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TITLE:
Relative Efficacy of AS03-Adjuvanted Pandemic Influenza A(H1N1) Vaccine in Children: Results of a Controlled, Randomized Efficacy Trial

ABSTRACT:
Background. The vaccine efficacy (VE) of 1 or 2 doses of AS03-adjuvanted influenza A(H1N1) vaccine relative to that of 2 doses of nonadjuvanted influenza A(H1N1) vaccine in children 6 months to <10 years of age in a multinational study conducted during 2010–2011. Methods. A total of 6145 children were randomly assigned at a ratio of 1:1:1 to receive 2 injections 21 days apart of A/California/7/2009(H1N1)-AS03 vaccine at dose 1 and saline placebo at dose 2, 2 doses 21 days apart of A/California/7/2009(H1N1)-AS03 vaccine (the Ad2 group), or 2 doses 21 days apart of nonadjuvanted A/California/7/2009(H1N1) vaccine (the NAd2 group). Active surveillance for influenza-like illnesses continued from days 14 to 385. Nose and throat samples obtained during influenza-like illnesses were tested for A/California/7/2009(H1N1), using reverse-transcriptase polymerase chain reaction. Immunogenicity, reactogenicity, and safety were assessed. Results. There were 23 cases of confirmed 2009 pandemic influenza A(H1N1) (A[H1N1]pdm09) infection for the primary relative VE analysis. The VE in the Ad2 group relative to that in the NAd2 group was 76.8% (95% confidence interval, 18.5%–93.4%). The benefit of the AS03 adjuvant was demonstrated in terms of the greater immunogenicity observed in the Ad2 group, compared with the NAd2 group. Conclusion. The 4–8-fold antigen-sparing adjuvanted pandemic influenza vaccine demonstrated superior and clinically important prevention of A(H1N1)pdm09 infection, compared with nonadjuvanted vaccine, with no observed increase in medically attended or serious adverse events. These data support the use of adjuvanted influenza vaccines during influenza pandemics. Clinical Trials Registration. NCT01051661.

Study Design and Subjects ::: METHODS:
This randomized, prospective phase 3 observer-blinded study was conducted in 17 centers in Australia, Brazil, Colombia, Costa Rica, Mexico, the Philippines, Singapore, and Thailand between 15 February 2010 and 19 August 2011. The study was approved by an institutional review board at each participating center. Written informed consent was obtained from parents or guardians of participating healthy children 6 months to <10 years (6 month to <10) of age before the children received the first vaccine dose.

Randomization ::: METHODS:
Participants were randomly assigned at a ratio of 1:1:1 to 1 of 3 treatment groups to receive 2 intramuscular injections 21 days apart. Group Ad1 received H1N1-AS03 at dose 1 and placebo (saline) at dose 2; group Ad2 received 2 doses of H1N1-AS03; group NAd2 received 2 doses of nonadjuvanted A/California/7/2009(H1N1) vaccine. The randomization procedure used a minimization algorithm accounting for center, age stratum (6 to <36 months or 3 to <10 years), and prior seasonal influenza vaccination status. These factors had equal weight in the minimization algorithm; that is, a participant's vaccine group allocation was based on the balance of the combination of all the minimization factors. Each age stratum was capped such that it could contribute no more than 75% to the total population.

Vaccines ::: METHODS:
Each dose of H1N1-AS03 vaccine contained 1.9 μg of hemagglutinin (HA) mixed with AS03B in a total delivery volume of 0.25 mL. AS03B is an oil-in-water emulsion containing squalene and DL-α-tocopherol (5.93 mg) in an aqueous phase [7].
The nonadjuvanted vaccine contained 30 μg of HA in 1 mL, and the volume (ie, HA dose) administered varied with age: children <3 years of age in the NAd2 group received two 0.25-mL doses (ie, 7.5 μg of HA), and children 3 to <10 years of age received two 0.5-mL doses (ie, 15 μg of HA). Placebo was 0.5 mL of saline. Single lots of the adjuvanted antigen, nonadjuvanted antigen, and adjuvant were used. Vaccines were administered into the deltoid (or anterior thigh, if the child was <12 months of age), using a needle length suitable for intramuscular administration.

