Antibody Persistence in Young Children 5 Years after Vaccination with Combined 
*Haemophilus influenzae* type b-*Neisseria meningitidis* Serogroup C or MenC Conjugate 
Vaccines Co-Administered with DTPa-Containing and Pneumococcal Conjugate 
Vaccines

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Running head: Antibody persistence to Hib-MenC-TT

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Keywords: *Haemophilus influenzae* type b, *Neisseria meningitidis* serogroup C, antibody persistence, MenC conjugate vaccine
Abstract

Background and aims: We evaluated antibody persistence in children up to 5 years after administration of a combined *Haemophilus influenzae* type b (Hib)-*Neisseria meningitidis* serogroup C (MenC) tetanus toxoid conjugate vaccine (Hib-MenC-TT) co-administered with a pneumococcal conjugate vaccine (NCT00891176).

Method: This is the follow-up of a randomised study (NCT00334334/00463437), in which healthy children were vaccinated (primary, 2–4–6 months of age; and booster, 11–18 months) with Hib-MenC-TT or a control MenC conjugate vaccine, co-administered with diphtheria-tetanus-acellular pertussis (DTPa)-based combination vaccines (DTPa/Hib for control groups) and a pneumococcal conjugate vaccine (10-valent pneumococcal non-typeable *H. influenzae* protein D conjugate vaccine [PHiD-CV] or 7-valent CRM197 conjugate vaccine [7vCRM]). MenC antibody titres were measured by serum bactericidal antibody assay using rabbit complement (rSBA) and antibodies against Hib polyribosylribitol phosphate (anti-PRP) by ELISA. Antibody persistence up to 5 years after booster vaccination is reported for 530 children aged ~6 years.

Results: The percentages of children with seroprotective rSBA-MenC titres were between 24.2% and 40.1% in all groups approximately 5 years after booster vaccination. More than 98.5% of children in each group retained seroprotective anti-PRP concentrations. No vaccine-related serious adverse events and no events related to lack of vaccine efficacy were reported.

Conclusion: Approximately 5 years after booster vaccination, the majority of children retained seroprotective anti-PRP antibody concentrations. The percentage of children retaining seroprotective rSBA-MenC titres was low (≤40%), suggesting that a significant proportion of children may be unprotected against MenC disease.
Introduction

Immunization of children with conjugate vaccines has proven to be a successful strategy to prevent infections caused by various encapsulated bacteria such as *Neisseria meningitidis* and *Haemophilus influenzae* type b (Hib) (1), and has resulted in a great decline in the incidence of meningococcal serogroup C (MenC) and Hib disease in the world (2, 3). In addition to immune memory, persisting antibodies have been suggested to be a more appropriate correlate of long-term protection against disease (4). Circulating antibodies are particularly important for sustained protection against invasive MenC disease (5).

In Europe, MenC is the second most important cause of invasive meningococcal disease (IMD) after meningococcal serogroup B. In 2012, according to the European Centre for Disease Prevention and Control, 17% of IMD in Europe was caused by MenC (6). In addition, recent estimates from Spain (covering the 2005–2011 period), Germany (2002–2010) and Poland (2002–2011) have found that MenC was responsible for 13.2%, 25.0% and 36.6% of confirmed IMD cases, respectively (7-9).

The Hib and MenC tetanus toxoid (TT) conjugate vaccine (Hib-MenC-TT, *Menitorix™*; GSK Vaccines) is a combination vaccine containing polyribosylribitol phosphate (PRP) from Hib and polysaccharide from MenC, each individually conjugated to TT as carrier protein. The vaccine was developed to provide protection to infants and toddlers against Hib and MenC diseases in a single injection (10). Hib-MenC-TT offers an alternative vaccination schedule to diphtheria-tetanus-acellular pertussis (DTPa)/Hib combined vaccines co-administered with MenC conjugate vaccines.

Hib-MenC-TT primary and booster vaccinations with different vaccination schedules and different combination vaccines were shown to be safe and immunogenic in infants and toddlers (11-27). Primary vaccination induced persistent antibodies against both antigens up to the second year of life (12-15). Persistence rates post-booster vaccination varied in
different studies (22, 23, 27), and there are no data available for antibody persistence after co-
administration with a pneumococcal conjugate vaccine.

As mass vaccination schedules expand, there is a risk that adding further antigens to the
schedule could lead to unexpected immune interferences (28, 29). If initial immune responses
are not as robust as initially expected, the antibody responses may not persist as long as
protection is needed. The purpose of this extension study was to explore whether differences
in initial antibody concentrations and titres to the co-administered antigens in the Hib-MenC
and pneumococcal conjugate vaccines translated into differences in long-term persistence.

In the original randomized study, 1548 healthy children were vaccinated with Hib-MenC-TT,
or a control MenC conjugate vaccine co-administered with a DTPa or DTPa/Hib-containing
vaccine, and a pneumococcal conjugate vaccine. After primary (at 2–4–6 months of age) and
booster (at 11–18 months of age) vaccination, immune responses were induced against all
vaccine components (16, 17). In the current follow-up study, we evaluated persistence of the
immunogenicity of the Hib-MenC-TT conjugate vaccine up to 5 years after booster
vaccination, with the evaluation of MenC antibody persistence as primary objective. The
antibody persistence to the pneumococcal conjugate vaccines administered in the study will
be addressed in a separate publication.

