H1N1 Antibody Persistence 1 Year After Immunization With an Adjuvanted or Whole-Virion Pandemic Vaccine and Immunogenicity and Reactogenicity of Subsequent Seasonal Influenza Vaccine: A Multicenter Follow-on Study

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Background. We investigated antibody persistence in children 1 year after 2 doses of either an AS03B-adjuvanted split-virion or nonadjuvanted whole-virion monovalent pandemic influenza vaccine and assessed the immunogenicity and reactogenicity of a subsequent dose of trivalent influenza vaccine (TIV).

Methods. Children previously immunized at age 6 months to 12 years in the original study were invited to participate. After a blood sample was obtained to assess persistence of antibody against swine influenza A/H1N1(2009) pandemic influenza, children received 1 dose of 2010/2011 TIV, reactogenicity data were collected for 7 days, and another blood sample was obtained 21 days after vaccination.

Results. Of 323 children recruited, 302 received TIV. Antibody persistence (defined as microneutralization [MN] titer $\geq 1:40$) 1 year after initial vaccination was significantly higher in the AS03B-adjuvanted compared with the whole-virion vaccine group, 100% (95% confidence interval [CI], 94.1%–100%) vs 32.4% (95% CI, 21.5%–44.8%) in children immunized <3 years old and 96.9% (95% CI, 91.3%–99.4%) vs 65.9% (95% CI, 55.3%–75.5%) in those 3–12 years old at immunization, respectively ($P < .001$ for both groups). All children receiving TIV had post-vaccination MN titers $\geq 1:40$. Although TIV was well tolerated in all groups, reactogenicity in children 5 years old was slightly greater in those who originally received AS03B-adjuvanted vaccine.

Conclusions. This study provides serological evidence that 2 doses of AS03B-adjuvanted pandemic influenza vaccine may be sufficient to maintain protection across 2 influenza seasons. Administration of TIV to children who previously received 2 doses of either pandemic influenza vaccine is safe and is immunogenic for the H1N1 strain.

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During the first and second waves of infection in the 2009–2010 A/H1N1(2009) influenza pandemic, there were at least 18,337 deaths globally [1, 2]. The United Kingdom Department of Health purchased 2 pandemic monovalent influenza vaccines (a nonadjuvanted cell culture-derived whole-virion vaccine and an AS03B-adjuvanted egg culture-derived split-virion vaccine) that were used during the national pandemic influenza
vaccination program. In 2009, we compared the safety and immunogenicity of a 2-dose regimen of these vaccines in 943 children aged 6 months to 12 years old. We found the AS03ß-adjuvanted vaccine more immunogenic, especially in younger children (microneutralization [MN] titer ≥1:40 in children under 3 years of age was 98.2% vs 80.1%) [3].

In the initial stage of the 2009 pandemic, at least 25,000 children at increased clinical risk from influenza infection received a 2-dose regimen of monovalent H1N1 vaccine (R. Pebody, personal communication) [4, 5]. Subsequently, the United Kingdom recommended single-dose immunization for all children aged 6 months to 5 years after review of early reactogenicity data [3, 6]. Analyses have since demonstrated the AS03ß-adjuvanted vaccine to be effective against the pandemic H1N1 strain [7–10], even after a single dose in young children [11]. However, there are no published data that assess the persistence of antibody in children immunized against the novel H1N1 virus to help guide future pandemic vaccination policy [12].

Concerns have arisen over both the reactogenicity and immunogenicity of the seasonal trivalent influenza vaccine (TIV) in those who have previously received monovalent pandemic vaccine, but to date this has not been studied in a clinical trial [13–15]. Recent analyses have shown significantly lower immunogenicity to monovalent pandemic influenza vaccines in those who had received previous TIV, but it is unknown whether the reverse is true [13–15].

This follow-on study investigated both the persistence of anti-H1N1 antibody 1 year after immunization with the pandemic vaccines and the immunogenicity of the H1N1 component and reactogenicity of a subsequent dose of TIV in these children.