Study Objectives ::: METHODS:
The primary objective was to evaluate the efficacy of 2 doses of H1N1-AS03 relative to that of 2 doses of nonadjuvanted vaccine beginning 14 days after dose 1 and continuing until study

conclusion on day 385. Noninferiority in terms of relative vaccine efficacy (VE) was concluded if the lower limit of the 95% confidence interval (CI) for relative VE against real-time PCR–confirmed A(H1N1)pdm09 infection (Ad2 vs NAd2) was ≥33%. Superiority was concluded if the lower limit of the 95% CI for relative VE was >0.
Secondary study objectives with predefined criteria (and no type 1 error adjustment) included (1) VE for Ad1 relative to that of NAd2, using the same criteria specified for the primary objective; (2) relative VE for any pneumonia, any pneumonia in individuals within 6 weeks of real-time PCR–positive A(H1N1)pdm09 infection, and any influenza-like illness (ILI); and (3) assessment of specific antibody titers at day 42.
The adjuvant effect on immunogenicity was assessed by comparing A/California/7/09 hemagglutination-inhibiting (HI) antibody responses at day 42 in terms of geometric mean titer (GMT) ratios and differences in seroconversion rate (SCR) by group. Adjuvant effect was demonstrated if the lower limit of the 95% CI for GMT ratio was >1.0 and the lower limit of the 95% CI for the group difference in the seroconversion rate was >0.
Reactogenicity and safety of the study vaccines and antibody persistence at days 182 and 385 were summarized descriptively as part of the evaluation of secondary objectives.

Evaluation of Influenza Outcomes ::: METHODS:
Passive surveillance began on day 0 and active surveillance via telephone contacts began for all subjects approximately 2 weeks after dose 1 and continued every 1–2 weeks until day 385. Parents and legal guardians notified the investigator if the child developed an ILI (defined as a temperature ≥38.0°C by any route and at least 1 of the following: new or worsening cough, sore throat, nasal congestion, and/or rhinorrhea). Nasal and throat swab specimens were collected within 7 days after symptom onset. A 7-day symptom-free period was required between ILI episodes to consider the subsequent episode distinct from the initial episode. Detection of influenza A(H1N1)pdm09, using real-time PCR (and of all influenza virus strains, using multiplex PCR), is described in the Supplementary Materials. Only real-time PCR–positive samples underwent viral culture [8].

Immunogenicity Assessment ::: METHODS:
Blood samples were collected before vaccination and 21 days after receipt of dose 2 (ie, on day 42) from all subjects. HI antibody levels were measured in a random subset of approximately 60 children in each treatment group from each participating country. The HI assay used chicken erythrocytes [9–11], and the lowest dilution tested was 1:10. The titration end point was the highest dilution step that showed complete (ie, 100%) inhibition of hemagglutination. A further blood sample was collected at either day 182 or day 385 from consenting subjects.

Safety and Reactogenicity Assessment ::: METHODS:
Injection site and systemic symptoms were recorded on diary cards for 7 days after each dose. Solicited symptoms were based on the ability to report by age and were therefore different in younger children (age, <6 years) versus older children (age, ≥6 years). All other adverse events (AEs) were recorded from the first dose until day 42. Medically attended AEs, serious AEs (SAEs), and potential immune-mediated diseases were recorded until day 385. All solicited injection site reactions were considered causally related to vaccination. Potentially causal relationships between vaccination and all other AEs were assessed by the site investigator.