Materials and Methods

Ethical statement

The antibody persistence study (ClinicalTrials.gov identifier: NCT00891176) was conducted
in Germany, Poland, and Spain between May and December 2009 (Year 2), May and
November 2010 (Year 3) and April and November 2012 (Year 5). The protocol was approved
by the following Ethics Committees: Comité Ético de Investigación Clínica del Hospital
Universitario de Móstoles, Madrid; Comité Ético de Investigación Clínica del Hospital
Clínico San Carlos, Madrid; Komisja Bioetyczna przy Okregowej Izbie Lekarskiej, Krakow; Ethik-Kommission der Bayerischen Landesärztekammer, München; Ethik-Kommission der Landesärztekammer Baden-Württemberg, Stuttgart; and Landesamt für Gesundheit und Soziales Geschäftss der Ethik-Kommission des Landes Berlin. The study was conducted in accordance with the principles of Good Clinical Practice and the Declaration of Helsinki, although some deviations in terms of study documentation and adherence to the study protocol were identified. Some additional Good Clinical Practice deviations were noted at one of the study centres, which included the finding that annual reports and updates of protocol amendments and administrative changes had not been sent to regulatory authorities, as required by local law. However, after full investigation by an independent department and discussion with local regulatory agencies, it was determined that these findings did not have an impact on the safety of participants or on data integrity.

Study design and participants

Written informed consent was obtained from each child’s parent or guardian. Healthy children who completed primary and booster vaccinations in a previous study (ClinicalTrials.gov identifier: NCT00334334/00463437 (17)) and were in the blood sampling subset in the booster study, and whose parents or legal guardians were believed by the investigator to be able to comply with study requirements, were included in this open, long-term persistence study. Children were excluded from the persistence study if they had received a MenC, Hib, hepatitis B, or pneumococcal vaccine or had developed MenC, Hib, hepatitis B, or invasive pneumococcal disease since booster vaccination, or had received an immune-modifying drug in the previous 6 months, a blood product in the previous 3 months, or an investigational drug or vaccine in the last 30 days before blood sampling. In the previous primary/booster vaccination study, exclusion criteria included vaccination against diphtheria, tetanus, pertussis, polio, MenC, Hib, hepatitis B, or Streptococcus pneumoniae.
Immunization with vaccines (such as hepatitis B vaccine) where the first dose was given within the first two weeks of life was permitted, in accordance with national recommendations.

In the initial phase III, open, randomised, multicentre study (17), Hib-MenC-TT or a control MenC conjugate vaccine (MenC-CRM$_{197}$ [Meningitec$^\text{TM}$; Pfizer Inc.] or MenC-TT [NeisVac-C$^\text{TM}$; Baxter Healthcare SA]) were co-administered with a DTPa or DTPa/Hib-containing vaccine (all manufactured by GSK Vaccines) and one of two pneumococcal conjugate vaccines: 10-valent pneumococcal non-typeable *Haemophilus influenzae* protein D conjugate vaccine (PHiD-CV, Synflorix$^\text{TM}$; GSK Vaccines) or 7-valent pneumococcal CRM$_{197}$ conjugate vaccine (7vCRM, Prevenar$^\text{TM}$/Prevnar$^\text{TM}$; Pfizer Inc.). Hib-MenC-TT, pneumococcal conjugate vaccines, and DTPa-containing vaccines were administered at 2–4–6 months of age, with booster vaccination at 11–18 months of age (17). The MenC-TT and MenC-CRM$_{197}$ vaccines were administered at 2 and 4 months of age (in Poland, to comply with national recommendations, a third dose was offered at approximately 7 months of age) and at 11–18 months of age.

There were four study groups: children were randomised (1:1:1:1) to receive Hib-MenC-TT plus either PHiD-CV (Hib-MenC + PHiD-CV group) or 7vCRM (Hib-MenC + 7vCRM group), MenC-CRM$_{197}$ plus PHiD-CV (MenC-CRM group), or MenC-TT plus PHiD-CV (MenC-TT group) (**Table 1**). In Germany and Poland, groups administered Hib-MenC-TT were given DTPa-hepatitis B-inactivated polio vaccine (DTPa-HBV-IPV, Infanrix penta$^\text{TM}$/Pediarix$^\text{TM}$; GSK Vaccines) and groups administered MenC conjugate vaccines were given DTPa-HBV-IPV/Hib (Infanrix hexa$^\text{TM}$; GSK Vaccines) as booster vaccination. Because hepatitis B booster vaccination is not recommended in Spain, the Spanish Hib-MenC-TT groups received DTPa-IPV (Infanrix-IPV$^\text{TM}$; GSK Vaccines) while the control groups received DTPa-IPV/Hib (Infanrix-IPV/Hib$^\text{TM}$; GSK Vaccines) as booster vaccination.
In Poland, children received a single dose of hepatitis B vaccine at birth, according to national recommendations.

