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Review

An indirect comparison meta-analysis of AS03 and MF59 adjuvants in pandemic influenza A(H1N1)pdm09 vaccines

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ABSTRACT

Background: Although oil-in-water adjuvants improve pandemic influenza vaccine efficacy, AS03 versus MF59 adjuvant comparisons in A(H1N1)pdm09 pandemic vaccines are lacking.

Methods: We conducted an indirect-comparison meta-analysis extracting published data from randomised controlled trials in literature databases (01/01/2009–09/09/2018), evaluating immunogenicity and safety of AS03- or MF59-adjuvanted vaccines. We conducted comparisons of log-transformed haemagglutination inhibition geometric mean titre ratio (GMTR; primary outcome) of different regimens of each adjuvant versus unadjuvanted counterparts. Then via test of subgroup differences, we indirectly compared different AS03 versus MF59 regimens.

Results: We identified 22 publications with 10,734 participants. In adults, AS03-adjuvanted vaccines (3.75 μg haemagglutinin) achieved superior GMTR versus unadjuvanted vaccines (all four comparisons); MD = 0.56 (95%CI 0.33 to 0.80, $p < 0.001$) to 1.18 (95%CI 0.72 to 1.65, $p < 0.001$). MF59 (full-dose)-adjuvanted vaccines (7.5 μg haemagglutinin) were superior to unadjuvanted vaccines (three of four comparisons); MD = 0.47 (95%CI 0.19 to 0.75, $p = 0.001$) to 0.80 (95%CI 0.44 to 1.16, $p < 0.001$). Adult indirect comparisons favoured AS03 over MF59 (six of eight comparisons; $p < 0.001$ to $p = 0.088$).

Paediatric indirect comparisons favoured MF59-adjuvanted vaccines (two of seven comparisons; $p = 0.011$, 0.079). However, unadjuvanted control group seroconversion rate was lower in MF59 than AS03 studies ($p < 0.001$ to $p = 0.097$).

There was substantial heterogeneity, and adult AS03 studies had lower risk of bias.

Conclusions: Despite limited studies, in adults, AS03-adjuvanted vaccines allow antigen sparing versus MF59-adjuvanted and unadjuvanted vaccines, with similar immunogenicity, but higher risk of pain and fatigue (secondary outcomes) than unadjuvanted vaccines. In children, adjuvanted vaccines are also superior, but the better adjuvant is uncertain.

Contents

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1. Introduction

Over the last century there were influenza pandemics in 1918, 1957, 1968 and 2009, caused by different influenza A subtypes and with varying clinical severity. The 1918 influenza A(H1N1) pandemic virus infected nearly half the world's population, causing 50 million deaths, whereas influenza A(H1N1)pdm09 that caused the 2009 pandemic was less severe [1]. These differences illustrate the unpredictability of timing, location, morbidity and mortality of influenza pandemics [2].

While various public health control interventions are needed in the early stages of a pandemic, vaccines remain a mainstay of ongoing pandemic control [3]. Although influenza vaccines reduce disease rates [4], there is difficulty in producing and delivering sufficient vaccine quantity in the early pandemic phases [5]. Even with a one year production lead time, optimistic estimates place the potential global population coverage of a two-dose pandemic vaccine course at 43% [6].

Reducing the amount of influenza haemagglutinin (HA) antigen needed per vaccine dose, while maintaining the same efficacy, would allow more vaccinations with limited antigen availability. Suitable vaccine adjuvants can achieve this [7]. They improve vaccine immunogenicity, allowing use of a lower antigen dose with similar efficacy [8], and can be readily stockpiled [9]. However, in pandemic influenza, traditional adjuvants like alum are no more immunogenic than unadjuvanted vaccines [10]. Therefore, alternatives like oil-in-water adjuvants have been explored. The oil-in-water adjuvants regularly used in influenza vaccines are AS03 (GlaxoSmithKline plc), and MF59 (Novartis International AG) [8]. A dose of AS03 ($AS03_A$)-adjuvanted vaccine contains 10.69 mg squalene, 11.86 mg α-tocopherol, 4.86 mg polysorbate 80, and 3.75 μg HA [11]. A dose of MF59 (MF59(f))-adjuvanted vaccine contains 9.75 mg squalene, 1.175 mg polysorbate 80, 1.175 mg sorbitan trioleate, and 7.5 μg HA [12]. Half-dose preparations are also available for both adjuvants, with MF59 (MF59(h)) using half the volume with the same concentration [13], and AS03 ($AS03_B$) using either half the volume with the same concentration [14], or the same volume with half the concentration [11]. Both adjuvants are associated with an increase in mild adverse events compared to unadjuvanted vaccines [9], and there have been rare reports of narcolepsy with one brand of AS03-adjuvanted vaccine (Pandemrix, GlaxoSmithKline Biologicals S.A) in paediatric populations in Sweden and Finland [15].

Only one clinical trial has directly compared vaccines with these adjuvants, looking at pre- and post-vaccination haemagglutination inhibition (HAI) geometric mean titres (GMT) in avian A (H7N9) influenza vaccines. The AS03-adjuvanted vaccines were superior to MF59-adjuvanted vaccine, which was in turn superior to unadjuvanted vaccines [9]. Meta-analyses also suggested that the adjuvanted vaccines met regulatory criteria at lower doses than unadjuvanted vaccines in A(H5N1) (although, with 1.9ug HA only AS03 met criteria) [16], and A(H1N1) influenza [10,17]. However, there have been no direct adjuvant comparisons for A (H1N1)pdm09 pandemic influenza vaccines [9].

Therefore, an indirect comparison meta-analysis of the short-term immunogenicity and safety of AS03- and MF59-adjuvanted vaccines versus each other, and versus unadjuvanted A(H1N1) pdm09 monovalent influenza vaccines, was undertaken. It was hypothesised that adjuvanted vaccines would be HA antigen dose sparing, without jeopardising immunogenicity or safety. This study was prompted by recent concerns over potential future pandemic vaccine availability, especially in lower income countries [18].

2. Method

An indirect-comparison meta-analysis of randomised controlled trials was performed to compare the immunogenicity and safety of AS03-adjuvanted, MF59-adjuvanted and unadjuvanted A (H1N1)pdm09 monovalent influenza vaccines.