Vaccine Efficacy ::: Statistical Methods ::: METHODS:
Efficacy was assessed in the according-to-protocol (ATP) efficacy cohort, which included all evaluable subjects who received 2 doses, who were successfully contacted at least once after the first vaccination, and who complied with protocol-defined procedures. The relative VE was calculated as 1 − relative risk (RR), where the RR is defined as the risk of real-time PCR–confirmed cases among subjects receiving H1N1-AS03 versus the risk of real-time PCR–confirmed cases among subjects receiving NAd2. RR was estimated via a Cox proportional hazard regression model (time to first event), with vaccine group as a fixed variable, and was adjusted by covariates of age, seasonal influenza vaccine history, and country. Subjects meeting censoring criteria (ie, receipt of protocol-forbidden vaccines or medication or receipt of nonprotocol vaccines containing A/California/7/09-like H1N1 antigen) were included in the analysis until the date of censoring or were excluded if the censoring criteria were met before the disease end point occurred. The relative VE (with 95% CI) was calculated for the 14–385-day

(primary end point) and 0–385-day surveillance periods. Secondary analyses were done on the total vaccinated cohort, which included all children who received at least 1 vaccine dose. During study preparation the future behavior of the pandemic was uncertain, but we projected a substantive third wave in 2010. On the basis of 1800 evaluable subjects per group, an assumed attack rate of 20% among unvaccinated subjects, and an assumed VE for nonadjuvanted H1N1 vaccine of 40%, if 360 real-time PCR–confirmed influenza cases were identified during the surveillance period, a lower limit of ≥33% for the 95% CI for the relative VE could be demonstrated with >99.9% power, if the VE in the H1N1-AS03 group was assumed to be 60% relative to that of a notional placebo. Type 1 error adjustment was not made for secondary objective evaluations.

Immunogenicity End Points ::: Statistical Methods ::: METHODS:
The following parameters were calculated (with 95% CIs) based on A/California/7/09 HI titers: GMT; seroconversion rate, defined as the percentage of initially seronegative subjects (titer, <1:10) with a postvaccination titer of ≥1:40 or the percentage of initially seropositive vaccinees (titer, ≥1:10) with a ≥4-fold increase in the postvaccination titer; seroprotection rate, defined as the percentage of subjects with titers of ≥1:40 [12, 13]; and seroconversion factor, defined as the ratio of the postvaccination titer to the prevaccination titer.

Reactogenicity End Points ::: Statistical Methods ::: METHODS:
Reactogenicity data were summarized by vaccine group and age stratum (from 6 months to <6 years and from 6 to <10 years) because a different AE intensity scale was used for children of different ages.

Study Subjects ::: RESULTS:
Each study center contributed between 105 (1.7%) and 886 (14.4%) of the total 6145 enrolled and vaccinated subjects. Of these, 5900 (96%) completed the study to day 42, and 5851 (95%) completed the study to day 385. Two children in the Ad1 group withdrew before day 42 because of an AE or SAE: 1 child died of asthma and pneumonia 20 days after dose 1, and 1 child had a nonserious upper respiratory tract infection. Two children in Ad2 were withdrawn before day 385 because of SAEs: 1 child drowned, and 1 died from an intestinal obstruction associated with parasitic gastroenteritis and aspiration pneumonia (95 days after dose 2). No event leading to withdrawal was considered related to vaccination. Subject flow through the study and reasons for withdrawal and elimination from ATP cohorts are given in Supplementary Figure 1.
The mean age (±SD) of all children at enrollment was 4.3 ± 2.64 years (range, 0.5–9 years); 49.8% (3058/6145) were female. The study groups in each analysis cohort were comparable in terms of demographic characteristics (Supplementary Table 1).

Vaccine Efficacy ::: RESULTS:
There were 3731 nasopharyngeal swab specimens collected from 4653 ILI episodes (81%). Multiplex PCR–confirmed influenza virus infection (any strain from days 14 to 385) occurred in 9.7% (95% CI, 8.4%–11.1%) of children in the Ad2 group, 8.4% (95% CI, 7.2%–9.8%) in the Ad1 group, and 9.3% (95% CI, 8.1%–10.7%) in the NAd2 group.
During the entire study follow-up period, 28 children had real-time PCR–confirmed A(H1N1)pdm09 infection (Table 1): 11 were in the Philippines, 7 were in Thailand, 5 were in Australia, 3 were in Mexico, and 1 each was in Singapore and Costa Rica. Three children developed influenza before day 14. One child (in the NAd2 group) received the second vaccination 4 days after the protocol-specified visit window. One subject (in the NAd2 group) was censored upon receiving seasonal trivalent vaccine 8 months after dose 1 and 3 months before onset of A(H1N1)pdm09 disease. Therefore, among 5803 children included in ATP time-to-event efficacy analysis (days 14–385), 23 had real-time PCR-confirmed A(H1N1)pdm09 infection, giving an attack rate in each group of 0.15% (in the Ad2 group), 0.34% (in the Ad1 group), and 0.68% (in the NAd2 group) in the ATP cohort (Figure 1).

