A relatively high burden of influenza is experienced by young children. In order to successfully tackle the burden of influenza in children, effective vaccines are necessary. Accumulated evidence on the efficacy and effectiveness of traditional inactivated split or subunit trivalent influenza vaccines points towards no significant protection in the youngest children, who are largely unprimed. Adjuvanted influenza vaccines have been developed to improve the immune response, and could possibly overcome limitations of traditional influenza vaccines in the youngest age groups. In this review, evidence from recent clinical trials of adjuvanted versus nonadjuvanted influenza vaccines in children younger than 3 years of age will be discussed. Important findings from identified studies will be highlighted, and ongoing challenges concerning the use of adjuvanted influenza vaccine in young children will be discussed.

Keywords: adjuvant (immunological) • alum • AS03 • infants • influenza vaccine • MF59 • oil-in-water • toddlers • virosome

Background

The burden of influenza in children

A relatively high burden of influenza is experienced by young children [1–4]. Young children often have not been exposed to influenza viral antigens, meaning that they have not been vaccinated or infected by influenza, thus are immunologically naïve. As a result, they have limited protection against infection with influenza viruses. Serological evidence suggests that by approximately the age of six, most children will have encountered at least one type of influenza virus [5]. As a consequence they will have built up some immunity through the presence of cross-reacting antibodies against drifted strains. With increasing age, it is primarily children with underlying cardiopulmonary, neurological or immunological disorders that are most vulnerable to the consequences of influenza infection [6]. As influenza viruses drift, individuals could be at a renewed risk of infection as pre-existing antibodies no longer confer protection. In order to overcome this, vaccines would ideally offer broad protection against heterotypic strains during influenza epidemics and pandemics.

Dependent on the predominant circulating strain, there is large variability in actual morbidity by year, region and age group. The burden is a combination of morbidity and mortality resulting from influenza infection, but also of indirect effects in those with underlying chronic conditions. For example, influenza infection is associated with exacerbations of wheezing and asthma [7]. In addition, the impact on primary and secondary healthcare resources, parental absenteeism from work and child absenteeism from school should also be considered. The latter can be considerable, as...
demonstrated in a recent study in Hong Kong [8]. This diversity and variability of the influenza burden should be kept in mind when considering estimates expressing different aspects of the burden.

An estimated 20–30% of the pediatric population is affected during annual influenza outbreaks [2]. This translates into considerable use of healthcare resources, caused by outpatient visits, prescription of antibiotics and antipyretics, and hospitalizations [9]. Rates of influenza-related hospitalization have been reported ranging from 10 to over 100 per 10,000 children under 1 year of age. Although less hospitalization occurs in older children, influenza remains one of the most important causes for hospitalization [2]. Influenza infection can lead to complications in children such as encephalitis, acute encephalopathy, Guillain–Barré syndrome (GBS), myocarditis and cardiac failure [10–11]. Global mortality due to influenza in children under 5 years of age in 2008 was estimated at between 28,000 and 111,500 deaths [12], most of which occurred in developing countries. Noteworthy, it has been found that approximately half of influenza deaths in children occur in previously healthy children [13]. With effective vaccination, all these deaths could have been prevented.

**Need for better influenza vaccines**

Vaccination is the mainstay in the prevention of influenza and in order to successfully tackle the burden of influenza in children, effective vaccines are necessary. Split or subunit trivalent inactivated influenza vaccines (TIVs) are available for seasonal influenza vaccination programs in most countries. It has long been known that these vaccines induce a limited immune response in immunologically naive persons [14]. Currently, accumulated evidence on the efficacy and effectiveness of traditional inactivated split or subunit TIVs points towards no significant protection in the youngest children, who are largely unprimed [15,16]. This could indicate that inactivated split or subunit vaccines do not adequately prime immune-naive persons. Priming is the activation and expansion of antigen specific T cells that are able to establish memory and exert effector functions [17], and is essential for a lasting protective immune response. The possible inability to prime can have large implications if these vaccines were relied on in a pandemic scenario in which there is limited or no cross-reactive antibody present in the population. In children between 2 and 6 years there is evidence of protection, albeit moderate [15,16,18].

A range of inactivated split or subunit vaccines have been licensed around the world, several of which are also licensed for use in children. Special pediatric formulations exist, which consists of half the adult dose. As pointed out, the evidence to support the use of split or subunit TIVs in young children is limited and does not point towards a clear benefit [15,16,18]. The evidence to support use of a half dose in this group is more limited. A recent study demonstrated that the full dose provided superior immunogenicity compared with the half dose in infants and toddlers (6–23 months), without increased reactogenicity [19], bringing the existence of half-dose recommendations into question. It is generally recommended that young children who have not been previously vaccinated with influenza vaccine, and are likely to be unprimed, receive two doses. Some studies have shown that two doses could result in effective protection in young children [20–24], yet evidence is limited. Another option for improving the response to TIVs is increasing the presentation of antigens to antigen-presenting-cells such as dendritic cells. This can be achieved through intradermal vaccination. Intradermal influenza vaccines are licensed for use in adults and elderly in the USA and in Europe, but not for use in children [101,102]. There is only limited data in children, but studies have demonstrated that intradermal influenza vaccine increases the immune response compared with intramuscular vaccination in primed children over the age of three and in children aged 6–12 months old [25,26].

