vaccine B.V., a partnership between Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc., Kenilworth, NJ, USA and Sanofi Pasteur, Inc., Swiftwater, PA. USA [NA]; MCM Vaccine B.V., a partnership between Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc., Kenilworth, NJ, USA and Sanofi Pasteur, Inc., Swiftwater, PA. USA [NA]; MCM Vaccine B.V., a partnership between Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc., Kenilworth, NJ, USA and Sanofi Pasteur, Inc., Swiftwater, PA, USA [NA].

ORCID

Tomáš Marček http://orcid.org/0000-0003-0920-4192
Camilo J. Acosta http://orcid.org/0000-0002-2313-3493

1. Maman K, Zollner Y, Greco D, Duru G, Sendyona S, Remy V. The value of childhood combination vaccines: from beliefs to evidence. Hum Vacc Immunother. 2015;11:2132–41. doi:10.1080/21645515.2015.1044180
2. Wallace AS, Mantel C, Mayers G, Mansoor O, Gindler JS, Hyde TB. Experiences with provider and parental attitudes and practices regarding the administration of multiple injections during infant vaccination visits: lessons for vaccine introduction. Vaccine. 2014;32:5301–10. doi:10.1016/j.vaccine.2014.07.076
3. Kurosky SK, Davis KL, Krishnarajah G. Effect of combination vaccines on completion and compliance of childhood vaccinations in the United States. Hum Vacc Immunother. 2017;13:2494–502. doi:10.1080/21645515.2017.1362515
4. Marshall GS, Happe LE, Lunacsek OE, Szymanski MD, Woods CR, Zahn M, Russell A. Use of combination vaccines is associated with improved coverage rates. Pediatr Infect Dis J. 2007;26:496–500. doi:10.1097/INF.0b013e31805d7f17
5. Kalies H, Grote V, Verstraeten T, Hessel L, Schmitt HJ, von Kries R. The use of combination vaccines has improved timeliness of vaccination in children. Pediatr Infect Dis J. 2006;25:507–12. doi:10.1091/01消息222413.47544.23
6. Happe LE, Lunacsek OE, Vluzin D, Marshall GS. Impact of a pentavalent combination vaccine on immunization timeliness in a state Medicaid population. Pediatr Infect Dis J. 2009;28:98–101. doi:10.1097/INF.0b013e31818d7d047
7. Happe LE, Lunacsek OE, Marshall GS, Lewis T, Spencer S. Combination vaccine use and vaccination quality in a managed care population. Am J Manag Care. 2007;13:506–12.
8. GlaxoSmithKline Biologicals sa. INFANRIX-hexa summary of product characteristics. Rixensart (Belgium), 2000.
9. Sanofi Pasteur Europe. HEXYON summary of product characteristics. Leiden (The Netherlands): European Medicines Agency; 2013.
10. MCM vaccine B. V. VAXELIS summary of product characteristics. Leiden (The Netherlands): European Medicines Agency; 2019.
11. European Medicines Agency. Assessment report. Hexyon. 2013.
12. European Medicines Agency. 2015. Assessment report for paediatric studies submitted according to article 46 of the regulation (EC) No 1901/2006. Infanrix® Hexa.
13. Vesikari T, Becker T, Vertruyen AF, Poschet K, Flores SA, Pagnoni MF, Xu J, Liu GF, Stek JE, Boisnard F, et al. A phase III randomized, double-blind, clinical trial of an investigational hexavalent vaccine given at two, three, four and twelve months. Pediatr Infect Dis J. 2017;36:209–15. doi:10.1097/INF.0b013e3189a1406.
14. Silfverdal SA, Icardi G, Vesikari T, Flores SA, Pagnoni MF, Xu J, Liu GF, Stek JE, Boisnard F, Thomas S, et al. A phase III randomized, double-blind, clinical trial of an investigational hexavalent vaccine given at 2, 4, and 11–12 months. Vaccine. 2016;34:3810–16. doi:10.1016/j.vaccine.2016.05.054.
15. Collett D. Modelling binary data. London, UK: Chapman & Hall/CRC; 2003.
16. Plotkin SA. Correlates of protection induced by vaccination. Clin Vaccine Immunol. 2010;17:1055–65. doi:10.1128/CVI.00131-10.
17. Storsaeter J, Hallander HO, Gustafsson L, Olin P. Levels of anti-pertussis antibodies related to protection after household exposure to Bordetella pertussis. Vaccine. 1998;16:1907–16. doi:10.1016/s0264-410x(98)00227-8.
18. Kohberger RC, Jemiolo D, Noriega F. Prediction of pertussis vaccine efficacy using a correlates of protection model. Vaccine. 2008;26:3516–21. doi:10.1016/j.vaccine.2008.04.016.
19. But DY, Lai CL, Lim WL, Fung J, Wong DK, Yuen MF. Two-year follow-up of a prospective randomized trial of hepatitis B vaccines without booster dose in children: final report. Vaccine. 2008;26:6587–91. doi:10.1016/j.vaccine.2008.09.034.
20. Bialek SR, Bower WA, Novak R, Helgenberger L, Auerbach SB, Williams IT, Bell BP. Persistence of protection against hepatitis B virus infection among adolescents vaccinated with recombinant hepatitis B vaccine beginning at birth: a 15-year follow-up study. Pediatr Infect Dis J. 2008;27:881–85. doi:10.1097/INF.0b013e3181770ba.
21. Fitzsimons D, Francois G, Hall A, McMahon B, Meheus A, Zanetti A, Duval B, Jilg W, Böcher WO, Lu S-N, et al. Long-term efficacy of hepatitis B vaccine, booster policy, and impact of hepatitis B virus infection. Vaccine. 2005;23:4158–66. doi:10.1016/j.vaccine.2005.03.017.