METHODS

Study Design
This was a multicenter, open-label, phase IV study following on from a randomized trial. At visit one, a blood sample assessed persistence of antibodies to H1N1. A single, 0.5-mL intramuscular dose of TIV was administered into the deltoid. A second blood sample was taken 21 days (−7 to +14 days) later. For those not wishing to receive the study vaccine, parents could consent to the first blood test alone. For 7 days after administration of TIV, parents used daily diary cards to record the axillary temperature, injection site reactions, systemic symptoms, and use of antipyretic medication (Supplementary Table 1). All medical consultations between study immunization and the second study visit were recorded. Serum analysis was undertaken centrally, using standard methods [3, 16, 17]. The study was registered at ClinicalTrials.gov (NCT01239537), conducted in accordance with the Declaration of Helsinki and Good Clinical Practice, and approved by the UK Medicines and Healthcare Products Regulatory Agency (EudraCT No. 2010-022817-24), the Oxfordshire Research Ethics Committee A (10/H0604/81), and local National Health Service (NHS) organizations.

Participants
Between 11 November and 2 December 2010, 323 children who completed the original study [3] and were now between 17 months and 13 years old were recruited at 5 UK sites. Inclusion, exclusion, and temporary exclusion criteria are shown in Supplementary Table 2A and for the original study in Supplementary Table 2B. The original study recruited healthy children; however, high-risk children, such as asthmatics, were not excluded unless considered immunosuppressed. Allocation was to the same group as in the original study, in which they had been stratified for age (<3 or ≥3 years old) and randomized to receive either pandemic vaccine (consort diagram, Figure 1) [3]. All vaccinated children received a single dose of the TIV (Fluarix™; GlaxoSmithKline). Parents or guardians gave written informed consent, and children aged ≥7 years gave verbal assent.

Vaccine
The TIV Fluarix™ (GlaxoSmithKline Biologicals) contained inactivated split-virion influenza virus propagated in fertilized hens’ eggs with 15 µg of hemagglutinin antigen for each strain in a 0.5-mL dose [18]. The 3 World Health Organization-recommended strains were A/California/7/2009 (H1N1)–derived strain, A/Perth/16/2009 (H3N2)-like strain, and B/Brisbane/60/2008 [18, 19].

Study End Points
The primary end points were as follows: (1) persistence of microneutralizing antibody titers against pandemic virus, measured as the percentage of children with MN titers ≥1:40 at 1 year compared with 3 weeks after 2 doses of pandemic monovalent influenza vaccine [3]; (2) immunogenicity of TIV against pandemic virus 3 weeks after vaccination, measured as the percentage of children who seroconverted and had a postvaccination MN titer ≥1:40 or hemagglutination inhibition (HI) titer ≥1:32 (against H1N1 strain) or who were seropositive before vaccination and had a 4-fold increase in titer; and (3) reactogenicity after TIV, measured as the percentage of children experiencing fever, local reactions, and nonfebrile systemic symptoms within 7 days after vaccination. Different systemic symptoms were solicited in those under and over 5 years to reflect differences in ability to articulate symptoms (Supplementary Table 1; secondary end points, Supplementary Table 3).

Statistical Analysis
Data were analyzed in groups based on a child’s vaccine allocation and original study age [3]. For analysis of reactogenicity, age was divided into <5 and ≥5 years of age at the time of TIV. Proportions with titers above MN and HI thresholds, with
Figure 1. Enrollment and follow-up of study participants. Groups were allocated as in the original study, first split into those receiving either the whole-virion or the AS03B-adjuvanted vaccine and then further divided into those <3 and ≥3–12 years of age at the time of the original study [3]. Annotations (a), (b), and (c) highlight the participants on whom analysis was performed: (a) indicates persistence of antibody levels; (b) and (c), immunogenicity and reactogenicity after trivalent influenza vaccine (TIV) analysis, respectively. In the original study, this participant accidently received the incorrect vaccine [3] (whole-virion instead of AS03B-adjuvanted vaccine, as per randomization) and was therefore moved to the whole-virion group for follow-on in this study. Participant was mistakenly put into the older age group in the original study but was ≥3 years of age by 1 day, should have been in the younger age group, and was moved there for analysis in this follow-on study [3]. Six participants who did not complete all of the study procedures in the original study and therefore should have been excluded from this follow-on study were approached in error [3].
RESULTS

Of 943 children enrolled in the original study, 894 were eligible for enrollment, of whom 323 were recruited (36%) (Figure 1) [3]. Three hundred four provided a sample for antibody persistence, of whom 302 received TIV (2 withdrew before receipt of TIV), and 19 took part in the persistence study alone.