In addition to inactivated influenza vaccines, live attenuated influenza vaccines are available in several parts of the world. Live attenuated influenza vaccines have been found to be more effective than inactivated influenza vaccines in children [27,28]. However, live attenuated influenza vaccines cannot be given to children under the age of two as its use has been associated with increased rates of medically attended wheezing and hospitalization [28,29,103,104].

Clearly, the current situation of influenza vaccination is poignant. For the age group with the highest attack rates there is a lack of effective vaccines. Adjuvanted influenza vaccines have been developed to improve the immune response and could possibly overcome limitations of traditional influenza vaccines in the youngest age groups.

**Adjuvanted influenza vaccines**

Adjuvants are components included in vaccine formulations in order to potentiate the immune response. Experience with adjuvanted influenza vaccines goes back to the 1950s, when mineral-in-oil adjuvanted influenza vaccines were used on a large scale. These were abandoned as their use was associated with severe local reactions, including cysts and abscess formation [30]. Other adjuvancing systems have been studied; however, it was not until the end of the 20th century that the first adjuvanted influenza vaccines were licensed. In 1997 an oil-in-water (MF59™) seasonal adjuvanted influenza vaccine was licensed for use in older adults in Europe [31]. In that same year a
virosomal adjuvanted influenza vaccine was licensed for use in all age groups [32]. Towards the end of the 20th century, increased awareness of potential pandemic threats in a world, with only limited production capacity for influenza vaccines, made way for the development and licensing of new adjuvanted pandemic influenza vaccines [33]. These were eventually used on a very large scale during the 2009/2010 pandemic, also in children [34].

A variety of different formulations of adjuvanted influenza vaccines have been studied over the past decades [35]. Several pre-pandemic, pandemic and seasonal adjuvanted influenza vaccines are licensed including aluminum-, oil-in-water- and virosomal-adjuvanted vaccines [36,37]. It has been demonstrated that oil-in-water adjuvants can potentiate the immune response to influenza vaccine thereby reducing the amount of hemagglutinin antibody (HA) needed [36]. Studies have also shown that the MF59 adjuvant induces a broader immune response providing protection against drifted strains and increase the diversity and affinity of antibodies [35,38,39]. No two adjuvants are the same, and the interaction between the virosomal antigens and adjuvants can be different for different antigens. Therefore safety and efficacy of each adjuvanted vaccine needs to be considered separately. An overview of adjuvanted influenza vaccines and recommended dosage for children aged 6–36 months is given in Table 1.

In the present paper, evidence from recent clinical trials of adjuvanted influenza vaccines versus non-adjuvanted influenza vaccines in the younger, unprimed children (up to 3 years old) is reviewed in order to evaluate whether adjuvanted vaccines might be able to address the limitations of current inactivated non-adjuvanted inactivated split or subunit vaccines. Data on comparative immunogenicity, efficacy and safety will be brought together to form a picture whether adjuvanted vaccines form a safe and efficacious option for protecting the youngest children against influenza and which existing gaps would need to be addressed.

The authors conducted a search of electronic databases (PubMed, EMBASE) in order to identify relevant studies comparing adjuvanted with nonadjuvanted influenza vaccines in infants and children. The medical subject heading terms 'influenza vaccine' and 'adjuvants, immunologic' were combined. The search was limited to articles concerning infants and preschool aged children up to 3 years of age. Publications up to November 2012 were included. Pertinent articles were retrieved and reference lists were scanned to identify any further publications. Furthermore, electronic public assessment reports on the website of the European Medicines Agency were consulted for data on pandemic, pre-pandemic and seasonal inactivated adjuvanted influenza vaccines.

### Immunogenicity of adjuvanted versus nonadjuvanted vaccines

The hemagglutination inhibition (HI) assay and virus neutralization or microneutralization assay are bioassays widely used to measure the immune response to the influenza virus or vaccine in the serum. A limitation of both assays is the large intralaboratory variability, which results from poor standardization [40,41]. Comparisons across studies are therefore not reliable, and only head-to-head comparative studies should be considered.

Based upon findings from a challenge study in healthy adults with attenuated strains [42], a cut-off value of HI-titer ≥1:40 is commonly used as a predictor for 50% protection in adults and elderly. Although no HI-based correlate for children has ever been defined, the cut-off of HI ≥1:40 or ≥1:32 is also widely used as a measure of seroprotection to express the immune response against influenza vaccination in children. A recent study in children, however, found that an HI titer ≥1:110 correlated to 50% protection in children aged 6–72 months, and that the cut-off of 1:40 correlated with a mere 22% protection [43]. It has not been established whether these findings can be extrapolated to other situations, that is, influenza seasons, virus strains and populations. A virus neutralization- or microneutralization-based correlate for protection has not been validated. Due to above named limitations, measures of seroprotection or seroconversion can be misleading, and are likely to hamper a proper assessment of the benefits of a vaccine in the youngest age groups. Rather, the focus should be on more qualitative comparisons of the immune response; such as geometric mean titers and ratios (post- compared with pre-immunization). In any case, without standardization of assays and the availability of a validated correlate of protection for children, it is not possible to translate immunogenicity findings from different studies into actual effects on protection against infection or disease offered by the vaccine.