The efficacy of Ad2 over NAd2 for prevention of real-time PCR-confirmed A(H1N1)pdm09 infection from days 14 to 385 was 76.8% (95% CI, 18.5%–93.4%). Both noninferiority and superiority of Ad2 versus NAd2 were thus demonstrated according to the prespecified statistical criteria (Table 2). The relative VE without adjustment for covariates of age, seasonal influenza vaccine history, and country was 77.1% (95% CI, 19.6%–93.5%).

Efficacy, noninferiority, and superiority of the 2-dose adjuvanted regimen were confirmed in the analysis of the total vaccinated cohort: the efficacy estimate for Ad2 over NAd2 was 78.5% (95% CI, 25.1%–93.8%; days 14–385).
Secondary analyses showed that Ad2 was noninferior to NAd2 for efficacy in preventing culture-confirmed influenza (days 14–385) and in preventing real-time PCR–confirmed A(H1N1)pdm09 infection in the subgroup aged 3 to <10 years (Table 1). Noninferiority was also observed for these end points in the total vaccinated cohort analysis (data not shown).
The noninferiority criteria were not met for Ad1 versus NAd2 for the ATP population or at any analysis interval (days 14–385 or 0–385) but were met for real-time PCR–confirmed A(H1N1)pdm09 infection (relative VE, 50.1% [95% CI, –23.5% to 79.9%]) in the total vaccinated cohort (days 14–385).

Immunogenicity ::: RESULTS:
The ATP immunogenicity subset included 1175 children at day 42, 1693 at day 182, and 1526 at day 385. At day 42, the seroprotection rate was 100% for Ad2, 98.7% for Ad1, and 92.8% for NAd2 (Table 3). Center for Biologics Evaluation and Research and Committee for Medicinal Products for Human Use criteria (defined in Table 3) were fulfilled by all treatment groups (overall and by each age stratum) at day 42.

Adjuvant effect (Ad2 vs NAd2 groups) was demonstrated in terms of seroconversion rate (lower limit of the 95% CI for the difference between groups, 7.2%) and GMT ratios (lower limit for 95% CI, 4.9). Adjuvant effect was also demonstrated in both age strata (lower limit for 95% CI for the difference in seroconversion rate between groups, 10.3% for ages 6 to <36 months and 1.4% for ages 3 to <10 years; lower limit for the 95% CI of the GMT ratio, 7.7 for ages 6 to <36 months and 2.4 for ages 3 to <10 years; Figure 2).

Adjuvant effect was also observed for Ad1 versus NAd2 in terms of seroconversion rate overall (lower limit of the 95% CI for the difference between groups, 0.9%) but not for GMT ratios. Adjuvant effect was observed for children aged 6 to <36 months in terms of seroconversion rate (lower limit of the 95% CI for the difference between groups, 8.6%) and GMT ratios (lower limit for the 95% CI on the GMT ratio, 1.3).
At day 385, the seroprotection rate was 97.3% for Ad2, 78.5% for Ad1, and 67.9% for NAd2 (Table 3). HI GMTs were highest in the Ad2 group at all postvaccination time points (Table 3).

Reactogenicity and Safety ::: RESULTS:
Pain at the injection site was the most frequently reported symptom in all groups after each dose (Figure 3) and was more frequent for Ad2 than NAd2. Grade 3 injection site reactions were reported by no more than 4.3% of children after either dose.