Persistence of Antibody Against A/H1N1(2009) Virus

The mean interval from second dose of pandemic vaccine to persistence blood test was 387 days (range, 365–429 days). Three hundred eighteen samples were available for analysis (Figure 1 and Supplementary Table 4A) The persistence of antibody levels 1 year after vaccination, defined by MN titer ≥1:40, was significantly higher in the AS03B-adjuvanted vaccine group than the whole-virion vaccine group, particularly in the younger age group (P < .001 in both age groups) (Table 1). In the original study, 100% of children in both age groups given AS03B-adjuvanted vaccine had an MN titer ≥1:40 3 weeks after vaccination (95% CIs, 94.1%–100% and 96.3%–100% in the younger and older groups, respectively). One year after vaccination, these figures were 100% (95% CI, 94.1%–100%) and 96.9% (95% CI, 91.3%–99.4%). In those originally given whole-virion vaccine, 82.4% (95% CI, 71.2%–90.5%) and 94.5% (95% CI, 87.6%–98.2%) had an MN titer ≥1:40 3 weeks after the initial vaccination, but by 1 year these had fallen to 32.4% (95% CI, 21.5%–44.8%) and 65.9% (95% CI, 55.3%–75.5%) in the younger and older groups, respectively (Table 1). These findings are illustrated by the reverse cumulative distribution curves (Figure 2).

Table 1. Antibody Persistence (by Microneutralization [MN] and Hemagglutination Inhibition [HI] Titters) 3 Weeks and 1 Year After 2 Doses of a Nonadjuvanted Whole-Virion or an AS03B-Adjuvanted Split-Virion Monovalent Pandemic Influenza Vaccine, by Age Group

<table>
<thead>
<tr>
<th>Age Group, y</th>
<th>Total No.</th>
<th>3 wk After 2nd Pandemic Dose</th>
<th>1 y Later</th>
<th>From ≥40 to &lt;40</th>
<th>From &lt;40 to ≥40</th>
<th>MN 1 y Later, Geometric Mean (95% CI)</th>
<th>3 wk After 2nd Pandemic Dose</th>
<th>1 y Later</th>
<th>From ≥32 to &lt;32</th>
<th>From &lt;32 to ≥32</th>
<th>HI Change Between Time Points</th>
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<tbody>
<tr>
<td>Whole-Virion Vaccine</td>
<td></td>
<td></td>
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<tr>
<td>&lt;3</td>
<td>68</td>
<td>56 (82.4)</td>
<td>22 (32.4)</td>
<td>35</td>
<td>1</td>
<td>33.6 (23.8–47.5)</td>
<td>40 (58.8)</td>
<td>43 (63.2)</td>
<td>8</td>
<td>11</td>
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<tr>
<td>3–12</td>
<td>91</td>
<td>86 (94.5)</td>
<td>60 (65.9)</td>
<td>26</td>
<td>0</td>
<td>66.9 (53.1–84.2)</td>
<td>82 (90.1)</td>
<td>72 (79.1)</td>
<td>14</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>159</td>
<td>142 (89.3)</td>
<td>82 (51.6)</td>
<td>61</td>
<td>1</td>
<td>49.8 (40.7–61.0)</td>
<td>122 (76.7)</td>
<td>115 (72.3)</td>
<td>22</td>
<td>15</td>
<td></td>
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<tr>
<td>AS03B-Adjuvanted Vaccine</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>&lt;3</td>
<td>61</td>
<td>61 (100)</td>
<td>61 (100)</td>
<td>0</td>
<td>0</td>
<td>411.9 (332.5–510.2)</td>
<td>61 (100)</td>
<td>60 (98.4)</td>
<td>1</td>
<td>0</td>
<td></td>
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<tr>
<td>3–12</td>
<td>98</td>
<td>98 (100)</td>
<td>95 (96.9)</td>
<td>3</td>
<td>0</td>
<td>287.6 (230.5–358.9)</td>
<td>98 (100)</td>
<td>95 (96.9)</td>
<td>3</td>
<td>0</td>
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<tr>
<td>All</td>
<td>159</td>
<td>159 (100)</td>
<td>156 (98.1)</td>
<td>3</td>
<td>0</td>
<td>330.1 (281.3–387.4)</td>
<td>159 (100)</td>
<td>155 (97.5)</td>
<td>4</td>
<td>0</td>
<td></td>
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</tbody>
</table>