#### Aluminum adjuvanted vaccines

Aluminum salts do not appear to potentiate the immune response to influenza antigens [36]. In one study the addition of aluminum salts was actually found to decrease the immune response [44]. In Hungary, an inactivated whole-virus trivalent aluminum phosphate gel (ALPO4) adjuvanted influenza vaccine is licensed [45], of which also an H5N1 and an H1N1pdm09 variant exist [46,47]. No comparative immunogenicity, safety or efficacy data in young children could be found, therefore it is not clear whether there is a benefit of ALPO4 compared with a whole-virus vaccine.

One safety and immunogenicity study with the whole virion H5N1 aluminum phosphate vaccine (6 µg + ALPO4), in 12 children aged 9–17 years, was...
identified [37]. As there was no nonadjuvanted comparator it is unclear what the added benefit of the aluminum adjuvant for this vaccine is. Only limited safety data in children <36 months could be found [105]. Nolan et al. report on the immunogenicity and safety of two formulations of aluminum phosphate adjuvanted H5N1 vaccines (two doses of 30 µg HA + ALPO4 per or 45µg HA + ALPO4 given 20 days apart) in children aged 6–9 years [48].

Table 1. Overview of adjuvanted influenza vaccines with recommended dosage for children aged 6–35 months.

<table>
<thead>
<tr>
<th>Brand name</th>
<th>Manufacturer</th>
<th>Culture medium</th>
<th>Product description</th>
<th>HA amount (per 0.5 ml)</th>
<th>Adjuvating system</th>
<th>Dosage (6–35 months)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Adjuvanted seasonal influenza vaccines</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fluad®</td>
<td>Novartis</td>
<td>Egg</td>
<td>Influenza vaccine (surface antigen, inactivated, MF59-adjuvanted) containing (H1N1)pdm09-derived strain, H3N2-derived strain and B-like strain</td>
<td>3 × 15 µg</td>
<td>MF59C.1 adjuvant</td>
<td>1 dose of 0.25 ml. For children who have not previously been vaccinated, a second dose should be given after an interval of at least 4 weeks</td>
</tr>
<tr>
<td>Inflexal® V</td>
<td>Crucell</td>
<td>Egg</td>
<td>Influenza vaccine (surface antigen, inactivated, virosome adjuvanted) containing (H1N1)pdm09-derived strain, H3N2-derived strain and B-like strain</td>
<td>3 × 15 µg</td>
<td>Virosomes</td>
<td>Clinical data are limited. Dosages of 0.25 or 0.5 ml may be given. For children who have not previously been vaccinated, a second dose should be given after an interval of at least 4 weeks</td>
</tr>
<tr>
<td><strong>Adjuvanted H1N1pdm09 vaccines</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fluviral® P</td>
<td>Omnivest</td>
<td>Egg</td>
<td>Inactivated, whole reassortant virus A/California/7/2009 (H1N1) V-like strain</td>
<td>6 µg</td>
<td>Aluminum phosphate gel</td>
<td>1 dose of 0.25 ml</td>
</tr>
<tr>
<td>Arepanrix®</td>
<td>GlaxoSmithKline</td>
<td>Egg</td>
<td>Inactivated, split-influenza, reassortant, A/California/7/2009 (H1N1) V-like strain</td>
<td>3.75 µg</td>
<td>AS03 adjuvant</td>
<td>1 dose of 0.25 ml†</td>
</tr>
<tr>
<td>Pandemrix®</td>
<td>GlaxoSmithKline</td>
<td>Egg</td>
<td>Inactivated, split-influenza, reassortant, A/California/7/2009 (H1N1) V-like strain</td>
<td>3.75 µg</td>
<td>AS03 adjuvant</td>
<td>1 dose of 0.25 ml†</td>
</tr>
<tr>
<td>Humenza®</td>
<td>Sanofi Pasteur</td>
<td>Egg</td>
<td>A/California/7/2009 (H1N1)-like strain (NYMC X-179A)</td>
<td>3.8 µg</td>
<td>AF03 adjuvant</td>
<td>1 dose of 0.25 ml†</td>
</tr>
<tr>
<td>Focetria®</td>
<td>Egg</td>
<td></td>
<td>Inactivated, surface-influenza antigens (HA and neuraminidase), reassortant, A/California/7/2009 (H1N1) V-like strain</td>
<td>7.5 µg</td>
<td>MF59C.1 adjuvant</td>
<td>1 dose of 0.5 ml at an elected date. A second dose of vaccine should be given after an interval of at least 3 weeks</td>
</tr>
<tr>
<td>Celtura®</td>
<td>Novartis</td>
<td>MDCK cells</td>
<td>A/California/7/2009 (H1N1)-like strain (X-179A)</td>
<td>3.75 µg</td>
<td>MF59C.1 adjuvant</td>
<td>1 dose of 0.25 ml at an elected date. A second dose of vaccine should be given after an interval of at least 3 weeks</td>
</tr>
</tbody>
</table>

†Withdrawn from the market.
†The Summary of Product Characteristics indicates that the immune response is increased with a second dose, and this can be considered taking into account the increased reactogenicity seen with a second dose.