**Study objectives**

The primary objective of this follow-up study was to evaluate the MenC antibody persistence after vaccination in all treatment groups that received the Hib-MenC-TT conjugate vaccine, in terms of percentage of children with serum antibody levels above the assay cut-off up to 60 months after booster vaccination (72–76 months of age). In this report, antibody persistence results are presented for blood samples taken from children at approximately 3, 4 and 6 years of age. The secondary objectives included the evaluation of antibody persistence with respect to Hib capsular polysaccharide (anti-PRP) and hepatitis B surface antigen (anti-HBs) antibody concentrations.

**Immunogenicity assessments**

Blood samples of approximately 5 mL were collected at 36–40 months of age (i.e. approximately 24 months after booster vaccination), 48–52 months of age (i.e. approximately 36 months after booster vaccination) and at 72–76 months of age (i.e. approximately 60 months after booster vaccination).

Antibodies against MenC at Year 5 were measured by serum bactericidal activity assay performed at Public Health England (PHE), using rabbit complement (rSBA) with a cut-off of 1:8 as indication of protection (30). Earlier time points were tested with an in-house rSBA-MenC assay performed at GSK Vaccines (16), which prevents a direct comparison of the Year 5 results with the initial results from earlier study time points (17).

Anti-PRP antibodies were assessed by an in-house ELISA performed at GSK Vaccines, for which concentration levels ≥0.15 µg/mL and ≥1 µg/mL are considered indicative of short- and long-term protection, respectively (31, 32).

Antibodies against hepatitis B were assessed at GSK Vaccines’ laboratories using the
commercial chemiluminescence immunoassay Centaur™ (Siemens Healthcare Diagnostics). Anti-HBs seroprotection was defined as an antibody concentration above 10 milli-
international units (mIU)/mL (33).

Safety assessments
Serious adverse events (SAEs) for the primary and booster phases of this study were reported
earlier (34). In this study, SAEs which were assessed by the investigator as potentially
vaccine- or study procedure-related or due to lack of vaccine efficacy were documented
retrospectively following booster vaccination and recorded at every visit/contact during the
study. Per definition, an SAE was any untoward medical occurrence that resulted in death,
was life-threatening, required hospitalization or resulted in disability/incapacity. Although it
was unlikely for SAEs related to vaccination to occur so late after vaccination, this study was
designed to ensure that any event that was only manifested long after vaccination and which
the investigator thought could have been related to the vaccination or any event related to
lack of vaccine efficacy during this long-term persistence phase and that fulfil the definition
of an SAE would be recorded.

Statistical analyses
Results are presented for the according-to-protocol (ATP) cohorts for antibody persistence
post-booster vaccination, which included all eligible children who received the full primary
and booster vaccination course corresponding to their group, were compliant with study
procedures and who had available assay results.

For each group, the percentages of participants retaining antibody concentrations or titres
above the predefined thresholds and geometric mean antibody titres and concentrations
(GMTs and GMCs) were calculated with associated 95% confidence intervals (CIs).

Exploratory statistical analyses were performed in which potential differences between
groups were defined when the asymptotic standardised 95% CI for the difference in
percentages of children with titres or concentrations above proposed cut-offs between the two groups did not contain the value “0”, or when the 95% CI for the GMT/GMC ratios between groups did not contain the value “1”. This analysis was performed using a one-way analysis of variance model on the logarithm in base 10 (log10) transformation of the titres or concentrations using the vaccine group and country as the only covariates.

The results of all exploratory group comparisons should be interpreted with caution considering that there was no adjustment for multiplicity for these comparisons and the clinical relevance of the differences was not pre-specified.

The statistical analyses were performed using the Statistical Analysis Systems (SAS®; Cary, NC, USA) version 9.22 on Windows.

Results

Study participants

Of the 1437 children who were part of the total enrolled cohort for the booster phase, 581, 561 and 539 were included in the total enrolled cohorts for persistence at 24, 36 and 60 months post-booster vaccination, respectively. The ATP cohorts for antibody persistence included 571 children at 24 months post-booster vaccination, 543 children at 36 months post-booster vaccination and 530 children at 60 months post-booster vaccination. Reasons for exclusion from the ATP cohorts and the distribution per study group are given in Figure 1.

Across the four vaccine groups, the mean age of the children in the ATP cohorts for persistence was similar between groups, and the majority was of European descent (Table 2). The proportions of males and females were similar across all groups, with a higher proportion of males in the MenC-TT group and a higher proportion of females in the Hib-MenC + PHiD-CV group. The gender and racial distribution in the ATP cohorts for persistence were consistent with the primary study (17).
Immunogenicity against MenC

Approximately 5 years post-booster vaccination, the percentage of children with an rSBA-MenC titre ≥8 as measured by the PHE assay was between 24.2% and 40.1% in all groups, with the highest value observed in the MenC-TT group (Table 3). GMTs ranged from 7.2 in the MenC-CRM group to 11.9 in the MenC-TT group. At Year 3, rSBA-MenC titres ≥8 as measured by the GSK assay were retained by at least 72.4% of children in each group (Supplementary Table 1).

Based on exploratory analyses, the percentage of children with rSBA-MenC titre ≥8 at Year 5 was higher in the MenC-TT and Hib-MenC + PHiD-CV groups compared to MenC-CRM group, and in the MenC-TT group compared to the Hib-MenC + 7vCRM group (Table 4). The rSBA-MenC GMT was higher in the MenC-TT group compared to MenC-CRM group. No difference between MenC-TT and Hib-MenC + PHiD-CV groups was observed. Similar observations were made at Year 3 (Supplementary Table 2).