A literature search was conducted with the inclusion criteria: peer-reviewed, English language, randomised controlled trial, human, healthy, non-pregnant, all ages, reporting data 21 days after final dose of an intramuscular A(H1N1)pdm09 vaccine. This included HAI GMT, HAI seroconversion rate, or vaccine effectiveness, and adverse effects.

Medline, Embase, Emcare, Public Health, CINAHL, Web of Science and clinicaltrials.gov were examined for articles that met these criteria published between 2009 (when A(H1N1)pdm09 emerged [19]) and September 9th 2018 (Supplementary File 1 shows search strategy). Reference lists of the included studies were also searched. Two reviewers (MIH and ACYS) independently assessed the eligibility of titles, abstracts and full texts with another reviewer (DJM) resolving discrepancies. Summary estimates of data were sought, and no study authors were contacted. This review was reported according to PRISMA guidelines and registered in PROSPERO (CRD42018099026) [20].

Two reviewers (MIH and ACYS) independently reviewed articles and extracted all data into standardised spreadsheets. The primary outcome was vaccine immunogenicity, represented by mean difference (MD) in HAI geometric mean titre ratio (GMTR) of post versus pre-vaccination titres, as well as risk ratio (RR) for HAI seroconversion rate. The secondary outcome was safety, represented by RR of adverse effects, specifically injection site pain (including tenderness), fatigue (including malaise and sleepiness), and fever, as well as incidental reports of narcolepsy.

The number of patients, adjuvanted and unadjuvanted vaccine dosages, number of doses administered, funding source, age range, mean age, percentage of female participants, influenza antigen source, study location, split versus whole virion vaccine, HAI GMTR (or baseline and 21-day post-vaccination HAI GMT where the ratio was not reported, allowing us to calculate this ratio) with 95% confidence intervals (95%CI), influenza seroconversion rate, and rates of pain, fatigue and fever were extracted.

The analysis was complicated by multiple doses used within studies, both with the adjuvant and in the control group. Analyses therefore had to compare AS03 and MF59 at different doses for the different control groups. Separate analyses were conducted for adult (≥18 years of age) and paediatric (<18 years of age) data. Where age subsets were reported within the adult or paediatric groups, these were combined into a single adult or paediatric group.

HAI GMTR data including 95% CIs were log-transformed using the natural logarithm, as these transformed values are generally accepted to follow a normal distribution curve [21], thus allowing us to calculate the standard error. Pooled estimates across studies (comparing adjuvanted to the unadjuvanted control group) for difference in mean log HAI GMTR, difference in proportion who seroconverted, and proportion with adverse effects, comparing adjuvanted versus unadjuvanted vaccines were calculated using a random effects model with inverse variance weighting [22].

As both the AS03- and MF59-containing influenza vaccine trials used unadjuvanted vaccines with either 7.5 or 15 μg (or both) of HA as a common comparator group, separate analyses were conducted for the 7.5 and 15 μg unadjuvanted vaccine comparison groups, and for one and two doses of vaccine. Forest plots were used to demonstrate the relative treatment effect (mean difference of GMTR and risk ratio of seroconversion rate) of AS03-adjuvanted versus unadjuvanted vaccines, with pooling across studies, along with 95%CI for each pooled comparison. The relative treatment effect of MF59-adjuvanted versus unadjuvanted vaccines was also displayed in a similar way in these forest plots. The amount of heterogeneity was estimated using the I^2 value [23].

We did not directly compare the pooled GMTRs and seroconversion rates of the MF59 and AS03 studies without considering their common comparator group, as this would increase bias [24]. Instead, we compared the pooled estimates for AS03-adjuvanted vaccine versus its control group (AS03 pooled mean difference), with pooled estimates of MF59-adjuvanted vaccine versus its control group (MF59 pooled mean difference), using a test of subgroup differences, as outlined by Higgins, Deeks, and Altman [24], thus providing an indirect comparison between AS03 and MF59 for different doses of adjuvanted vaccine. For each comparison, this provided a test of the more immunogenic adjuvant (GMTR and seroconversion rate) with corresponding p value. We interpret weak evidence as a p value 0.05 to <0.1, evidence as a p value 0.01 to <0.05, and strong evidence as a p value < 0.01 [25,26].

Meta-regressions were conducted for mean age versus GMTR, and mean age versus seroconversion rate in the paediatric unadjuvanted comparator group, and for adjuvanted vaccine GMTR mean difference versus mean age and proportion female in adults.

We used the Cochrane risk of bias tool [27] to assess bias risk within studies, and subcategorised performance bias into subject and vaccine administrator blinding, as in many studies adjuvanted vaccines looked different. A funnel plot was used to assess publication bias (if more than 10 studies in each comparison group). Sensitivity analyses were carried out, where possible, based on funding source, antigen source, age, percentage female, study location, and split versus whole virion vaccine. All analyses were conducted in R version 3.4.3 [28] using the meta package [29].

3. Results

We identified 1378 references, including 774 duplicates. Thirty-four articles passed title and abstract screening and underwent full-text review. Twenty-two studies met the inclusion criteria (12 adult and 10 paediatric; Fig. 1, Supplementary File 2). One incomplete study was identified (NCT00964951; withdrawn before patient recruitment).

All studies reported day 21 and/or 42 GMTR, and/or baseline and post-vaccination GMT, as well as HAI seroconversion rate (percentage of subjects with a minimum four-fold rise to ≥1:40 [12] (one study ≥1:32)), with one adult and one paediatric study reporting vaccine effectiveness (there was an insufficient number of papers to perform a meta-analysis on effectiveness). Twenty-one studies with 10,734 participants using either 7.5 or 15 μg HA unadjuvanted vaccine in the comparator group were included in the analyses. Adult AS03 studies used the same $AS03_A$ with 3.75 μg HA, while paediatric AS03 studies used the same $AS03_A$ with 3.75 μg HA, and/or $AS03_B$ with 1.875/1.9 μg HA (either half the volume or half the concentration). All MF59 studies used the same MF59(f) with 7.5 μg HA, and/or the same MF59(h) with 3.75 μg HA. Supplementary File 2 shows individual study demographics.