HA: Hemagglutinin antibody; MDCK: Madine-Darby canine kidney.
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too, no nonadjuvanted control arm was included. Finally, one study was found in which aluminum-adjuvanted whole-virion influenza H5N1 vaccine was compared with nonadjuvanted influenza vaccine in children aged 6 months to 17 years [49]. Children received two injections of vaccine containing either 30 µg HA + aluminium or 7.5 µg HA without adjuvant. Of the children in the 30 µg HA + aluminium vaccine arm, 79% achieved an HI titer ≥1:32, whilst 46% in the 7.5 µg HA arm achieved a titer ≥1:32. From a design perspective it is surprising that the HA content of the adjuvanted vaccine is higher than that of the nonadjuvanted comparator. The finding that the children who received 30 µg HA + aluminium adjuvant had higher immune responses than those receiving 7.5 µg HA cannot be attributed to the adjuvant as it could simply be a result of the higher HA content.

■ Virosomes

Kanra et al. reported on an open-label randomized controlled trial in which the safety and immune response to a virosomal adjuvanted vaccine and a nonadjuvanted split influenza vaccine were compared in 454 children aged 6–71 months [50]. Those previously vaccinated were considered primed and received a single dose. Unprimed children received a second dose after 4 weeks and children up to 36 months of age received a half dose. The immunogenicity was assessed with an HI assay prior to vaccination and 4 weeks after the last dose received. Although point estimates were higher for the virosomal adjuvanted vaccine, differences were small and not statistically significant. No statistically significant difference between the adjuvanted and unadjuvanted vaccine in increase in geometric mean titres (GMTs) was found. Seroconversion and seroprotection (percentage with HI ≥1:40) were also broadly similar between the two vaccines. A recently published study showed that a single adult dose (15 µg) of virosomal adjuvanted influenza vaccine elicited a similar immune response as two half doses (7.5 µg), given 4 weeks apart in children aged 6–36 months [51].

■ Oil-in-water adjuvanted vaccines

AF03

During the 2009/2010 H1N1 pandemic, an inactivated split virion H1N1pdm09 AF03 adjuvanted vaccine was licensed in Europe. This vaccine was not used. A study in 401 children aged 6–36 months looked at immunogenicity and safety of different dosages of this vaccine [52,106]. Children received either two doses of 1.9 µg HA + 1/2 AF03, 3.8 µg HA + 1/2 AF03, 3.8 µg HA + AF03 or 7.5 µg HA. The response following the first dose was modest, and improved with a second dose. Antibody titers were 5–7 times higher following adjuvanted vaccine compared with the nonadjuvanted vaccine. This also translated into better persistence of antibodies.

AS03

Two publications were identified that reported the immunogenicity and tolerability or safety of AS03 adjuvanted H1N1pdm09 vaccine (Pandemrix™) compared with a nonadjuvanted influenza vaccine in children aged 6–36 months [53,54].

Langley et al. randomized 323 children aged 6 months to <9 years of age to receive two doses of nonadjuvanted influenza A(H1N1)pdm09 vaccine (15 or 7.5 µg HA) or AS03 adjuvanted influenza A(H1N1)pdm09 vaccine (3.75 µg HA/AS03A or 1.9 µg HA/AS03B), 21 days apart [54]. The immune response was measured as HI antibody response and as microneutralization response and was evaluated according to the Committee for Medicinal Products for Human Use (CHMP) criteria. Overall, immune responses were improved with the adjuvanted vaccine compared with the nonadjuvanted vaccine. CHMP criteria were met for all vaccine groups except the half dose unadjuvanted group, however this carries little meaning as explained above.

In children aged 6–11 months antibody titers were five to ten-times higher with the adjuvanted compared with the nonadjuvanted vaccines following the first dose. A second dose further increased antibody titres in all vaccine groups, resulting in titers three to 22 times higher in the adjuvanted compared with the nonadjuvanted groups. In children aged 12–35 months a similar pattern was seen, with higher responses in the adjuvanted versus the nonadjuvanted vaccine groups. Notably, the response to the half-dose unadjuvanted vaccine was higher in this age group compared with younger children. After 6 months, antibody levels remained higher for the adjuvanted vaccine groups compared with the nonadjuvanted vaccine groups. Note, that as only a modest number of young children was included (5–25 per vaccine group), there is limited power to detect differences and confidence intervals overlap.

In the study by Waddington et al. the immunogenicity and safety of a two dose regimen of AS03 adjuvanted split virion H1N1pdm09 vaccine was compared with a whole-virion cell culture-derived H1N1pdm09 vaccine (Celvapan, Baxter) in children aged from 6 months to 12 years [55]. A single dose of the AS03 adjuvanted vaccine contained 1.9 µg HA, whilst a single dose of the whole-virion vaccine contained 7.5 µg. In all children the AS03 adjuvanted vaccine elicited higher antibody titers than the whole virion vaccine. In children aged 6–36 months the GMT after a second dose of AS03 adjuvanted vaccine was 461.0 compared with 44.0 for the whole-virion vaccine. The fold rise in HI titer from baseline was also higher for the AS03 adjuvanted vaccine.
(107.4 vs 9.5). In line with the increase in GMTs, seroconversion rates were also consistently higher for the adjuvanted vaccine compared with the whole virion vaccine. Note that a whole virion is not comparable to a split or subunit vaccine. Whole-virion vaccines not only contain surface proteins but also matrix proteins, as well as genomic RNA. It has been suggested that whole-virion vaccines have a ‘built-in adjuvant’ through the remaining RNA in the vaccine [56]. In an extension of this study the T-cell responses were evaluated 1 year after vaccination with the AS03 adjuvanted split virion and the nonadjuvanted whole virion H1N1pdm09 vaccines. An important observation in this study was that children who received an AS03 adjuvanted split virion H1N1pdm09 vaccine had higher T-cell responses to internal influenza antigens 1 year after vaccination, compared with children who received a whole virion nonadjuvanted H1N1pdm09 vaccine [55].