Immunogenicity against Hib PRP

Approximately 5 years post-booster vaccination, the percentage of children with anti-PRP concentrations ≥0.15 μg/mL remained high in all groups (≥98.5%) (Table 5). Among all groups, at least 57.5% of children had anti-PRP concentrations ≥1 μg/mL, with the highest percentages in the Hib-MenC-TT recipients (Hib-MenC + PHiD-CV and Hib-MenC + 7vCRM groups). Anti-PRP GMCs at Year 5 ranged from 1.65 in the MenC-TT group to 2.95 in the Hib-MenC + PHiD-CV group.

Based on exploratory analyses, there was no significant potential difference between groups in percentages of children achieving anti-PRP antibody concentrations ≥0.15 μg/mL. Anti-PRP antibody GMCs were lower in the control MenC vaccines recipients than in the Hib-MenC-TT groups, regardless of MenC vaccine type, and there was no difference in anti-PRP antibody GMCs between the two MenC vaccine groups (Table 6).
**Immunogenicity against hepatitis B**

Considering the anti-HBs results as tested by chemiluminescence assay, at least 72.8% of children had antibody concentrations ≥10 mIU/mL approximately 60 months post-booster vaccination (Table 7). Anti-HBs antibody GMCs declined by 60 months post-booster vaccination and were between 45.7 and 85.4 mIU/mL at 60 months post-booster vaccination.

**Safety**

No SAEs considered by the investigator to be potentially vaccine- or study procedure-related or due to lack of vaccine efficacy were reported following administration of the booster vaccination.

**Discussion**

This study reports the antibody persistence in healthy children up to 5 years after the Hib and MenC full vaccination course. At the time the study was conducted, children in the United Kingdom (UK) used to receive concomitant doses with Hib-MenC and pneumococcal conjugate vaccines as primary vaccinations. Our data are important in comparing persistence post-booster vaccination, since children in the UK currently receive booster doses of Hib-MenC and pneumococcal conjugate vaccines simultaneously at 12–13 months of age (35).

For MenC, the percentages of children retaining seroprotective rSBA-MenC titres at Year 5 post-booster vaccination were 24.2%, 25.4%, 38.5% and 40.1% in the MenC-CRM, Hib-MenC + 7vCRM, Hib-MenC + PHiD-CV and MenC-TT groups, respectively. In contrast, retention of anti-PRP antibodies was nearly universal in all groups: at least 98.5% of children in each group were observed to have anti-PRP concentrations ≥0.15 μg/ml, 5 years post-booster vaccination.

The percentages of children with seroprotective rSBA-MenC titres ≥8 were considerably lower than the 72.4%–96.4% rates reported three years after booster vaccination. However,
the assays were performed in different laboratories with different procedures, and therefore
the results cannot be directly compared. It has been observed that the GSK rSBA-MenC
assay used previously has a higher sensitivity than the PHE rSBA-MenC assay used in the
current follow-up study, especially to naturally acquired antibodies (36). In addition, the PHE
laboratory uses the discontinuous method to interpolate titres from the standard curve as
opposed to GSK, which uses a continuous method. The discontinuous method of titre
calculation is known to result in lower titres than the continuous method.
The proportions of children with seroprotective rSBA-MenC titres ≥8 in our study were
consistent with the 22%–43% rates reported in a previous study in the UK (measured with the
same rSBA assay at PHE) at 24 months after the administration of a Hib-MenC-TT booster
dose in toddlers (12–14 months of age) (19). In this study, infants had been primed with two
doses of MenC-CRM or MenC-TT at 2 and 3, or 2 and 4 months of age (19). In contrast, in a
study in Spain, where the GSK Vaccines rSBA-MenC assay was used, higher percentages
were observed more than 5 years after Hib-MenC-TT booster vaccination in toddlers
following Hib-MenC-TT or MenC-TT priming in infancy in a 2-4-6-month schedule (78%–
97%) (23). However, these results cannot be directly compared with the current study results
as different rSBA assay procedures were used.
The low MenC titres at 5 years post-vaccination suggested that individuals may no longer be
protected or contribute to herd immunity. The introduction of a MenC adolescent booster
dose at around 14 years has been adopted in the UK in 2013 based on advice from the Joint
Committee on Vaccination and Immunisation (37). The adolescent booster vaccination at 12
years of age was also incorporated in the vaccination calendar of all Spanish autonomous
regions in 2014 (7).
The exploratory analyses revealed possible differences between groups in MenC antibody
persistence. The percentage of children with an rSBA-MenC titre ≥8 at Year 5 post-booster
vaccination was higher in the MenC-TT and Hib-MenC + PHiD-CV groups compared to MenC-CRM group, and in the MenC-TT group compared to the Hib-MenC + 7vCRM group. MenC GMTs were observed to be higher in the MenC-TT group versus the MenC-CRM group, which is consistent with previous observations suggesting that TT was a better carrier protein for MenC priming than CRM₁⁹⁷ in terms of seroprotection and GMTs (19, 38).