Fig. 2 shows log-transformed GMTR comparisons of different doses of AS03 and MF59 (adult and paediatric) versus their corresponding 15 μg HA control groups in single dose studies. Supplementary File 3 shows similar data for two dose studies, while Supplementary Files 4 and 5 show similar data for 7.5 μg HA control groups. Hence, for each dose of adjuvanted vaccine (e.g. $AS03_A$ with 3.75 μg HA), there were 4 comparisons (one and two dose versus 15 μg HA unadjuvanted, and one and two dose versus 7.5 μg HA unadjuvanted, as shown in the four forest plots).

Based on the four forest plots, when comparing the mean difference between each adjuvant and its control group (the mean

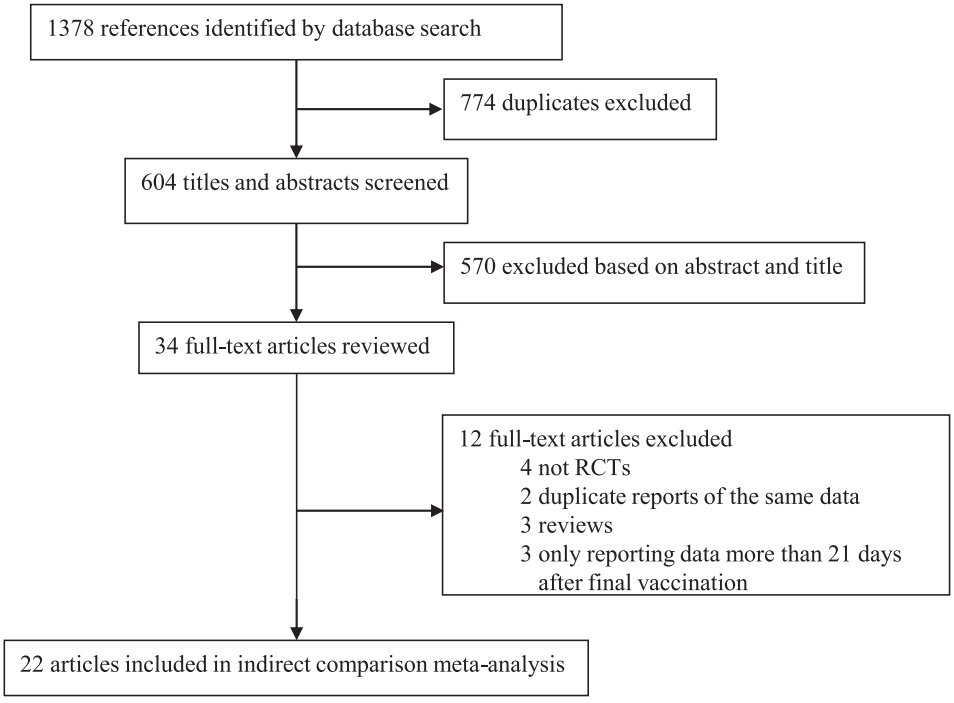

Fig. 1. Study selection.

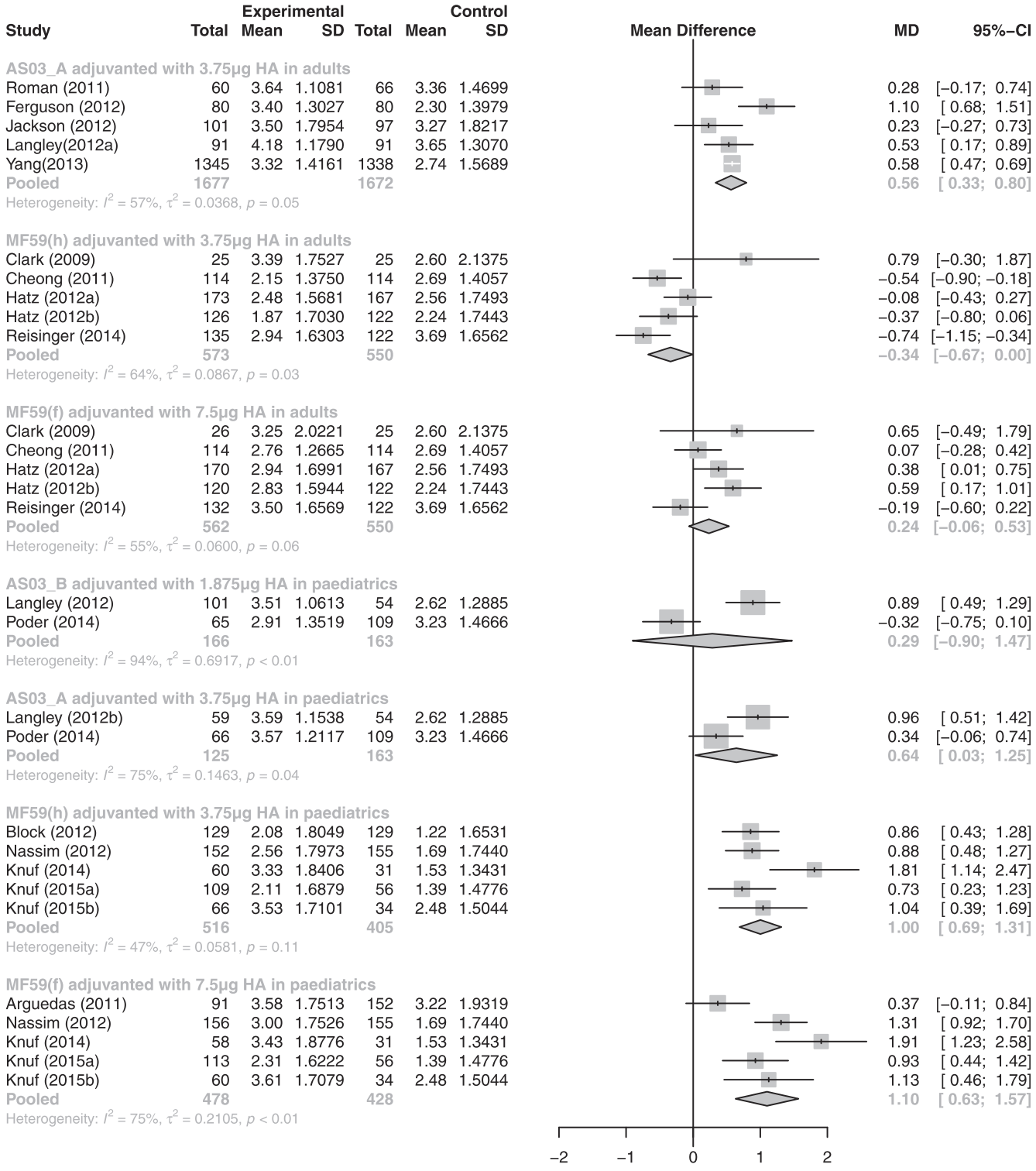

Fig. 2. Forest plot of log transformed geometric mean titre ratios from single dose data of adjuvanted vaccines versus 15 µg HA unadjuvanted vaccine (random-effects model), showing pooled mean difference and 95%CI. MF59(h) refers to a half dose of MF59 and MF59(f) refers to a full dose of MF59. The upper 95% confidence interval of MF59(h) adjuvanted with 3.75 µg HA in adults is −0.004.