MF59
A dose finding study by Block et al. clearly demonstrated that MF59 enhances the immune response in children aged 6–36 months [56]. In their study, 654 healthy children aged from 6 to <36 months of age were randomized to receive two half doses of MF59-adjuvanted vaccine (3.75 µg HA + 1/2 MF59); two half doses of nonadjuvanted vaccine (7.5 µg HA); two full doses with half the amount of MF59 adjuvant (7.5 µg HA + 1/2 MF59) or two full doses of nonadjuvanted vaccine (15 µg HA). Antibody responses were measured by the HI assay. On day 22, 3 weeks after the first dose, seroprotection rate (HI titer ≥1:40) was 79% (95% CI: 71–86%) and 86% (95% CI: 79–91%) in half-and full-dose adjuvanted group. The response was lower for nonadjuvanted vaccines, 37% (95% CI: 29–46%) and 50% (95% CI: 41–59%) for the half- and full-dose respectively. A total of 3 weeks after the second dose, the response increased to 100% (95% CI: 97–100%) in both adjuvanted groups and to 70% (95% CI: 61–78%) and 81% (95% CI: 74–88%) for the half- and full-dose nonadjuvanted group, respectively. The geometric mean ratio was also higher for both adjuvanted vaccines. An important observation in this study was that after 6 months children immunized with the MF59-adjuvanted vaccine formulations had persisting antibodies, whilst this was not the case for children in the nonadjuvanted arms. An indication that MF59-adjuvanted influenza vaccine also enhances cross-reactivity comes from the study by Vesikari et al., in which not only HI titers against vaccine strains were measured but also against mismatched strains [57]. In their study they randomized 281 unprimed children aged 6–36 months to receive either two doses of an MF59 adjuvanted inactivated split vaccine (7.5 µg HA per strain) or a nonadjuvanted inactivated split vaccine (7.5 µg HA per strain). After 1 year subjects received a repeat vaccination. Antibody titers were measured with the HI assay. GMTs and fold increase was significantly higher after MF59-adjuvanted vaccination compared with nonadjuvanted vaccination for all three vaccine strains. Although titers decreased over the following year, they remained significantly higher for the MF59 adjuvanted vaccine. The booster response was also stronger in those receiving the adjuvanted vaccine. When tested against mismatched strains, postvaccination titers and fold increase was significantly higher with the adjuvanted vaccine 3 weeks after the second dose for all three strains (A/H1N1, A/H3N2 and B). In an extension to this study, 89 children were revaccinated in the following season. Children who had received an adjuvanted vaccine in the previous season had higher prevaccination HI antibody titers than those who received a nonadjuvanted vaccine. A total of 3 weeks after being revaccinated, the immune responses were significantly higher following the adjuvanted vaccine compared with the nonadjuvanted vaccine [58].

Efficacy of adjuvanted versus nonadjuvanted vaccines
Only one study was identified in which the efficacy of an adjuvanted influenza vaccine was compared with the efficacy of a nonadjuvanted influenza vaccine, and control vaccine, in young children. This large, randomized, controlled trial evaluated the protective efficacy of an MF59-adjuvanted seasonal influenza vaccine compared with that of a nonadjuvanted seasonal influenza vaccine and a non-influenza vaccine control in unprimed children aged 6–72 months [59]. The study was conducted over two seasons. In the first season 654 children were randomized to receive adjuvanted influenza, nonadjuvanted subunit influenza or control (meningococcal) vaccine in a 2:2:1 ratio. Efficacy was determined against influenza illness confirmed by realtime PCR assay. Children up to 36 months received two half doses, older children received full doses. In the first season there were insufficient cases of influenza to determine vaccine efficacy (VE). In the second year the VE against all strains was 86% (95% CI: 73–92) for the MF59-adjuvanted vaccine. The VE for the nonadjuvanted split vaccine was 40% (95% CI: 11–60). For the subgroup aged 6–36 months this was 79% (95% CI: 55–90) versus 40% (95% CI: 6–66) for the adjuvanted and nonadjuvanted vaccine, respectively. Note that for children under 2 years of age, there was no significant VE for the two half doses of nonadjuvanted vaccine (VE: 11%; 95% CI: -89–58%) whilst the VE
for the adjuvanted vaccine remained relatively high at 77% (95% CI: 37–92). The VE against matched strains was slightly higher.

Although the publication states that the study was conducted according to Good Clinical Practice (GCP) guidelines, during an inspection it was found that the study was not compliant with GCP standards. Several critical issues are discussed in the CHMP withdrawal assessment report [107] pertaining to the validity and adequacy of the PCR used, but also the reliability of recorded adverse events and suspected influenza cases [60]. In response, the authors re-analyzed all samples using validated methods, but also re-analyzed the efficacy excluding one critical investigational site. The reanalysis yielded a higher VE for the adjuvanted vaccine and similar results concerning the exclusion of the questioned site. The authors pointed out that the VE for the nonadjuvanted vaccine is similar to those reported in the same age groups in published studies, providing external validation. In addition, serological findings from this study are in line with the efficacy findings. Nonetheless, as the GCP issues have not been resolved and there is no full insight into the extent to which the different issues affect the validity of the findings, uncertainty surrounding the findings of this trial will remain.