Unless otherwise specified, data represent No. (% of children). Abbreviation: CI, confidence interval.

a Age groups based on age at original study [3].

b P < .001 for all comparisons between 1-year values for whole-virion and AS03B-adjuvanted vaccines.
The proportion of children with titers above the putative protective threshold were comparable by both HI and MN titers (HI $\geq 1:32$ and MN $\geq 1:40$), except in the serum samples from those in the younger age group who had received the whole-virion vaccine, in which the percentage of children with an HI $\geq 1:32$ increased from 58.8% (95% CI, 46.2%–70.6%) 3 weeks after initial vaccination to 63.2% (95% CI, 50.7%–74.6%) at 1 year (Table 1 and Figure 2). The HI titers of 11 of 68 younger and 4 of 91 older children in the whole-virion group were low ($<1:32$) 3 weeks after the second dose of pandemic vaccine but higher ($\geq 1:32$) 1 year later (Table 1); however, MN titers did not rise in these children (Table 1). When considering only children who responded well to the initial whole-virion vaccination (HI titer $\geq 1:32$ at 3 weeks), the percentages at 1 year with HI titers $\geq 1:32$ dropped consistently from 100% in both age groups to 80% (95% CI, 64.4%–90.9%) and 82.9% (95% CI, 73%–90.3%) in the younger and older groups, respectively (Supplementary Table 5).

**Immunogenicity of Trivalent Influenza Vaccine**

Three hundred two children received the 2010/2011 TIV (Figure 1 and Supplementary Table 4B). Across all groups the average HI GMT increased by 10.7 to 16.7 fold increase in HI GMT (Table 2). All children had a post-vaccination MN $\geq 1:40$ and an HI titer $\geq 1:32$ (Table 2). However, HI GMTs were significantly higher ($P < .001$) in those who initially received AS03$\_B$-adjuvanted vaccine compared with those receiving whole-virion vaccine, and these data are illustrated by the reverse cumulative distribution curves (Table 2 and Figure 3).

**Reactogenicity of Trivalent Influenza Vaccine**

Diaries were returned for 295 children (Figure 1). There were no serious adverse reactions. Redness and severe local symptoms were more frequent in children <5 years old who had previously received the AS03$\_B$-adjuvanted vaccine than the whole-virion vaccine ($P < .05$) (Supplementary Table 6). For all other solicited local and systemic symptoms, there were no significant differences between the vaccine groups. There were 2 significant differences ($P < .05$) between age groups: pain was reported more frequently in those $>5$ years of age, and fever $\geq 38^\circ C$ was reported more frequently in those $<5$ years of age, regardless of preceding pandemic vaccine (Supplementary Tables 6 and 7).

**Long-Term Safety Monitoring**

No solicited adverse events (ie, influenzalike illness, adverse events of special interest) were reported to have occurred in the year after receipt of either pandemic influenza vaccine (Supplementary Table 8).
DISCUSSION

Persistence of Antibody

Almost all children who received the AS03\textsubscript{B}-adjuvanted vaccine retained MN titers $\geq 1:40$ 1 year later, compared with 52% of those previously immunized with whole-virion vaccine (Table 1). Other studies have shown persistence of anti-H1N1 titer at 6 months after AS03\textsubscript{A}-adjuvanted vaccine [15, 20]. However, the pediatric study used the full adult dose (0.5 mL) [15], not the half dose recommended since September 2009 [21], and the data do not extend to a second influenza season (AS03\textsubscript{A} and AS03\textsubscript{B} differ only in that “B” denotes pediatric adjuvant dose). A recent analysis of the vaccine effectiveness of either monovalent pandemic influenza vaccination in 2009/2010, TIV in 2010/2011, or both in protecting against A/H1N1 (2009) virus during the winter of 2010–2011 suggested that single-dose pandemic vaccine protection may not last across seasons, although only 20% of those studied were children [22]. These data are supported by a recent Canadian study showing that only 46% of children maintained protective levels of antibody 1 year after receiving a single dose of AS03\textsubscript{B}-adjuvanted pandemic influenza vaccine [23]. Our data suggest that a 2-dose regimen of AS03\textsubscript{B}-adjuvanted pandemic vaccine may have been more effective across influenza seasons.