differences are also summarised in Table 1), we have found the following results. In adults, compared to unadjuvanted vaccines, there was a higher immune response as measured by GMTR for $AS03_A$ with 3.75 µg HA in all four comparisons (Fig. 2, Supplementary Files 3–5; MD = 0.56, 95%CI 0.33 to 0.80, p < 0.001 to 1.18, 95%CI 0.72 to 1.65, p < 0.001), MF59(f) with 7.5 µg HA in three comparisons (MD = 0.47, 95%CI 0.19 to 0.75, p = 0.001 to 0.80, 95%CI 0.44 to 1.16, p < 0.001), and MF59(h) in one comparison (MD = 0.38, 95%CI 0.02 to 0.73, p = 0.036), but a lower GMTR response to MF59(h) compared to unadjuvanted vaccine in another comparison (MD = -0.34 95%CI −0.67 to −0.004, p = 0.048).

In children, there was a higher GMTR for $AS03_B$ with 1.875/1.9 µg HA in two of three comparisons with more than one

Table 1
Indirect comparisons of log-transformed geometric mean titre ratio, and seroconversion rate of AS03 versus MF59-adjuvanted vaccines (test of subgroup differences).[a]

Comparison	Control group unadjuvanted vaccine HA dose (μg) and corresponding forest plot(s)	Pooled mean difference of log-transformed GMTR of AS03-adjuvanted versus unadjuvanted vaccines	Pooled mean difference of log-transformed GMTR of MF59-adjuvanted versus unadjuvanted vaccines	Indirect comparison showing adjuvant with better GMTR versus unadjuvanted vaccine (p value)[b]	Pooled risk ratio of SCR of AS03-adjuvanted versus unadjuvanted vaccines	Pooled risk ratio of SCR of MF59-adjuvanted versus unadjuvanted vaccines	Indirect comparison showing adjuvant with better SCR versus unadjuvanted vaccine (p value)[c]
Adult							
1 dose							
3.75 μg HA + $AS03_A$ vs 3.75 μg HA + MF59(h)	7.5(sup. file 4)[d]	0.94 (0.36 to 1.52)	0.27 (−0.11 to 0.64)	AS03 (0.055)	1.33 (1.08 to 1.62)	1.12 (0.99 to 1.27)	AS03 (0.173)
	15(Figs. 2, 3)	0.56 (0.33 to 0.80)	−0.34 (−0.67 to −0.004)	**AS03 (<0.001)**	1.14 (1.05 to 1.23)	0.97 (0.84 to 1.12)	AS03 (0.055)
3.75 μg HA + $AS03_A$ vs 7.5 μg HA + MF59(f)	7.5(sup. file 4)[d]	0.94 (0.36 to 1.52)	0.73 (0.34 to 1.11)	AS03 (0.545)	1.33 (1.08 to 1.62)	1.20 (0.99 to 1.46)	AS03 (0.492)
	15 (Figs. 2, 3)	0.56 (0.33 to 0.80)	0.24 (−0.06 to 0.53)	AS03 (0.088)	1.14 (1.05 to 1.23)	1.13 (1.01 to 1.28)	AS03 (0.963)
2 dose							
3.75 μg HA + $AS03_A$ vs 3.75 μg HA + MF59(h)	7.5(sup. file 5)[d]	1.18 (0.72 to 1.65)	0.38 (0.02 to 0.73)	**AS03 (0.006)**	1.31 (0.99 to 1.73)	1.11 (0.997 to 1.25)	AS03 (0.302)
	15(sup. file 3)[d]	0.82 (0.60 to 1.05)	−0.09 (−0.32 to 0.15)	**AS03 (<0.001)**	1.07 (0.98 to 1.17)	1.03 (0.93 to 1.14)	AS03 (0.527)
3.75 μg HA + $AS03_A$ vs 7.5 μg HA + MF59(f)	7.5(sup. file 5)[d]	1.18 (0.72 to 1.65)	0.80 (0.44 to 1.16)	AS03 (0.196)	1.31 (0.99 to 1.73)	1.22 (1.10 to 1.35)	AS03 (0.661)
	15(sup. file 3)[d]	0.82 (0.60 to 1.05)	0.47 (0.19 to 0.75)	AS03 (0.056)	1.07 (0.98 to 1.17)	1.13 (1.05 to 1.21)	MF59 (0.402)
Paediatric							
1 dose							
1.875 μg HA + $AS03_B$ vs 3.75 μg HA + MF59(h)	15(Figs. 2, 3)	0.29 (−0.90 to 1.47)	1.00 (0.69 to 1.31)	MF59 (0.253)	1.13 (0.89 to 1.43)	1.51 (1.37 to 1.67)	**MF59 (0.026)**
1.875 μg HA + $AS03_B$ vs 7.5 μg HA + MF59(f)	15(Figs. 2, 3)	0.29 (−0.90 to 1.47)	1.10 (0.63 to 1.57)	MF59 (0.212)	1.13 (0.89 to 1.43)	1.46 (1.19 to 1.79)	MF59 (0.103)
3.75 μg HA + $AS03_A$ vs 3.75 μg HA + MF59(h)	15(Figs. 2, 3)	0.64 (0.03 to 1.25)	1.00 (0.69 to 1.31)	MF59 (0.305)	1.15 (0.99 to 1.33)	1.51 (1.37 to 1.67)	**MF59 (0.002)**
3.75 μg HA + $AS03_A$ vs 7.5 μg HA + MF59(f)	15(Figs. 2, 3)	0.64 (0.03 to 1.25)	1.10 (0.63 to 1.57)	MF59 (0.245)	1.15 (0.99 to 1.33)	1.46 (1.19 to 1.79)	MF59 (0.057)
2 dose							
1.875 μg HA + $AS03_B$ vs 3.75 μg HA + MF59(h)	7.5(sup. file 5)[d]	1.95 (1.64 to 2.26)	1.54 (1.24 to 1.84)	AS03 (0.062)	1.21 (1.12 to 1.31)	1.35 (1.19 to 1.52)	MF59 (0.151)
	15(sup. file 3)[d]	1.05 (0.82 to 1.28)	1.45 (1.24 to 1.66)	**MF59 (0.011)**	1.01 (0.95 to 1.08)	1.18 (1.10 to 1.27)	**MF59 (0.002)**
1.875 μg HA + $AS03_B$ vs 7.5 μg HA + MF59(f)	15(sup. file 3)[d]	1.05 (0.82 to 1.28)	1.44 (1.07 to 1.81)	MF59 (0.079)	1.01 (0.95 to 1.08)	1.12 (1.05 to 1.20)	**MF59 (0.024)**