Safety of adjuvanted versus nonadjuvanted vaccines

■ Data from clinical trials
Clinical trials are useful for describing and comparing the reactogenicity of vaccine formulations, but are usually too small to detect and evaluate (rare) vaccine-related adverse events as these are fortunately uncommon. This section, therefore, focuses on the comparative tolerability or reactogenicity of adjuvanted versus nonadjuvanted influenza vaccines. The collection and presentation of safety data varies between studies and publications. Although calls have been made to harmonize study protocols and the presentation of safety data in clinical study publications, this is not yet reality [61–63]. Comparisons of safety between different types of vaccines should ideally come from head-to-head trials.

Nonadjuvanted split and subunit influenza vaccines have a long track record of safe use, including in the youngest age groups. Accumulated evidence shows that these vaccines are well tolerated, with only a small minority of children reporting mild transient systemic reactions including malaise, fever and myalgia. Systemic symptoms are most prominent in children younger than 36 months of age, possibly as these are unprimed to the viral antigens. Most frequently reported adverse events include fever, rash, injection-site reactions and febrile seizures [64–69].

Little can be concluded on the relative safety of aluminium-adjuvanted influenza vaccines compared with nonadjuvanted inactivated split or subunit vaccines in children <36 months of age. Results from head-to-head comparisons were not reported in the published studies [37–48], or the nonadjuvanted comparator vaccine had a different HA content [49]. This prevents any conclusions regarding the added risk resulting from the addition of an aluminium adjuvant to influenza vaccines.

Based upon available data from one clinical study in young children, the tolerability profile of the virosomal adjuvanted vaccines is quite similar compared with that of a nonadjuvanted split influenza vaccine. With regards to solicited adverse events, no difference between the two vaccines was found. The virosomal adjuvanted vaccine has a lower content of ovalbumin and is therefore expected to induce fewer allergic reactions [32]. Although the vaccine has been safely administered to children with egg allergy [70], large postmarketing safety studies would be needed to support such a claim.

The overall picture for the oil-in-water adjuvanted vaccines is that there is an increase in reactogenicity compared with the nonadjuvanted vaccines. Although there are no head-to-head comparisons between the different types of adjuvanted influenza vaccines, there are clearly differences. For both AS03- as AF03-adjuvanted vaccines, fever appears to increase with the second dose. This was especially evident in a study with the AS03-adjuvanted H1N1pdm09 vaccine [71]. Here, fever defined as a temperature ≥37.5°C, was reported by 20% of children aged 6–35 months following the first dose. Following the second dose, 67% reported a temperature ≥37.5°C. In a study with the AF03-adjuvanted H1N1pdm09 vaccine, 8% of children aged 6–11 months reported fever (≥38.0°C) following the first dose. This increased to 33% following the second dose [106]. This was not seen in children aged 12–35 months. For the MF59-adjuvanted vaccine there is an increase in local reactions, but no apparent increase in systemic reactions. In the largest randomised controlled trial by Vesikari et al., sufficient children were included to evaluate less frequent adverse events [59]. Febrile convulsions were reported in five children (out of 993) who received non-adjuvanted split influenza vaccine and in five children (out of 1099) who received adjuvanted influenza vaccine, indicating no increased risk.

■ Safety of adjuvanted influenza vaccines: lessons learned during the 2009/2010 pandemic
Both MF59 and AS03 adjuvanted influenza vaccines were used on a large scale during the 2009/2010 H1N1 pandemic. Children belonged to one of the main target groups for vaccination, including children younger than 3 years. Considering that in Europe alone over 37 million people had been vaccinated by April 2010 [72], and for the countries which reported Pandemrix™
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was used by 74% [34] – it can be assumed that the exposure to adjuvanted influenza vaccines in the youngest age group was substantial. An in-depth review on the safety of influenza A(H1N1)pdm09 vaccines, including an evaluation of postmarketing data for adjuvanted and nonadjuvanted vaccines, is presented elsewhere and is not the focus of the present article [63]. Yet, when discussing the use of adjuvanted influenza vaccines in young children the authors think it is important to highlight the experience with adjuvanted influenza vaccines during the 2009/2010 H1N1 pandemic.

As there was only limited safety data prior to the start of vaccination campaigns, especially in children, active monitoring of safety took place with focus on adverse events of special interest including neuritis, convulsions, anaphylaxis, encephalitis, vasculitis, GBS, Bell’s palsy, demyelinating disorders and laboratory-confirmed vaccination failure [72]. Evaluations of background rates of these adverse events of special interests were performed in order to be able to use observed to expected analyses for rapid signal detection [108]. Following the observed increased risk of GBS associated with swine flu vaccination in 1976 in the USA, studies were started around the world to prospectively evaluate the risk of GBS following vaccination [73–78]. Largely, studies pointed out that there was no increased risk of adverse events of special interests following vaccination with adjuvanted influenza vaccines [72–74,79–81]. However, a cohort study in Sweden found a small increased risk of Bell’s Palsy, paraesthesia, and inflammatory bowel disease associated with AS03 adjuvanted influenza vaccine [82], and a small but significant increase in the risk of GBS was seen in Quebec (Canada) following vaccination with AS03 adjuvanted influenza vaccine [78].