22. Hammit L, Hennesey TW, Fiore AE, Zanis C, Hummel KB, Dunaway E, Bullock L, McMahon BJ. Hepatitis B immunity in children vaccinated with recombinant hepatitis B vaccine beginning at birth: a follow-up study at 15 years. Vaccine. 2007;25:6958–64. doi:10.1016/j.vaccine.2007.06.059.

23. Kao JT, Wang JH, Hung CH, Yen YH, Hung SF, Hu TH, Lee CM, Lu SN. Long-term efficacy of plasma-derived and recombinant hepatitis B vaccines in a rural township of Central Taiwan. Vaccine. 2009;27:1858–62. doi:10.1016/j.vaccine.2009.01.027.

24. Poovorawan Y, Chongsrisawat V, Theamboonlers A, Leroux-Roels G, Kuriyakose S, Leysen J, Maquet J, Evidence of protection against chronic and chronic hepatitis B infection 20 years after infant vaccination in a high endemicity region. J Viral Hepat. 2011;18:369–75. doi:10.1111/j.1365-2893.2010.01312.x.

25. Roznovsky L, Orsagova I, Kloudova A, Tvrdik J, Kabieszova L, Lochman J, Krkocek J, Hoxakova L, Zvejkova I, Pliskova L. Long-term protection against hepatitis B after newborn vaccination: 20-year follow-up. Infection. 2010;38:395–400. doi:10.1007/s10152-009-0397-9.

26. Su F-H, Cheng S-H, Li C-Y, Chen J-D, Hsiao C-Y, Chien C-C, Yang Y-C, Hung –H-H, Chu F-Y. Hepatitis B seroprevalence and anamnestic response amongst Taiwanese young adults with full vaccination in infancy, 20 years subsequent to national hepatitis B vaccination. Vaccine. 2007;25:8085–90. doi:10.1016/j.vaccine.2007.09.013.

27. van der Sande MA, Weight PA, Mendy M, Zaman S, Kitty S, Sam O, Kahn A, Jeffries D, Akum AA, Hall AJ, et al. Long-term protection against HBV chronic carriage of Gambian adolescents vaccinated in infancy and immune response in HBV booster trial in adolescence. PLoS One. 2007;2:e753. doi:10.1371/journal.pone.0000753.

28. van der Sande MA, Weight P, Mendy M, Rayco-Solon P, Hutt P, Fulford T, Doherty C, McConkey S, Jeffries D, Hall A, et al. Long-term protection against carriage of hepatitis B virus after infant vaccination. J Infect Dis. 2006;193:1528–35. doi:10.1086/jid.2006.193.issue-11.

29. Alfaleh F, Alshereh S, Alansari S, Aljeffri M, Almazrou Y, Shaffi A, Abdo AA. Long-term protection of hepatitis B vaccine 18 years after vaccination. J Infect. 2008;57:404–9. doi:10.1016/j.jinf.2008.08.008.

30. Leuridan E, Van Damme P. Hepatitis B and the need for a booster dose. Clin. Infect. Dis. 2011;53:68–75. doi:10.1093/cid/cir270.

31. Bruce MG, Bruden D, Hurlbut D, Zanis C, Thompson G, Rea L, Toomey M, Townshend-Bulson L, Rudolph K, Bullock L, et al. Antibody levels and protection after hepatitis b vaccine: results of a 30-year follow-up study and response to a booster dose. J Infect Dis. 2016;214:16–22. doi:10.1093/infdis/jiv748.

32. Simons BC, Spradling BR, Bruden DJ, Zanis C, Case S, Choromanski TL, Apodaca M, Brodgon HD, Dwyer G, Snowball M, et al. A longitudinal hepatitis B vaccine cohort demonstrates long-lasting hepatitis B virus (HBV) cellular immunity despite loss of antibody against HBV surface antigen. J Infect Dis. 2016;214:273–80. doi:10.1093/infdis/jiw142.

33. Zanetti AR, Romano L, Giambi C, Pavan A, Carnelli V, Baitelli G, Malchiodzi G, Valerio E, Marchisio MA, et al. Hepatitis B immune memory in children primed with hexavalent vaccines and given monovalent booster vaccines: an open-label, randomised, controlled, multicentre study. Lancet Infect Dis. 2010;10:755–61. doi:10.1016/S1473-3099(10)70195-X.