[a] Comparisons with p-value < 0.05 are shown in bold. Pooled GMTR and SCR data for adjuvanted vaccines is shown in Supplementary File 12.
[b] Comparisons of the pooled estimates of mean difference of GMTR for AS03-adjuvanted vaccine versus its control group, with the pooled estimates of mean difference of GMTR for MF59-adjuvanted vaccine versus its control group, showing the adjuvant with better GMTR and corresponding p value.
[c] Comparisons of the pooled estimates of risk ratio of seroconversion rate for AS03-adjuvanted vaccine versus its control group, with the pooled estimates of risk ratio of seroconversion rate for MF59-adjuvanted vaccine versus its control group, showing the adjuvant with better seroconversion rate and corresponding p value.
[d] Forest plots showing seroconversion data not shown.

study in each arm (Fig. 2, Supplementary Files 3–5; MD = 1.05, 95% CI 0.82 to 1.28, p < 0.001 to 1.95, 95%CI 1.64 to 2.26, p < 0.001), $AS03_A$ with 3.75 μg HA in the only comparison (MD = 0.64, 95%CI 0.03 to 1.25, p = 0.039), MF59(h) with 3.75 μg HA in all four comparisons (MD = 1.00, 95%CI 0.69 to 1.31, p < 0.001 to 1.54, 95%CI 1.24 to 1.84, p < 0.001), and MF59(f) with 7.5 μg HA in both comparisons (MD = 1.10, 95%CI 0.63 to 1.57, p < 0.001 to 1.44, 95%CI 1.07 to 1.81, p < 0.001).

Fig. 3 shows seroconversion rates of single dose adjuvanted vaccines versus 15 μg HA unadjuvanted vaccine (commonest regimen in studies; forest plots of the other three seroconversion rate comparisons (two dose versus 15 μg HA unadjuvanted, and one and

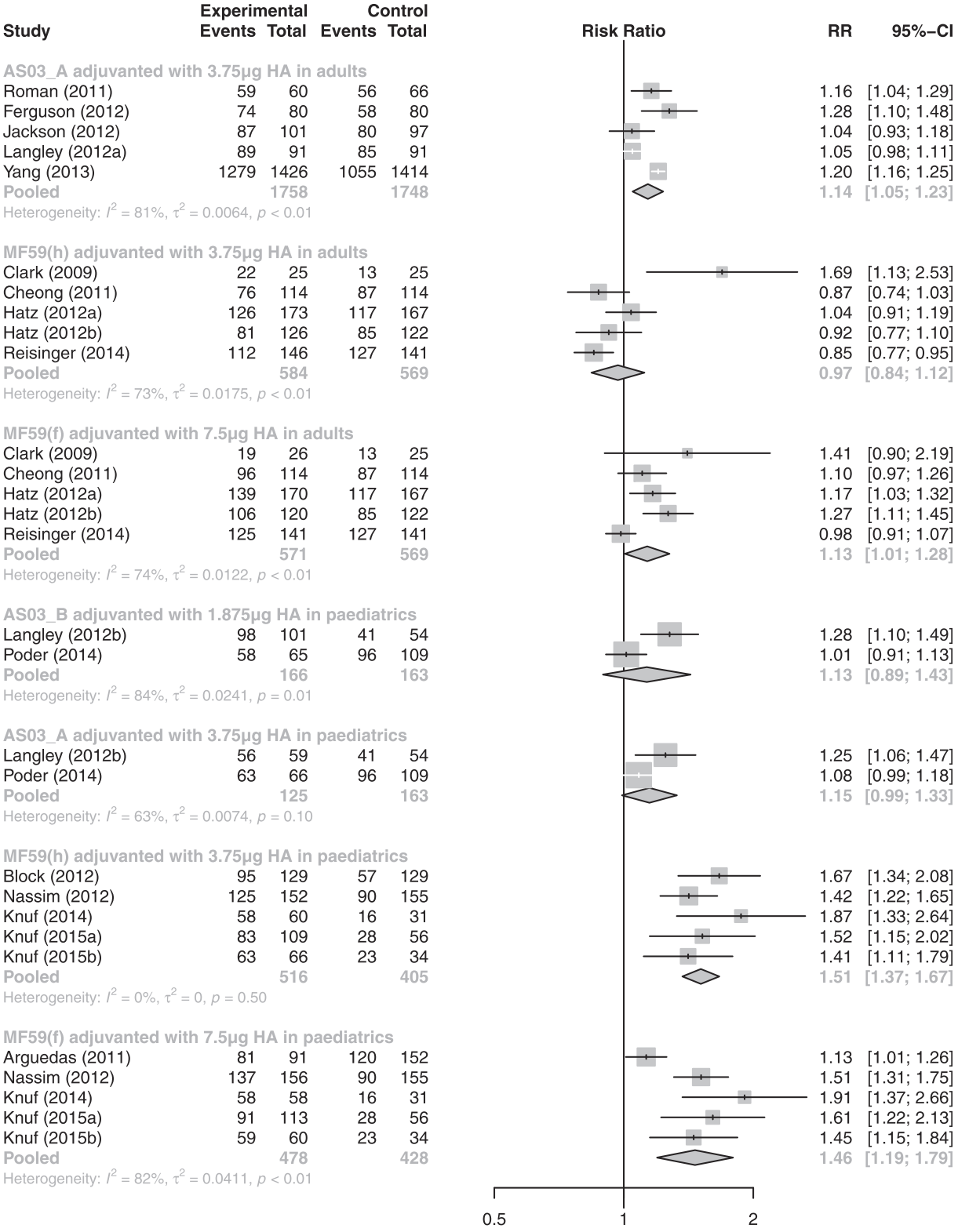

Fig. 3. Forest plot of seroconversion rates (risk ratio) from single dose data of adjuvanted vaccines versus 15 μg HA unadjuvanted vaccine (random-effects model), showing pooled risk ratio and 95%CI. MF59(h) refers to a half dose of MF59 and MF59(f) refers to a full dose of MF59.