In the initial stages of a pandemic, there is high likelihood of both a limited supply of pandemic antigen and logistical difficulties in administering a 2-dose immunization regimen. However, the lower antibody responses with a single dose reported by some [23] may limit protection for a second wave compared with the 2-dose regimen reported here. In a subsequent pandemic, policymakers might therefore plan to initially give a single dose of an adjuvanted influenza vaccine to all age groups for the first wave, which is of proven effectiveness [7–11], followed by a booster dose of the vaccine at the time of the second pandemic wave, thus providing capacity during the first wave and time for vaccine production for the subsequent booster dose.

Although there is less published data, MN titers are likely to be a better correlate of protection against influenza than HI titers [24]. In the younger vaccine group who received the whole-virion vaccine in 2009, the proportion with MN titers $\geq 1:40$ fell from 82.4% 3 weeks post-vaccination to 32.4% at 1 year. Unexpectedly, the proportion with HI titers $\geq 1:32$ remained comparable (58.8%–63.2%) during the same period (Figure 2 and Table 1). This disparity between MN and HI titers was not seen in other groups or in previous studies [3, 16, 25, 26]. Although the higher HI titers are perhaps most likely to have been caused by wild-type H1N1 infection in those who had a previously poor response to the initial vaccine course (Table 1), it is difficult to propose a biologically plausible explanation as to why their MN titers decreased. Although no influenza-like
illnesses were reported, it is possible that these were mild or subclinical H1N1 infections, a possibility compatible with national seroepidemiological evidence that showed 26% of >5-year-olds and 53% of 5–14-year-olds were infected in the second wave [26]. Differences between MN and HI trends could also occur because MN measures a broader range of antibodies that neutralize the virus, whereas HI measures a limited set of epitopes involved in hemagglutination. One possible explanation is that, in some vaccinated children, responses to hemagglutinin predominate on exposure to infection. Further work will be required to assess the correlation between MN titers and protection from influenza.

Immunogenicity of Seasonal Trivalent Influenza Vaccine
Findings of other studies have suggested that prior receipt of TIV may reduce the immunogenicity of pandemic monovalent influenza vaccines [14, 15]. Despite no control group, our data do not suggest that the reverse is true. In all groups, the GMTs were higher than those seen 3 weeks after the original vaccination course. A higher anti-H1N1 titer before TIV was associated with a higher titer after vaccination (data not shown). In the younger age group, previous receipt of an AS03B-adjuvanted vaccine seems to enhance the serological response to a dose of a nonadjuvanted vaccine 1 year later, even allowing for the pre-vaccination titers (P = .03) (data not shown). This result could be explained by an enhanced priming mechanism in the adjuvanted vaccine group (Figure 3). Therefore, it will be informative to determine whether there was any effect of vaccine group on responses to the nonpandemic influenza strains included in the TIV.

Reactogenicity of Seasonal Trivalent Influenza Vaccine
Mild reactogenicity was seen in all groups after TIV. Four children <5 years of age had a temperature of ≥39°C, 1 in the AS03B-adjuvanted and 3 in the whole-virion vaccine groups. No febrile convulsions were reported.

Long-Term Safety Monitoring
No adverse events of special interest occurred in the year after pandemic vaccination, although the study was not powered to detect rare events. Despite recent European reports of narcolepsy in children and young adults who received AS03B-adjuvanted vaccine [27, 28], there has been no UK signal detected from passive surveillance [29].