In August 2010, a signal of narcolepsy associated with Pandemrix appeared in Sweden and Finland in children and adolescents aged 5–19. Epidemiological investigations have since then confirmed the signal [83–85,109,110], and more European countries have reported an increase in narcolepsy associated with the use of Pandemrix [86,111]. After 2 years, it remains unclear what the exact explanation is for the increased incidence of narcolepsy associated with Pandemrix, and much work needs to be done before we can fully understand what happened. The absolute risk is small (~1 in 20,000 vaccinations), yet considering the severity of the disease and the ages it affects, a small risk can have a considerable impact. With suitable alternatives available the European Medicines Agency restricted the use of Pandemrix in children in 2011 [112]. No association between MF59-adjuvanted influenza vaccines and narcolepsy has been seen [87], although absolute exposure of the affected age groups is expected to be lower than is the case for Pandemrix. Moreover, a study in China found no association with nonadjuvanted influenza vaccines but did find an association between onset of narcolepsy and infection with influenza A(H1N1)pdm09 virus [88].

Conclusion

It has long been known that traditional split- and subunit influenza vaccines do not perform well in younger, unprimed children. This has been confirmed by few studies showing that the efficacy of nonadjuvanted split or subunit influenza vaccines in this group is limited [15,16,18,39]. Fortunately, so are the safety concerns. These vaccines have proven to be well tolerated with adverse events reported in a small minority. Several clinical trials comparing different adjuvanted influenza vaccines with nonadjuvanted influenza vaccines in young children were identified. No strong evidence was found that either aluminum salts or virosomes significantly enhance the immune response in this age group. However, identified studies clearly demonstrated that oil-in-water adjuvants improve the immune response to influenza vaccines, leading to higher antibody titers when measured with either the HI or virus neutralization assay. Not only were titers greater directly after vaccination, antibodies persisted for longer and demonstrated better response against heterologs or drifted strains. These are potentially important benefits for the youngest children, but also for other age groups, as this could mean that annual revaccination against influenza would not be necessary. However, what an increase in the antibody response means in terms of protection against infection or disease is still unknown as there is no validated correlate of protection. This uncertainty makes it difficult to weigh benefits against identified risks. For a proper benefit–risk analysis, studies evaluating the efficacy against relevant clinical outcomes are needed. Only one such study is known [52].

In this study it was found that the increased immune response does translate into improved efficacy. The largest gain was for children younger than 24 months, where there was no apparent efficacy of the nonadjuvanted split vaccine (VE: 11%; 95% CI: 89–58) whilst the efficacy of the MF59 adjuvanted vaccine, Fluarix®, remained high (VE: 77%; 95% CI: 37–92). This forms an indication that where nonadjuvanted vaccines are failing to adequately prime, adjuvanted vaccines do achieve this. However, as this study was not performed according to GCP guidelines some uncertainty on these findings remains. Clearly, much more work could be done on the evaluation of adjuvanted-influenza vaccines and more large-scale studies evaluating the efficacy against clinically relevant outcomes in immunologically naïve children would be welcomed. The finding that adjuvanted influenza vaccines confer some degree of cross-protection against drifted strains opens up the possibility of alternative
vaccination approaches, that is, annual revaccination might no longer be needed.

The gains in immunogenicity and efficacy provided by the different oil-in-water adjuvants evaluated do come at a price. With the MF59-adjuvanted seasonal and pandemic vaccines, this cost appears to be limited to a small increase in local reactogenicity compared with the non-adjuvanted vaccines. With the AS03 adjuvant, an increase in febrile reactions is seen following the second dose in several clinical studies, and in 2009 this led to a warning from European Medicines Agency [113]. A similar trend was seen in the limited data available for AF03 adjuvanted influenza A(H1N1)pdm09 vaccine, with an increase in febrile reactions in children aged 6–12 months.

For adjuvanted influenza vaccines ideally more work would be done to investigate the most optimal schedule and antigen–adjuvant balance. Especially where a second dose is associated with increases in febrile reactions, careful consideration of the need for a second dose with an adjuvanted vaccine would be needed. Although influenza infection in young children does lead to complications, hospitalizations and even death, in most children the disease is self-limiting. Therefore the tolerability and safety of the vaccine should be optimal. The dose recommendations for the AS03 and AF03 adjuvanted pandemic-influenza vaccines for children state that there is a further immune response to a second dose of 0.25 ml administered after an interval of 3 weeks, but that the use of a second dose should take the increased reactogenicity into consideration [114–116]. This advice should be improved if these vaccines were to be used in the future.

As highlighted earlier, there is some evidence indicating that two doses of unadjuvanted traditional split or subunit influenza vaccines could be effective in protecting young children against influenza [20–24]. This underlines the necessity of proper evaluation of different dosing regimens for the traditional, nonadjuvanted, split or subunit influenza vaccines in unprimed children before disregarding these vaccines as an option for protecting young children.