two dose versus 7.5 μg HA unadjuvanted) are not shown). In adults, only $AS03_A$ (RR = 1.14, 95%CI 1.05 to 1.23, p = 0.002), and MF59(f) (RR = 1.13, 95%CI 1.01 to 1.28, p = 0.039) induced higher seroconversion rates compared to 15 μg HA unadjuvanted vaccine. In paediatrics, only MF59(h) (RR = 1.51, 95%CI 1.37 to 1.67, p < 0.001) and MF59(f) (RR = 1.46, 95%CI 1.19 to 1.79, p < 0.001) adjuvanted vaccines induced higher seroconversion rates compared to 15 μg HA unadjuvanted vaccine.

Table 1 also presents the test for subgroup differences comparing AS03 and MF59. This test compares the pooled mean difference of GMTR for AS03-adjuvanted vaccine versus its control group, with the pooled mean difference of GMTR for MF59-adjuvanted vaccine versus its control group. The table shows which adjuvant (if any) had the better GMTR mean difference (and thus by indirect comparison, better GMTR). Seroconversion rate (SCR) indirect comparisons of AS03 versus MF59 are presented similarly.

In adults, for log-transformed HAI GMTR, there was strong evidence of AS03 having higher GMTRs than MF59(h) with 3.75 μg HA in three of four indirect comparisons (p < 0.001 to 0.006), with weak evidence in the fourth (p = 0.055). There was weak evidence of AS03 having higher GMTRs than MF59(f) with 7.5 μg HA in two of four comparisons (p = 0.056, 0.088). In paediatrics, for one-dose regimens, there was no evidence of difference between adjuvants in all four comparisons. Two-dose data was mixed, with weak evidence of AS03 having higher GMTRs than MF59 in one comparison (p = 0.062), but weak-to-moderate evidence of MF59 having higher GMTRs than AS03 in the other two (p = 0.011, 0.079).

Between-adjuvant indirect comparisons of seroconversion rate in adults (Table 1) showed weak evidence of higher seroconversion with AS03 than MF59 in one of eight comparisons (p = 0.055). In paediatric studies, there was strong evidence higher seroconversion with MF59 than AS03 in two of seven comparisons (p = 0.002 for both), with evidence in another two (p = 0.024, 0.026), and weak evidence in one (p = 0.057).

Table 2 shows the pooled GMTR as well as seroconversion rate of the AS03 and MF59 control groups (many studies used the same control group for different doses of AS03 and MF59). Unlike in adults, where there was no significant difference in all but one of the pooled MF59 control group GMTRs and seroconversion rates versus their corresponding AS03 control groups, in paediatrics, most of the MF59 control group pooled GMTRs and seroconversion rates were lower than the AS03 control groups.

Also, the median of the mean ages in each study was lower in MF59 (4.6 years) than AS03 (9.15 years) studies in the single dose versus 15 μg HA comparator paediatric cohort, in which meta-regression (AS03 and MF59 studies combined) showed a positive association between mean age and GMTR (p = 0.001) as well as seroconversion rates (p < 0.001) in the comparator group.

Table 3 shows adverse effect risk ratios for adjuvanted versus unadjuvanted vaccines for injection site pain, fatigue, and fever. In adults, all adjuvanted vaccines caused a significantly higher risk of injection site pain than their unadjuvanted counterparts (RR = 1.70, 95%CI 1.25 to 2.31, p = 0.001 to 2.90, 95%CI 2.37 to 3.54, p < 0.001). AS03-adjuvanted vaccines caused higher risk of fatigue than unadjuvanted vaccines (RR = 1.21, 95%CI 1.03 to 1.41, p = 0.019 to 1.57, 95%CI 1.18 to 2.08, p = 0.002), but MF59-adjuvanted vaccines did not.

Table 3 also shows an indirect comparison of adverse effect risk ratios of AS03- versus MF59-adjuvanted vaccines using a test of subgroup differences. In adults, there was strong evidence of AS03 causing higher risk of injection site pain in one of four comparisons (p = 0.004), and weak evidence in another (p = 0.062), with no difference in fatigue. There was no difference in fever rates between any vaccine.

In paediatric studies (Table 3), compared to 15 μg HA unadjuvanted vaccines, MF59(f) (RR = 1.50, 95%CI 1.22 to 1.84, p < 0.001), but not MF59(h) (RR = 1.14, 95%CI 0.87 to 1.49, p = 0.360) caused higher rates of injection site pain, and there was weak evidence of MF59(h) causing higher rates of fatigue (RR = 1.29, 95%CI 0.98 to 1.71, p = 0.069). There were no other differences in adverse effects.

Other paediatric adverse effects data, including comparison of adjuvants, was difficult to determine because two of ten [39,40] paediatric studies expressed adverse effects as percentages in different age subgroups, without stating the number of subjects in each subgroup. In addition, the age ranges of the subgroups chosen for adverse effects (6–35 months [39]; 0.5–6 years [40]), were different to the age ranges chosen for determination of immunogenicity (6–11 months [39]; 0.5–3 years [40]). A third study [44] only reported adverse effects as "any", "local" or "systemic". All three paediatric studies suggested increased local adverse effects with adjuvanted vaccines in most age groups, with the two AS03 studies showing increased rates of fever ≥39 °C. This did not leave enough paediatric AS03 studies with 7.5 or 15 μg HA unadjuvanted vaccine control groups, or MF59 studies with the 7.5 μg HA unadjuvanted vaccine control group to conduct a pooled analysis. There were no reports of narcolepsy in any age group in these short-term studies.