A clear effect of the co-administered pneumococcal conjugate vaccines could not be observed, since there were no significant potential differences in terms of MenC GMTs between PHiD-CV and 7vCRM recipients. Moreover, the control vaccines MenC-CRM and MenC-TT were only co-administered with PHiD-CV; no groups receiving these control MenC vaccines together with 7vCRM were included in this study.

High seroprotection rates against the Hib antigen were observed in all groups. There was no significant potential difference between groups in the percentage of children with anti-PRP antibody concentration ≥0.15 μg/mL, and no decline compared to earlier time points. Comparison of anti-PRP antibody GMCs between groups showed better long-term persistence 5 years post-booster vaccination in the Hib-MenC-TT recipients compared to the DTPa/Hib recipients, regardless of the co-administered pneumococcal conjugate vaccine, which is consistent with a previous report (23). There was only a slight decrease in the Year 5 anti-PRP antibody GMC values compared to the previous time points (up to 3 years post-booster vaccination). The high anti-PRP antibody persistence in all groups suggests that an additional Hib booster dose is not needed.

Infants and toddlers are routinely vaccinated against hepatitis B in many countries with the expectation that such vaccination will protect against infections that may be acquired years in the future. The purpose of assessing the hepatitis B antibody persistence was to explore the percentage of subjects retaining seroprotective antibody concentrations 5 years after vaccination with the various regimens. Our results showed that the anti-HBs seroprotection
rates 5 years post-booster vaccination ranged from 73% to 84% in all groups. These percentages appeared to be smaller compared to earlier time points (2 and 3 years post-booster vaccination). However, statistical comparisons were not performed and therefore, the anti-HBs data from our study must be interpreted with caution.

In conclusion, 5 years post-booster vaccination, protective anti-PRP antibody concentrations persisted in at least 98.5% of children in all groups, but the protective rSBA-MenC antibody titres were shown to persist only in 24.2–40.1% of the children, with the highest values in the Hib-MenC + PHid-CV and MenC-TT groups. These results are consistent with previously reported Hib-MenC-TT vaccine data. No SAEs considered possibly related to vaccination were reported during the 5-year persistence phase of the study.

**Funding information**

GlaxoSmithKline Biologicals SA was the funding source and was involved in all stages of the clinical trial conduct and analysis. GlaxoSmithKline Biologicals SA also took in charge all costs associated with the development and the publishing of the present manuscript. All authors had full access to the data. MVW and DK are employees of the GSK group of companies. YB was an employee of the GSK group of companies and is co-inventor of a patent which, if granted, will be owned by the GSK group of companies. YB and MVW own stock options and stock grants in the GSK group of companies. JCT, JB, RK and DG have no conflict of interest.

**Acknowledgements**

The authors thank the children and their families for participating in this trial, and all investigators and their clinical teams for their contribution to this study. The authors also thank Melissa McNeely (XPE Pharma & Science c/o GSK Vaccines) for publication
management and Vasile Coman (XPE Pharma & Science) for drafting the manuscript.

Authors’ contributions

JCT, JB, RK, DG, DK and MVW conceived and designed the experiments. JCT, JB, RK and DG performed the experiments. DK and MVW analyzed the data. JCT, JB, RK, DG, DK, YB and MVW contributed with reagents, materials and/or analysis tools. JCT, YB, JB, RK, DG, DK and MVW wrote the paper.

Trademarks

Menitorix, Priorix, Hiberix, Infanrix-IPV and Infanrix-IPV/Hib are trademarks of the GSK group of companies. Meningitec is a trademark of Nuron Biotech. NeisVac-C is a trademark of Pfizer. ADVIA Centaur is a trademark of Siemens Healthcare Diagnostics.

References


15. Habermehl, P., G. Leroux-Roels, R. Sanger, G. Machler, and D. Boutriau. 2010. Combined Haemophilus influenzae type b and Neisseria meningitidis serogroup C (HibMenC) or serogroup C and Y-tetanus toxoid conjugate (and HibMenCY) vaccines are well-tolerated and immunogenic when administered according to the 2,3,4 months schedule with a fourth dose at 12-18 months of age. Hum Vaccin 6:640-651.


25. Khatami, A., M. D. Snape, B. Ohene-Kena, K. Young, C. Oeser, L. J. Michaelis,


### Table 1. Study groups

<table>
<thead>
<tr>
<th>Group</th>
<th>MenC vaccine</th>
<th>Pneumococcal vaccine</th>
<th>Co-administered vaccine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hib-MenC + PHiD-CV</td>
<td>Hib-MenC-TT</td>
<td>PHiD-CV</td>
<td>DTPa-HBV-IPV or DTPa-IPV&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Hib-MenC + 7vCRM</td>
<td>Hib-MenC-TT</td>
<td>7vCRM</td>
<td>DTPa-HBV-IPV or DTPa-IPV&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>MenC-CRM&lt;sup&gt;a&lt;/sup&gt;</td>
<td>MenC-CRM</td>
<td>PHiD-CV</td>
<td>DTPa-HBV-IPV/Hib or DTPa-IPV/Hib&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>MenC-TT&lt;sup&gt;a&lt;/sup&gt;</td>
<td>MenC-TT</td>
<td>PHiD-CV</td>
<td>DTPa-HBV-IPV/Hib or DTPa-IPV/Hib&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Primary vaccination phase: doses at 2, 4, and 6 months. Booster phase: vaccines administered at 11–18 months.