The percentage of children in each age stratum reporting systemic solicited symptoms was similar among treatment groups and after doses 1 and 2 (Figure 3), with the exception of fever (oral or axillary temperature, ≥38.0°C) in the Ad2 group, which increased from 10.2% (95% CI, 8.6%–11.9%) after dose 1 to 19.0% (95% CI, 16.9%–21.2%) after dose 2 in subjects aged 6 months to <6 years (an increase was observed in the subgroups aged 6 to <36 months and 3 to <6 years; Supplementary Table 2) and from 4.8% (95% CI, 3.3%–6.6%) to 8.7% (95% CI, 6.7%–11.1%), respectively, in subjects aged 6 to <10 years. In the Ad2 group, grade 3 fever (oral or axillary temperature of ≥39.0°C) was reported for 1.9% (95% CI, 1.2%–2.8%) of children aged 6 months to <6 years after dose 1 and for 3.1% (95% CI, 2.2%–4.2%) after dose 2. Fever (defined as a temperature of >40.0°C) was only reported after dose 1: 2 episodes occurred in children in the Ad2 group, and 3 episodes occurred in each of the other groups (Supplementary Table 2).
There were 15 children who experienced febrile convulsions through day 385: 4 were in the Ad2 group, 6 were in the Ad1 group, and 5 were in the NAd2 group. Seven febrile convulsions were reported as SAEs (Table 4). None occurred within 42 days after vaccination, and none were considered vaccine related. One so-called convulsion (fever was absent) was reported through day 42 after vaccination (onset, 7 days after vaccination in a subject from the Ad2 group who had preexisting epilepsy). The event lasted 6 days and was not considered to be vaccine related.

Percentages of children with any AE within 42 days of vaccination requiring a medically attended visit were similar among groups (23.4% for the Ad2 group, 22.9% for the Ad1 group, and 22.8% for the NAd2 group; Table 4).
During the 42-day follow-up period, 0.5% of subjects in the Ad2 group, 0.9% in the Ad1 group, and 1.2% in the NAd2 group reported at least 1 grade 3 AE. None of the grade 3 symptoms reported in the Ad2 group were considered to be vaccine related. One case of grade 3 headache in the Ad1 group and 1 case each of grade 3 vomiting and gastroenteritis in the NAd2 group were assessed as potentially vaccine related.
Percentages of children experiencing at least 1 SAE from days 0 to 385 were 3.7% in the Ad2 group, 3.2% in the Ad1 group, and 3.3% in the NAd2 group. The 10 most frequently reported SAEs were similar across groups (Table 4). One SAE (which involved a subject in theAd1 group, who required an emergency department visit for gastroenteritis, with onset on the day of dose 2) was considered to be vaccine related. There were 3 fatal events (all in the Philippines), and none were considered vaccine related: 1 involved a 10-year-old child (in the Ad2 group), who drowned; 1 involved a 20-month-old child (in the Ad2 group), who died from intestinal obstruction, parasitic gastroenteritis, and aspiration pneumonia; and 1 involved a 6-month-old child (in the Ad1 group), who had a history of pneumonia and asthma and died 20 days after vaccination from community-acquired pneumonia and asthma (real-time PCR negative). No autopsy was done.
During the study period, 1 potential immune-mediated disease was reported for the Ad2 group (0.05% of subjects), and 3 and 4 for the Ad1 (0.15%) and NAd2 (0.2%) groups, respectively (Table 4).