Eight of 22 comparisons (with two or more studies; Fig. 2, Supplementary Files 3–5) for HAI GMTR versus unadjuvanted control groups showed substantial heterogeneity (I^2 = 61–94%).

Table 2
Pooled GMTRs and seroconversion rates of unadjuvanted vaccine control groups in studies of AS03 versus MF59-adjuvanted vaccines (test of subgroup differences).[a]

Control group unadjuvanted vaccine HA dose (μg)	Pooled AS03 control group GMTR	Pooled MF59 control group GMTR	P value for pooled AS03 versus MF59 control group GMTR	Pooled AS03 control group seroconversion rate	Pooled MF59 control group seroconversion rate	P value for pooled AS03 versus MF59 control group seroconversion rate
Adult 7.5 μg 1 dose	2.55 (2.03 to 3.06)	2.74 (2.47 to 3.02)	0.510	66% (48 to 81%)	70% (63 to 77%)	0.668
Adult 7.5 μg 2 dose	2.69 (2.28 to 3.11)	2.85 (2.46 to 3.24)	0.581	73% (51 to 88%)	74% (67 to 80%)	0.927
Adult 15 μg 1 dose	3.06 (2.62 to 3.50)	2.77 (2.25 to 3.29)	0.406	82% (74 to 87%)	74% (63 to 83%)	0.216
Adult 15 μg 2 dose	**3.50 (3.22 to 3.77)**	**3.00 (2.66 to 3.33)**	**0.024**	91% (78 to 97%)	84% (78 to 88%)	0.227
Paediatrics 7.5 μg 2 dose	2.79 (2.62 to 2.96)	2.60 (2.23 to 2.96)	0.352	82% (74 to 88%)	73% (63 to 81%)	0.097
Paediatrics 15 μg 1 dose	**2.94 (2.34 to 3.53)**	**(studies with MF59(h)) 1.63 (1.27 to 2.00)**	**<0.001**	**83% (68 to 92%)**	**(studies with MF59(h)) 53% (45 to 61%)**	**0.001**
		(studies with MF59(f)) 2.06 (1.32 to 2.80)	0.072		**(studies with MF59(f)) 62% (49 to 74%)**	**0.033**
Paediatrics 15 μg 2 dose	**3.56 (3.18 to 3.94)**	**(studies with MF59(h)) 2.84 (2.68 to 3.00)**	**0.001**	**97% (93 to 98%)**	**(studies with MF59(h)) 82% (75 to 87%)**	**<0.001**
		(studies with MF59(f)) 3.00 (2.71 to 3.29)	**0.022**		**(studies with MF59(f)) 85% (80 to 89%)**	**<0.001**

[a] Comparisons with p-value < 0.05 are shown in bold.

Table 3
Comparisons of rates of injection site pain, fatigue and fever between adjuvanted and unadjuvanted vaccines; and between adjuvants (test of subgroup differences)[a].

Adjuvanted vaccine	Control group unadjuvanted vaccine HA dose (μg)	Risk ratio for injection site pain versus unadjuvanted vaccine (95% CI), p-value	P-value for difference in injection site pain between AS03 and MF59	Risk ratio for fatigue versus unadjuvanted vaccine (95% CI), p-value	P-value for difference in fatigue between AS03 and MF59	Risk ratio for fever versus unadjuvanted vaccine (95% CI), p-value	P-value for difference in fever between AS03 and MF59
Adult							
$AS03_A$ + 3.75 μg HA	7.5	**2.90 (2.37 to 3.54), <0.001**	Reference Group	**1.57 (1.18 to 2.08), 0.002**	Reference Group	1.20 (0.49 to 2.95), 0.684	Reference Group
MF59(h) + 3.75 μg HA	7.5	**1.70 (1.25 to 2.31), 0.001**	**0.004 (AS03 worse)**	1.26 (0.29 to 5.55), 0.756	0.778	0.33 (0.01 to 7.80), 0.495	0.443
MF59(f) + 7.5 μg HA	7.5	**2.07 (1.55 to 2.77), <0.001**	0.062 (AS03 worse)	1.81 (0.14 to 22.80), 0.648	0.914	1.31 (0.09 to 19.49), 0.843	0.952
$AS03_A$ + 3.75 μg HA	15	**2.32 (1.60 to 3.36), <0.001**	Reference Group	**1.21 (1.03 to 1.41), 0.019**	Reference Group	1.37 (0.79 to 2.39), 0.262	Reference Group
MF59(h) + 3.75 μg HA	15	**1.75 (1.43 to 2.14), <0.001**	0.188	0.93 (0.71 to 1.24), 0.634	0.118	1.09 (0.32 to 3.72), 0.897	0.733
MF59(f) + 7.5 μg HA	15	**2.22 (1.81 to 2.71), <0.001**	0.832	1.12 (0.72 to 1.75), 0.625	0.751	0.73 (0.20 to 2.76), 0.648	0.393
Paediatric							
MF59(h) + 3.75 μg HA	15	1.14 (0.87 to 1.49), 0.360	Not Applicable	1.29 (0.98 to 1.71), 0.069	Not Applicable	1.14 (0.61 to 2.13), 0.674	Not Applicable
MF59(f) + 7.5 μg HA	15	**1.50 (1.22 to 1.84), <0.001**	(No AS03 reference)	1.09 (0.80 to 1.49), 0.587	(No AS03 reference)	1.43 (0.69 to 3.00), 0.338	(No AS03 reference)

[a] Comparisons with p-value < 0.05 are shown in bold.

Supplementary File 6 shows risk of bias within studies, suggesting higher risk of bias in all MF59, and paediatric AS03 studies. There were not enough studies to assess publication bias.

All following subgroup analyses were conducted on adult single dose versus 15 μg HA comparator group studies, with insufficient studies to do this with paediatric studies. These showed similar findings to the whole cohort, when industry-only funded studies (Supplementary File 8), non-egg-derived vaccines (Supplementary File 9), studies with a mean age below the weighted overall mean of 37.9 years (Supplementary File 10), or with percentage female lower than the weighted overall mean of 58.5% (Supplementary File 11) were excluded. In adults, meta-regressions of adjuvant (AS03 and MF59) GMTR mean difference versus age ($p = 0.235$) and proportion female ($p = 0.538$) were not significant. A subgroup analysis based on study location could not be performed as all but one AS03 study was North American, while only one MF59 study was located there, nor split versus whole virion vaccine, as most MF59 studies did not report this.