<sup>a</sup>MenC-CRM and MenC-TT vaccines were administered at 2 and 4 months of age; in Poland, to comply with national recommendations, children were offered a third dose of MenC vaccines at ±7 months of age.

<sup>b</sup>Because hepatitis B booster vaccination is not recommended in Spain, the Spanish Hib-MenC-TT groups received DTPa-IPV while the control groups received DTPa-IPV/Hib as booster vaccination.
Table 2. Demographic characteristics of the study children (ATP cohorts for antibody persistence at 24 (Y2), 36 (Y3) and 60 months (Y5) post-booster vaccination)

<table>
<thead>
<tr>
<th>Timepoint</th>
<th>N</th>
<th>Mean age ± SD (months)</th>
<th>Gender (%)</th>
<th>Ethnicity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y2</td>
<td>144</td>
<td>37.3 ± 1.2</td>
<td>140</td>
<td>37.3 ± 1.3</td>
</tr>
<tr>
<td>Y3</td>
<td>133</td>
<td>49.2 ± 1.5</td>
<td>136</td>
<td>48.7 ± 1.2</td>
</tr>
<tr>
<td>Y5</td>
<td>131</td>
<td>72.9 ± 1.0</td>
<td>134</td>
<td>72.9 ± 1.2</td>
</tr>
</tbody>
</table>

Gender (%)
- Y2: 57.6%
- Y3: 60.2%
- Y5: 58.8%

Ethnicity (%)
- Y2: 95.1%
- Y3: 95.5%
- Y5: 96.2%

N, total number of children per group.
Table 3. Serum bactericidal activity against MenC approximately 5 years post-booster vaccination (ATP cohort for antibody persistence at 60 months post-booster vaccination)

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>% ≥8 (95% CI)</th>
<th>GMT (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hib-MenC + PHiD-CV</td>
<td>130</td>
<td>38.5 (30.1–47.4)</td>
<td>10.1 (7.9–12.9)</td>
</tr>
<tr>
<td>Hib-MenC + 7vCRM</td>
<td>134</td>
<td>25.4 (18.3–33.6)</td>
<td>8.5 (6.6–11.1)</td>
</tr>
<tr>
<td>MenC-CRM</td>
<td>128</td>
<td>24.2 (17.1–32.6)</td>
<td>7.2 (5.8–8.9)</td>
</tr>
<tr>
<td>MenC-TT</td>
<td>137</td>
<td>40.1 (31.9–48.9)</td>
<td>11.9 (8.9–16.0)</td>
</tr>
</tbody>
</table>

N, total number of children with available results.
Table 4. Differences between groups (first group minus second group) in percentages of children with rSBA-MenC titres above the threshold and rSBA-MenC GMT ratios (first group over second group) approximately 5 years post-booster vaccination (exploratory analyses; ATP cohort for antibody persistence at 60 months post-booster vaccination)

<table>
<thead>
<tr>
<th>Group comparison</th>
<th>Difference in % of children achieving ≥8</th>
<th>GMT ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>MenC-CRM vs. MenC-TT</td>
<td>-15.93 (−26.79; −4.67)</td>
<td>0.68 (0.47; 0.96)</td>
</tr>
<tr>
<td>MenC-CRM vs. Hib-MenC + PHiD-CV</td>
<td>-14.24 (−25.26; −2.92)</td>
<td>0.73 (0.53; 1.01)</td>
</tr>
<tr>
<td>MenC-CRM vs. Hib-MenC + 7vCRM</td>
<td>-1.15 (−11.62; 9.39)</td>
<td>0.92 (0.67; 1.28)</td>
</tr>
<tr>
<td>MenC-TT vs. Hib-MenC + PHiD-CV</td>
<td>1.68 (−10.02; 13.32)</td>
<td>1.15 (0.79; 1.68)</td>
</tr>
<tr>
<td>MenC-TT vs. Hib-MenC + 7vCRM</td>
<td>14.77 (3.59; 25.62)</td>
<td>1.38 (0.95; 2.01)</td>
</tr>
</tbody>
</table>

*a 95% CIs of differences not including 0 were regarded as indicative that a significant potential difference might exist between groups. For GMT ratio, 95% CIs not including 1 indicated that a significant potential difference might exist. **BOLD** = comparison for which the exploratory analysis suggests that a potentially significant difference may exist.
Table 5. Anti-PRP antibody persistence (ATP cohort for antibody persistence at 60 months post-booster vaccination)