DISCUSSION:
This is the first prospective efficacy study commencing during an influenza pandemic and the first to assess an AS03-adjuvanted inactivated influenza vaccine in children. We found a clinically important and statistically significant improvement in the efficacy of AS03-adjuvanted vaccine, compared with efficacy of the nonadjuvanted influenza vaccine. Moreover, improved efficacy was shown using one-fourth to one-eighth of the standard dose of HA, and noninferiority was shown for a single dose of adjuvanted vaccine (one-eighth to one-sixteenth of the standard HA dose), although only in the total vaccinated cohort (exploratory analysis).
We observed lower than expected numbers of real-time PCR–confirmed cases of A(H1N1)pdm09 infection, most likely because of limited A(H1N1)pdm09 exposure during 2010, owing to the absence of the anticipated third pandemic wave during 2010 in the participating countries [14]. Furthermore, all study subjects received active influenza vaccination. We believe our active surveillance was adequate, as many cases of ILI were evaluated, and subsequent testing revealed a substantial incidence of infection with respiratory syncytial virus and seasonal influenza virus types A and B. Potential effects of variability between countries in active surveillance performance were minimized by the randomization process. Overall influenza incidence rates in each group had overlapping 95% CIs, which does not support the proposition that the adjuvant groups experienced an unqualified general reduction in risk. Nevertheless, despite the low number of influenza cases, the relative VE of the adjuvanted vaccine after 2 doses was higher than estimated and was sufficient overall to reach a definitive conclusion. However, the low case numbers led to wide CIs for relative VE estimates and increased the uncertainty regarding our exploration of secondary end points and subanalyses by age, although point estimates for relative VE were the same for subjects younger and those older than 3 years.
This study commenced during the 2009–2010 influenza A(H1N1) pandemic in expectation of a third pandemic wave, and it was not considered ethically acceptable to include a placebo group. Even though the study could not provide absolute VE values, these can be estimated using other sources from the literature. If an absolute VE of 40% for 2 doses of nonadjuvanted H1N1 vaccine is assumed (from the study by Vesikari et al [5], against mainly influenza A[H3N2]), the estimated absolute VE for Ad2 in our study is 86%; if an absolute VE for plain H1N1 antigen of 59% is assumed (efficacy estimate from the same influenza A[H1N1] antigen in a quadrivalent formulation) [15], the estimated absolute VE for Ad2 is 90%, which is consistent with short-term vaccine effectiveness estimates (86%–100%) reported in case-control studies involving children and adults who received 1 dose of H1N1-AS03 [16–18]. Adjuvant benefit in terms of immunogenicity, with higher and more persistent immune responses, was also demonstrated for 2 H1N1-AS03 doses over 2 nonadjuvanted vaccine doses, consistent with findings from previous clinical trials [6, 19].
Evidence from multiple clinical trials indicates transient increase in injection site reactions following AS03-adjuvanted versus nonadjuvanted influenza vaccines, but there has been no

evidence of an increased incidence of medically attended events or SAEs associated with AS03 use [7]. Consistent with these studies, we observed higher rates of injection site pain in H1N1-AS03 recipients than in nonadjuvanted vaccine recipients. However, grade 3 pain was reported for no more than 3.6% of children. Reported potential immune-mediated diseases were evenly distributed across groups. The incidence of mild fever increased after the second H1N1-AS03 dose, as observed in other studies of this vaccine in healthy children [20]. There was no clustering of febrile convulsion cases in temporal relationship to vaccination.

After commencement of our study, several retrospective studies suggested an association between vaccination with another A(H1N1)pdm09 vaccine (PandemrixTM) and the subsequent onset of narcolepsy in individuals <21 years of age and in adults [21–31]. These retrospective observational studies alone are insufficient to ascribe the risk solely to the vaccine, as other factors may play a role. The recent identification of an epitope in the H1N1 HA protein that mimics an epitope present within hypocretin [32] suggests that exposure of individuals with the HLA DQ0602 allele to H1N1 can result in an autoimmune response involving CD4+ T cells that could lead to the onset of narcolepsy. No narcolepsy cases were reported in the current study, which was too small to detect rare events such as narcolepsy.

Our results provide evidence of the potential benefit of AS03-adjuvanted pandemic influenza vaccines for control of future pandemics with respect to prevention of disease in a particularly vulnerable age segment. Similarly, the availability of effective adjuvanted influenza vaccines for children with reduced antigen content could offer opportunities for improved control of seasonal influenza. However, in either the seasonal or pandemic setting, use of AS03-adjuvanted influenza vaccines associated with injection site symptoms, fever, and a theoretical risk of rarer events will need to be balanced against the risk of death or severe illness caused by the seasonal or pandemic influenza viral strains.