Unfortunately, dose finding trials are naturally limited to immunogenicity studies and it is not known what the gains of an increased antibody titer translate to in terms of protection against infection and disease. Thus, the benefit of a full versus half dose, or two versus one dose, is not fully understood. Considering the shortcomings of current serological studies, collaborative effort is required to increase understanding into immune markers, their correlation to protection and to overcome limitations of existing assays to measure these markers.

The finding that the AS03-adjuvanted influenza A(H1N1)pdm09 vaccine was associated with an increase in the incidence in narcolepsy in children 5–19 years of age has led to the restriction of its use in Europe. Although this A(H1N1)pdm09 influenza vaccine will unlikely be used in the future, the association between Pandemrix and narcolepsy has undoubtedly cast a shadow over the use and development of adjuvanted influenza vaccines in children. Narcolepsy is a serious debilitating chronic condition, and it is imperative that the role of Pandemrix as a potential trigger is fully investigated and understood. The epidemiological studies, so far, have probably led to more questions than answers, and investigations are expanding globally in order to gain more insight in countries that used Pandemrix but did not have the same media coverage on the association with narcolepsy compared with many European countries [117]. Moreover, studies that can shed light on potential mechanisms are needed to start understanding how narcolepsy is triggered, and what role Pandemrix could have played.

It is clear that improved vaccines for young children are needed, and oil-in-water adjuvanted vaccines are an effective alternative, which could address an urgent need in the youngest, immunologically naive children. The limited studies available point towards greatly improved immunogenicity, both quantitative as qualitative, but also improved efficacy. There is a cost in the tolerability, which needs to be carefully considered for each vaccine separately when determining the optimal dosage and schedule. What should be underlined above all is that the uncertainties regarding rare but serious adverse events, such as the association between Pandemrix and narcolepsy, need to be addressed and fully investigated if we are to move forward with these vaccines for young children. Until we fully understand how these adjuvants work in children with immature, developing immune systems, basic research to increase our understanding is needed. At the same time, other options to increase the immune response and efficacy in young, unprimed children, including higher dosages of traditional inactivated split or subunit influenza vaccines and intradermal vaccination, should be further considered as these could also form effective alternatives; however, data to substantiate this are limited.

Future perspective

As more countries consider and implement influenza vaccination recommendations for healthy children, there is a growing need for improved influenza vaccine for the youngest, immunological-naive children. More basic research is needed into the mechanisms and effects of the different adjuvants and new types of adjuvants should be tested in clinical trials leading to a more diverse field of adjuvanted influenza vaccines. As different influenza vaccines increasingly become available, a different approach to influenza vaccination could be considered – no longer will entire populations be vaccinated with the same influenza vaccine but different types of vaccines with different dosages and vaccination schedules could be considered for different
target populations. Adjuvanted influenza vaccines will clearly play an important role in the future of influenza vaccination. Due to safety concerns around the use of adjuvanted influenza vaccines in children, safety should be monitored and more rapid benefit-risk models need to be developed. With increased investment and improved global collaboration large systems to follow the use this may be done in a collaborative manner to take advantages of heterogeneity and scale.

Executive summary

**Background**
- A relatively high burden of influenza is experienced by young children who are largely unprimed.
- For the age group with the highest attack rates there is a lack of effective vaccines.
- Adjuvanted influenza vaccines could possibly overcome limitations of traditional influenza vaccines in the youngest age groups.
- Adjuvanted influenza vaccines available include aluminum-, oil-in-water- and virosomal-adjuvanted vaccines.

**Immunogenicity of adjuvanted versus nonadjuvanted vaccines**
- No strong evidence was found that either aluminum salts or virosomes significantly enhance the immune response in children under 3 years of age.
- Few available studies demonstrated that oil-in-water adjuvants (AF03, MF59, AS03) improve the immune response to influenza vaccines.

**Efficacy of adjuvanted versus nonadjuvanted vaccines**
- One study showed that the MF59-adjuvant also improves protection against influenza infection in children under 3 years of age.

**Safety of adjuvanted versus nonadjuvanted vaccines**
- The improved immune response and efficacy comes at a cost of increased reactogenicity, which underlines the need for careful consideration of the optimal dosage and schedule.
- The association between Pandemrix and narcolepsy has undoubtedly cast a shadow over the use and development of adjuvanted influenza vaccines in children.
- Narcolepsy is a serious debilitating chronic condition, and it is imperative that the role of Pandemrix® as a potential trigger is fully investigated and understood.

**Discussion**
- Oil-in-water-adjuvanted vaccines are clearly an effective alternative that could address an urgent need in the youngest, immunological-naive children.
- Until we fully understand how these adjuvants work in children with immature, developing immune systems, basic research to increase our understanding is needed.

References

Papers of special note have been highlighted as: 
- of interest
- of considerable interest

Adjuvanted versus nonadjuvanted influenza vaccines in young children

Review: Clinical Trial Outcomes


A meta-analysis of the efficacy of influenza vaccines against specific outcomes, highlighting the lack of efficacy in the youngest children.


Review: Clinical Trial Outcomes

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This study demonstrates that MF59-adjuvanted influenza vaccine also enhances cross-reactivity, thereby possibly providing protection against drifted strains.


The only Phase III study evaluating the efficacy of adjuvanted influenza vaccine in young children against clinically relevant outcomes. Read in conjunction with [107].


A study into the association between narcolepsy and Pandemrix® in Finland. Read in conjunction with the [107].


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