<table>
<thead>
<tr>
<th>Group</th>
<th>Time point</th>
<th>N</th>
<th>% ≥0.15 µg/mL (95% CI)</th>
<th>% ≥1 µg/mL (95% CI)</th>
<th>GMC (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hib-MenC + PHiD-CV</td>
<td>Post-primary</td>
<td>130</td>
<td>98.5 (94.6; 99.8)</td>
<td>97.7 (93.4; 99.5)</td>
<td>13.71 (11.11; 16.93)</td>
</tr>
<tr>
<td></td>
<td>Post-booster: M1</td>
<td>130</td>
<td>100 (97.2; 100)</td>
<td>100 (97.2; 100)</td>
<td>90.53 (75.73; 108.23)</td>
</tr>
<tr>
<td></td>
<td>Post-booster: M24</td>
<td>121</td>
<td>100 (97.0; 100)</td>
<td>93.4 (87.4; 97.1)</td>
<td>4.21 (3.41; 5.21)</td>
</tr>
<tr>
<td></td>
<td>Post-booster: M36</td>
<td>122</td>
<td>100 (97.0; 100)</td>
<td>90.2 (83.4; 94.8)</td>
<td>3.76 (3.03; 4.66)</td>
</tr>
<tr>
<td></td>
<td>Post-booster: M60</td>
<td>130</td>
<td>100 (97.2; 100)</td>
<td>84.6 (77.2; 90.3)</td>
<td>2.95 (2.40; 3.62)</td>
</tr>
<tr>
<td>Hib-MenC + 7vCRM</td>
<td>Post-primary</td>
<td>132</td>
<td>100 (97.2; 100)</td>
<td>97.0 (92.4; 99.2)</td>
<td>10.70 (8.87; 12.90)</td>
</tr>
<tr>
<td></td>
<td>Post-booster: M1</td>
<td>130</td>
<td>100 (97.2; 100)</td>
<td>100 (97.2; 100)</td>
<td>65.65 (52.76; 81.69)</td>
</tr>
<tr>
<td></td>
<td>Post-booster: M24</td>
<td>125</td>
<td>100 (97.1; 100)</td>
<td>84.8 (77.3; 90.6)</td>
<td>3.77 (3.04; 4.69)</td>
</tr>
<tr>
<td></td>
<td>Post-booster: M36</td>
<td>125</td>
<td>99.2 (95.6; 100)</td>
<td>79.2 (71.0; 85.9)</td>
<td>2.80 (2.27; 3.46)</td>
</tr>
<tr>
<td></td>
<td>Post-booster: M60</td>
<td>132</td>
<td>100 (97.2; 100)</td>
<td>75.8 (67.5; 82.8)</td>
<td>2.56 (2.07; 3.17)</td>
</tr>
<tr>
<td>MenC-CRM</td>
<td>Post-primary</td>
<td>127</td>
<td>98.4 (94.4; 99.8)</td>
<td>86.6 (79.4; 92.0)</td>
<td>4.23 (3.36; 5.32)</td>
</tr>
<tr>
<td></td>
<td>Post-booster: M1</td>
<td>127</td>
<td>100 (97.1; 100)</td>
<td>99.2 (95.7; 100)</td>
<td>33.49 (27.11; 41.37)</td>
</tr>
<tr>
<td></td>
<td>Post-booster: M24</td>
<td>118</td>
<td>100 (96.9; 100)</td>
<td>78.0 (69.4; 85.1)</td>
<td>2.51 (1.99; 3.16)</td>
</tr>
<tr>
<td></td>
<td>Post-booster: M36</td>
<td>123</td>
<td>99.2 (95.6; 100)</td>
<td>67.5 (58.4; 75.6)</td>
<td>1.94 (1.56; 2.41)</td>
</tr>
<tr>
<td></td>
<td>Post-booster: M60</td>
<td>127</td>
<td>99.2 (95.7; 100)</td>
<td>66.9 (58.0; 75.0)</td>
<td>1.66 (1.34; 2.05)</td>
</tr>
<tr>
<td>MenC-TT</td>
<td>Post-primary</td>
<td>135</td>
<td>100 (97.3; 100)</td>
<td>95.6 (90.6; 98.4)</td>
<td>6.48 (5.48; 7.67)</td>
</tr>
<tr>
<td></td>
<td>Post-booster: M1</td>
<td>134</td>
<td>100 (97.3; 100)</td>
<td>100 (97.3; 100)</td>
<td>36.35 (30.22; 43.73)</td>
</tr>
<tr>
<td></td>
<td>Post-booster: M24</td>
<td>130</td>
<td>100 (97.2; 100)</td>
<td>77.7 (69.6; 84.5)</td>
<td>2.29 (1.84; 2.85)</td>
</tr>
<tr>
<td></td>
<td>Post-booster: M36</td>
<td>128</td>
<td>99.2 (95.7; 100)</td>
<td>62.5 (53.5; 70.9)</td>
<td>1.78 (1.42; 2.24)</td>
</tr>
<tr>
<td></td>
<td>Post-booster: M60</td>
<td>134</td>
<td>98.5 (94.7; 99.8)</td>
<td>57.5 (48.6; 66.0)</td>
<td>1.65 (1.30; 2.09)</td>
</tr>
</tbody>
</table>

N, total number of children with available results; Post-primary, 1 month after third dose at 6 months of age; Post-booster: M1, 1 month after booster vaccination; Post-booster: M24, approximately 24 months after booster vaccination; Post-booster: M36, approximately 36 months after booster vaccination; Post-booster: M60, approximately 60 months after booster vaccination.
Table 6. Differences between groups (first group minus second group) in percentages of children with anti-PRP antibody concentrations above the threshold and anti-PRP GMC ratios (first group over second group) approximately 5 years post-booster vaccination (exploratory analyses; according-to-protocol cohort for antibody persistence at 60 months post-booster vaccination)