Limitations and Applicability
First, the main limitation was lower recruitment than anticipated, possibly owing to low public anxiety regarding influenza during the recruitment window. Despite this, the demographics were similar between follow-on and original studies (Supplementary Table 9), apart from comparatively fewer <3-year-old children being recruited in the follow-on study.
and one site recruiting less well than in the original study. Although this loss of power is relevant when interpreting differences between groups, the resultant reduction in detectable difference is not marked (~3%), and actual differences seen between the vaccine groups were generally large. Second, we have currently assessed only the immunogenicity of the H1N1 component of TIV. Assessment of the other 2 TIV strains is planned when funding is available. The relevance of our results to adjuvanted polyvalent vaccines remains uncertain. Third, owing to funding limitations, this study was limited to the children in our original study, who all received 2 doses of pandemic influenza vaccine rather than the 1-dose regimen subsequently recommended for the AS03B-adjuvanted vaccine in the national program for healthy children in the United Kingdom [3, 6]. The majority of children in the United Kingdom who were vaccinated with adjuvanted pandemic vaccine received only 1 dose, and they are likely to have lower antibody persistence [30].

Eighty-one percent of the children known to have died in the 2009/2010 UK winter season from pandemic H1N1 infection were in clinical risk groups [31]. More than 25,000 at-risk children received a 2-dose vaccination regimen similar to that received by our cohort (R. P. Pebody, personal communication) [4]. While our original study did not specifically recruit at-risk children (although some were included, such as asthmatics), our data are directly applicable to the at-risk children who are most likely to die of pandemic H1N1 infection.

CONCLUSION

Children who received 2 doses of AS03B-adjuvanted pandemic influenza vaccine showed persistence of antibody above putative protective thresholds 1 year after vaccination compared with only approximately half of those who received the whole-virion vaccine. It is safe to administer trivalent seasonal influenza vaccine to children who received a 2-dose regimen of the pandemic influenza vaccines used in the United Kingdom in 2009, and this increased children’s antibody levels against the H1N1 component of the vaccine. This study provides serological evidence that a 2-dose regimen of vaccination with an AS03B-adjuvanted pandemic influenza vaccine may be sufficient to maintain protection across 2 influenza seasons. Pandemic planners may consider initially administering a single dose of adjuvanted pandemic vaccine to allow higher population coverage from limited dose supply, followed by a booster dose in the next season if the same strain is circulating.

Supplementary Data

Supplementary materials are available at Clinical Infectious Diseases online (http://www.oxfordjournals.org/our_journals/cid/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

Authors’ contributions. A. J. P., M. D. S., E. M., S. N. F., A. F., P. T. H., R. T., N. A., K. H., P. d. W., and W. T. W. designed the study with critical input from all authors. A. J. P. was chief investigator. M. D. S., S. N. F., A. F., P. T. H., and R. T. were local principal investigators. N. A. was responsible for statistical analysis. K. H. was responsible for laboratory analysis, and P. K. was responsible for data management, all with oversight and guidance by E. M. (lead investigator). B. T., T. M. J., and C. J. were project managers. S. K. was responsible for data monitoring, and J. C. was responsible for the computer database. W. T. W., P. d. W., C. O., M. C., L. M., C. H., P. M., C. I., T. M. J., R. T., P. T. H., S. N. F., M. D. S., A. F., and A. J. P. enrolled patients and/or contributed to data collection. A. J. P., M. D. S., E. M., S. N. F., A. F., P. T. H., R. T., N. A., K. H., P. d. W., and W. T. W. were involved in data interpretation. W. T. W. and S. N. F. drafted this report, which was subsequently reviewed by all authors. All authors have seen the final submitted report and agree with its contents. All authors had full access to all the data (including statistical reports and tables) in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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Potential conflicts of interest. Vaccines used in the original study were manufactured by GlaxoSmithKline and Baxter, both of which donated the vaccine but had no role in study planning or conduct. The vaccine used in this follow-on study was manufactured by GlaxoSmithKline, from whom it was purchased. A. J. P., A. F., M. D. S., P. T. H., and S. N. F. act as chief or principal investigators for clinical trials conducted on behalf of respective NHS trusts and/or universities, sponsored by vaccine manufacturers, but receive no personal payments from them. K. H. has been an investigator for clinical trials sponsored by vaccine manufacturers but received no personal payments from them. A. J. P., A. F., M. D. S., E. M., and S. N. F. have participated in advisory boards for vaccine manufacturers but receive no personal payments for this work. M. D. S., S. N. F., P. T. H., K. H., and A. F. have received financial assistance from vaccine manufacturers to attend conferences. All grants and honoraria are paid into accounts within the respective NHS trusts or universities or to independent charities. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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