4. Discussion

To our knowledge, this is the first meta-analysis comparing the immunogenicity and short-term adverse effects of AS03- and MF59-adjuvanted A(H1N1)pdm09 influenza vaccines. In adults, compared to unadjuvanted vaccines, $AS03_A$ with 3.75 μg HA achieved higher HAI GMTR, as did MF59(f) with 7.5 μg HA, but MF59(h) with 3.75 μg HA showed mixed results. Seroconversion data, although less conclusive, supported these findings. In between-adjuvant HAI GMTR comparisons in adults, $AS03_A$ with 3.75 μg HA achieved higher GMTR than MF59(h) with 3.75 μg HA, and trended towards higher GMTR versus MF59(f) with 7.5 μg HA. Seroconversion rate data trended towards higher levels with AS03 than MF59. This was not statistically significant, potentially due to high unadjuvanted-vaccine control group seroconversion rates. In adults, there was higher risk of injection site pain with all adjuvanted vaccines, and of fatigue with AS03 compared to unadjuvanted vaccines. There was a trend towards higher risk of injection site pain with AS03 versus MF59.

In paediatrics, adjuvanted vaccines achieved higher HAI GMTR than unadjuvanted vaccines. Seroconversion data supported this for MF59, but less so for AS03. There was no evidence of a between-adjuvant HAI GMTR difference in single-dose regimens, and mixed evidence in two-dose regimens, with an overall trend favouring MF59 over AS03. Seroconversion data favoured MF59 more strongly. However, these results must be interpreted cautiously, as they were largely driven by differences in GMTR and seroconversion rates between AS03 and MF59 15 μg HA unadjuvanted control groups, in turn possibly related to the lower age of the MF59 cohort (GMTR and seroconversion rate correlated with age in these paediatric studies). The adverse effects profile for paediatric populations was poorly described in three studies, [39,40,44] and thus could only be determined for MF59 versus 15 μg HA unadjuvanted vaccines, which suggested increased risk of injection site pain with 7.5 μg HA adjuvanted with MF59(f).

Limitations of this meta-analysis include the relative paucity of available studies, lack of vaccine administrator blinding, and inability to determine risk of bias due to inadequate reporting of study design (randomisation and blinding), and outcomes (dropout explanation and subgroup participant numbers). Combining results from studies with varying inclusion criteria, subject groups, antigens/assays from different settings, and different volumes (but same doses) of $AS03_B$-adjuvanted vaccine (paediatric studies only) also presents a potential limitation of our study, as does the fact that our results examine immunogenicity and not prevention of disease outcomes or population level vaccine effectiveness, given the paucity of randomised controlled trials comparing the latter

outcomes for adjuvanted and unadjuvanted vaccines in pandemic influenza prevention. Many studies were solely industry funded, but excluding these from the adult trials analysis produced similar results to the whole cohort. There was substantial variability among studies, as shown by the relatively high between-study heterogeneity, but this is taken into account by the standard random-effects model used [48].

The results in adults for A(H1N1)pdm09 vaccines are similar to the only randomised controlled trial comparing AS03 versus MF59 [9], undertaken with A(H7N9) vaccines. With at least 94 participants in each study arm, using 15 μg HA, AS03-adjuvanted vaccine showed superior GMTs to MF59-adjuvanted vaccine ($p < 0.001$), which was in turn superior to unadjuvanted vaccine. 3.75 μg HA adjuvanted with $AS03_A$ was not significantly different to 15 μg HA adjuvanted with $AS03_A$, emphasising the dose-sparing potential of AS03. There was little difference in overall adverse effects between AS03- and MF59-adjuvanted vaccines, although both were higher than with unadjuvanted vaccines, but with no increase in study dropouts. However, compared to our meta-analysis, unadjuvanted vaccines performed worse in this A(H7N9) study (2% seroprotection rate).

Although not identified in the short-term trials in our meta-analysis, a potential association between receipt of an AS03-adjuvanted vaccine (Pandemrix, GlaxoSmithKline Biologicals S.A) and narcolepsy in paediatric populations was identified in 2010 in Sweden and Finland [15], in up to 1/18,400 recipients [49]. It is unlikely that ascertainment bias from media reporting contributed to this observation [50]. It was not seen post-vaccination in 8 other countries [51], with little (only in <20 years old individuals) [52], or no association with Arepanrix (GlaxoSmithKline Biologicals S.A), a similar AS03-adjuvanted vaccine [51]. It was possibly caused by increased structurally altered viral nucleoprotein in Pandemrix, rather than the adjuvant itself [53,54].

In adults, using AS03 as an adjuvant would allow a quarter of the usual 15 μg HA antigen dose to be used, and half the dose needed with MF59(f) to achieve a similar or greater serological effect, albeit with more adverse effects. Thus, based on this review, use of $AS03_A$ as an adjuvant would allow a greater number of adults to be vaccinated with limited quantity of antigen early in a pandemic. Based on the short-term trials included here, the greater reductions in morbidity and mortality would outweigh the increased, mainly local (apart from fatigue), adverse effect profile of vaccines containing AS03. It would be difficult to substantiate this by further direct randomised controlled comparison trials using A(H1N1)pdm09 vaccines, given that this subtype is now a seasonal influenza strain and included in polyvalent vaccine preparations, but it should be studied in trials of potential or future pandemic influenza vaccine candidates, like A(H7N9) or A(H5N1). In paediatric studies, due to different control group seroconversion rates, and inadequate adverse effect reporting, the benefit of one adjuvant over another cannot be clearly determined, but both achieve higher immune response compared to unadjuvanted vaccines. Further research in children is needed, with careful post-marketing monitoring of adverse events.

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Authorship contributions

DJM designed the study with input from RMT and MIH. MIH performed the literature search, carried out the search strategy and first screening of articles. ACYS performed a secondary screening of articles returned in the search strategy. MIH and ACYS applied the inclusion criteria and extracted the data. MIH performed the meta-analysis. MIH wrote the final report with contributions from RMT, DJM, DED, and ACYS. All authors approved the final manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2019.06.039.

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