34. Zanetti A, Parlati A, Romano L, Desole MG, Ferrera G, Giurandella F, Zuliani M, Richard P, Thomas S, Fiquet A. Challenge with a hepatitis B vaccine in two cohorts of 4–7-year-old children primed with hexavalent vaccines: an open-label, randomised trial in Italy. Vaccine. 2012;30:5770–75. doi:10.1016/j.vaccine.2012.06.078.

35. Giambi C, Bella A, Barale A, Montu D, Marchisio M, Oddone M, Zito S, Rapicetta M, Chieffe P, Madonna E, et al. A cohort study to evaluate persistence of hepatitis B immunogenicity after administration of hexavalent vaccines. BMC Infect Dis. 2008;8:101. doi:10.1186/1471-2334-8-100.

36. Zinke M, Kappes R, Kindler K, Paulus-Koschik A, Goering U, Desselhoff J, Soemantari P, Grunerdt D, Laakmann KH, Gunasekaran R, et al. Immune memory to hepatitis B virus in 4–9-year old children vaccinated in infancy with four doses of hepatitis B vaccine. J HIV. 2009;5:592–98. doi:10.1016/j.hiv.9051.

37. Huang K, Lin S. Nationwide vaccination: a success story in Taiwan. Vaccine. 2000;18(Suppl 1):S53–8. doi:10.1016/s0264-410x(99)00460-0.

38. Van Damme P. Long-term protection after hepatitis B vaccine. J Infect Dis. 2016;214:1–3. doi:10.1093/infdis/jiv750.

39. Robert Koch-Institut. Statement of the German standing committee on vaccination at the RKI recommendations of the standing committee on vaccination (STIKO) at the Robert Koch institute - 2017/2018. Epidemiologisches Bull. 24 of August 2017. 2017;34:333–76.

40. Olivier C, Belohradsky BH, Stojanov S, Bonnet E, Petersen G, Liese JG. Immunogenicity, reactogenicity, and safety of a seven-valent pneumococcal conjugate vaccine (PCV7) concurrently administered with a fully liquid DTPa-IPV-Hib combination vaccine in healthy infants. Vaccine. 2008;26:3142–52. doi:10.1016/j.vaccine.2007.11.096.

41. Hansen B, Belmont M, Soung G, Song L, Egan PM, Capen R, Hogenesch H, Mancinelli R, Hem SL. Effect of the strength of adsorption of hepatitis B surface antigen to aluminum hydroxide adjuvant on the immune response. Vaccine. 2009;27:888–92. doi:10.1016/j.vaccine.2008.11.078.

42. Minervini G, McCarson BJ, Reisinger KS, Martin JC, Stek JE, Atkins BM, Nadig KB, Liska V, Schödel FP, Bhuyan PK. Safety and immunogenicity of a modified process hepatitis B vaccine in healthy neonates. Vaccine. 2012;30:1476–80. doi:10.1016/j.vaccine.2011.12.095.

43. Gilbert CL, Klopfer SO, Martin JC, Schoell FP, Bhuyan PK. Safety and immunogenicity of a modified process hepatitis B vaccine in healthy adults ≥50 years. Hum Vaccin. 2011;7:1336–42. doi:10.4161/hv.7.12.18333.

44. Gilbert CL, Stek JE, Villa G, Klopfer SO, Martin JC, Schoell FP, Bhuyan PK. Safety and immunogenicity of a recombinant hepatitis B vaccine manufactured by a modified process in renal pre-dialysis and dialysis patients. Vaccine. 2013;31:6521–26. doi:10.1016/j.vaccine.2014.09.015.

45. Vesikari T, Martin JC, Liss CL, Liska V, Schoell FP, Bhuyan PK. Safety and immunogenicity of a modified process hepatitis B vaccine in healthy infants. Pediatr Infect Dis J. 2011;30:e109–13. doi:10.1097/INF.0b013e31821ed1a4.

46. Van Damme P, Minervini G, Liss CL, McCarson B, Vesikari T, Boslego JW, Bhuyan PK. Safety, tolerability and immunogenicity of a recombinant hepatitis B vaccine manufactured by a modified process in healthy young adults. Hum Vaccin. 2009;5:92–97. doi:10.4161/hv.5.2.6587.

47. Edwards KM, Meade BD, Dercker MD, Reed GF, Rennels MB, Steinhoff MC, Anderson EL, Englund JA, Pichichero ME, Deloria MA. Comparison of 13 acellular pertussis vaccines: overview and serologic response. Pediatrics. 1995;96:548–57.

48. Plotkin SA. Complex correlates of protection after vaccination. Clin. Infect. Dis. 2013;56:1458–65. doi:10.1093/cid/cit048.
vaccine-induced human reactogenicity biomarkers in vitro. Vaccine. 2019;37:80–89. doi:10.1016/j.vaccine.2018.11.028.

50. Gorringe AR, Vaughan TE. Bordetella pertussis fimbriae (Fim): relevance for vaccines. Expert Rev Vaccines. 2014;13:1205–14. doi:10.1586/14760584.2014.930667.

51. European Centre for Disease Prevention and Control. Vaccine schedules in all countries of the European Union. 2018.

52. Centers for Disease Control and Prevention. Recommended immunization schedule for children and adolescents aged 18 years or younger, UNITED STATES, 2018. 2018.