<table>
<thead>
<tr>
<th>Group comparison</th>
<th>Difference in % of children achieving ≥0.15 µg/mL (95% CI)</th>
<th>Difference in % of children achieving ≥1 µg/mL (95% CI)</th>
<th>GMC ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MenC-CRM vs. MenC-TT</td>
<td>0.71 (-2.97; 4.59)</td>
<td>9.47 (-2.34; 20.99)</td>
<td>0.96 (0.70; 1.31)</td>
</tr>
<tr>
<td>MenC-CRM vs. Hib-MenC + PHDiCV</td>
<td>-0.79 (-4.34; 2.11)</td>
<td>-17.69 (-27.93; -7.31)</td>
<td>0.54 (0.41; 0.73)</td>
</tr>
<tr>
<td>MenC-CRM vs. Hib-MenC + 7vCRM</td>
<td>-0.79 (-4.34; 2.06)</td>
<td>-8.83 (-19.76; 2.20)</td>
<td>0.64 (0.47; 0.87)</td>
</tr>
<tr>
<td>MenC-TT vs. Hib-MenC + PHDiCV</td>
<td>-1.49 (-5.29; 1.41)</td>
<td>-27.15 (-37.34; -16.49)</td>
<td>0.56 (0.41; 0.75)</td>
</tr>
<tr>
<td>MenC-TT vs. Hib-MenC + 7vCRM</td>
<td>-1.49 (-5.29; 1.37)</td>
<td>-18.29 (-29.18; -6.99)</td>
<td>0.65 (0.47; 0.89)</td>
</tr>
</tbody>
</table>

95% CIs of differences not including 0 were regarded as indicative that a significant potential difference might exist between groups. For GMC ratio, 95% CIs not including 1 indicated that a significant potential difference might exist. **BOLD** = comparison for which the exploratory analysis suggests that a potentially significant difference may exist.
Table 7. Anti-HBs antibody persistence at Year 5 post-booster vaccination, as assessed by a chemiluminescence assay (ATP cohort for antibody persistence at 60 months post-booster vaccination)\textsuperscript{a}

<table>
<thead>
<tr>
<th>Group</th>
<th>Time point</th>
<th>N</th>
<th>% ≥10 mIU/ml (95% CI)</th>
<th>GMC, mIU/mL (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hib-MenC + PHid-CV</td>
<td>Post-booster: M24</td>
<td>122</td>
<td>88.5 (81.5; 93.6)</td>
<td>154.5 (106.8; 223.5)</td>
</tr>
<tr>
<td></td>
<td>Post-booster: M36</td>
<td>118</td>
<td>84.7 (77.0; 90.7)</td>
<td>92.3 (63.8; 133.6)</td>
</tr>
<tr>
<td></td>
<td>Post-booster: M60</td>
<td>125</td>
<td>72.8 (64.1; 80.4)</td>
<td>45.7 (32.2; 64.8)</td>
</tr>
<tr>
<td>Hib-MenC + 7vCRM</td>
<td>Post-booster: M24</td>
<td>119</td>
<td>93.3 (87.2; 97.1)</td>
<td>218.9 (152.4; 314.4)</td>
</tr>
<tr>
<td></td>
<td>Post-booster: M36</td>
<td>119</td>
<td>89.9 (83.0; 94.7)</td>
<td>142.5 (100.3; 202.6)</td>
</tr>
<tr>
<td></td>
<td>Post-booster: M60</td>
<td>128</td>
<td>84.4 (76.9; 90.2)</td>
<td>85.4 (60.4; 120.6)</td>
</tr>
<tr>
<td>MenC-CRM</td>
<td>Post-booster: M24</td>
<td>118</td>
<td>92.4 (86.0; 96.5)</td>
<td>211.1 (147.1; 303.1)</td>
</tr>
<tr>
<td></td>
<td>Post-booster: M36</td>
<td>120</td>
<td>86.7 (79.3; 92.2)</td>
<td>130.8 (91.9; 186.2)</td>
</tr>
<tr>
<td></td>
<td>Post-booster: M60</td>
<td>122</td>
<td>82.0 (74.0; 88.3)</td>
<td>66.7 (47.2; 94.1)</td>
</tr>
<tr>
<td>MenC-TT</td>
<td>Post-booster: M24</td>
<td>130</td>
<td>90.8 (84.4; 95.1)</td>
<td>189.2 (134.2; 266.7)</td>
</tr>
<tr>
<td></td>
<td>Post-booster: M36</td>
<td>127</td>
<td>88.2 (81.3; 93.2)</td>
<td>128.4 (91.3; 180.6)</td>
</tr>
<tr>
<td></td>
<td>Post-booster: M60</td>
<td>129</td>
<td>79.8 (71.9; 86.4)</td>
<td>64.7 (46.4; 90.2)</td>
</tr>
</tbody>
</table>

\textsuperscript{a}N, total number of children with available results; Post-booster: M24, approximately 24 months after booster vaccination; Post-booster: M36, approximately 36 months after booster vaccination; Post-booster: M60, approximately 60 months after booster vaccination.

\textsuperscript{b}Note that a booster vaccination of HBV was not given in Spain, in accordance with national recommendations.

Data shown here includes subjects from all countries combined.
Figure Legend

Figure 1. Disposition of study children and reasons for exclusion from ATP cohorts for persistence at 24, 36 and 60 months post-booster vaccination.
Figure 1.

N, number of study participants for specified group