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MF59®-adjuvanted influenza vaccine (FLUAD®) in children: Safety and immunogenicity following a second year seasonal vaccination

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ABSTRACT

After priming with two intramuscular doses of MF59®-adjuvanted (Sub/MF59) or split influenza vaccines during the 2006/07 season, 89 healthy children received a third booster dose of the respective vaccine (2007/08 Northern Hemisphere formulation) approximately 1 year later, and were followed up for 6 months post-third injection. Immunogenicity was evaluated on 81 of them by a hemagglutination inhibition (HI) assay before and 3 weeks after vaccination.

The Sub/MF59 influenza vaccine was safe and well tolerated following the booster vaccination. Pre-booster HI antibody titers were consistently higher in the Sub/MF59 group than in the comparator group, confirming significantly longer persistence of antibodies after priming with Sub/MF59 vaccine. Post-booster immune responses were significantly higher in the Sub/MF59 group compared with the split group, especially vs. the influenza B strain, which is epidemiologically relevant in the pediatric population. Altogether, these data further support the potential use of MF59®-adjuvanted influenza vaccine as a safe and highly immunogenic influenza vaccine for young children.

1. Introduction

Although recent epidemiologic observations suggesting that children have the highest attack rates of influenza [1,2], and that very young children are at substantially increased risk for influenza-related hospitalizations [3], seasonal vaccination of children is currently only recommended in six European countries [4]. The limited immunogenicity, efficacy and effectiveness of the currently available trivalent inactivated influenza vaccines (TIVs) in young children may have played a significant role in the slow and skeptical implementation of universal immunization strategy in Europe [5].

Improved immune responses to seasonal influenza vaccines have been shown with the use of MF59® as adjuvant with influenza vaccine [6]. A proof of concept, randomized, observer-blind study, performed in Finland during 2006/07 NH season, showed that unprimed children under 3 years of age displayed higher immune responses to the MF59®-adjuvanted vaccine compared with a conventional non-adjuvanted split vaccine. Higher seroprotection rates vs. the A/H3N2 vaccine strain were already obtained after the first vaccination. In addition, significantly higher percentages of children were seroprotected against the B vaccine strain and statistically significantly longer persistence of hemagglutination inhibition (HI) antibody titers was demonstrated in children receiving the adjuvanted vaccine, compared with the conventional TIV [7].

The aim of this study was to compare in healthy children from the original study, the clinical tolerability, safety and immunogenicity of a third consecutive dose of the MF59-adjuvanted vaccine, compared with the split TIV, administered approximately 1 year after priming.

2. Materials and methods

This observer-blind study was conducted in Finland. Parents of children who were primed with a MF59-adjuvanted (FLUAD®, Novartis Vaccines; Sub/MF59) or a split vaccine (Vaxigrip®, Sanofi Pasteur) in the proof of concept study (Northern Hemisphere [NH] season 2006/07), were invited to allow their children to participate in this extension study, designed to evaluate responses to a third dose of the respective vaccines, formulated for the NH 2007/08 campaign. The subjects were children from 16 to <48 months of age, in good health as determined by medical history, physical examination and clinical judgment of the investigator, whose parents had given written informed consent prior to study entry. The study was performed according to the ethical guidelines of the 1975 Declaration of Helsinki, Good Clinical Practice, and local laws. Before

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Table 1
Overview of children with solicited reactions, adverse events (AEs) and serious adverse events (SAEs), by vaccine and age group.

Type of reaction	Overall		<3 years		≥3 years	
	Sub/MF59 ($N=43$)[a]	Split ($N=46$)[a]	Sub/MF59 ($N=25$)[a]	Split ($N=23$)[a]	Sub/MF59 ($N=18$)[a]	Split ($N=23$)[a]
Any	34 (79%)	27 (59%)	18 (72%)	13 (57%)	16 (89%)	14 (61%)
Local	30 (70%)	21 (46%)	15 (60%)	10 (43%)	15 (83%)	11 (48%)
Systemic	18 (42%)	17 (37%)	11 (44%)	9 (39%)	7 (39%)	8 (35%)
Other[b]	9 (21%)	4 (9%)	5 (20%)	3 (13%)	4 (22%)	1 (4%)
Any AE	30 (70%)	35 (76%)	17 (68%)	16 (70%)	13 (72%)	19 (83%)
At least possibly or probably related AEs[c]	10 (23%)	2 (4%)	8 (32%)	1 (4%)	2 (11%)	1 (4%)
Fever	1 (2%)	0	1 (4%)	0	0	0
Cough	2 (5%)	0	1 (4%)	0	1 (6%)	0
Injection site pruritus	1 (2%)	1 (2%)	1 (4%)	0	0	1 (4%)
Induration	1 (2%)	0	1 (4%)	0	0	0
Irritability	1 (2%)	1 (2%)	1 (4%)	1 (4%)	0	0
Nasopharingitis	1 (2%)	0	1 (4%)	0	0	0
Respiratory tract infection	3 (7%)	0	3 (12%)	0	0	0
Rhinitis	2 (5%)	1 (2%)	1 (4%)	1 (4%)	1 (6%)	0
SAEs	0	0	0	0	0	0

[a] Vaccine group.
[b] Other reactions recorded were: body temperature, analgesic/antipyretic use.
[c] More than one adverse event could have been reported in a single subject.

the trial started, the study protocol and informed consent form were approved by the ethical committee of the Pirkanmaa Hospital District, Finland.

After obtaining informed consent from parents/legal guardians, children received a single intramuscular (IM) dose of 0.25 mL (children < 3 years) or 0.5 mL (≥3 years of age) of the corresponding vaccine. Each 0.5 mL dose contained 15 μg of hemagglutinin of each of the three vaccine strains [A/Solomon Islands/3/2006 (H1N1)-like, A/Wisconsin/67/2005 (H3N2)-like virus, B/Malaysia/2506/2004-like virus].

For the 2007/08 NH season, the only change in the vaccine formulation form the previous campaign was the A/H1N1-like strain (A/Solomon Islands/3/2006). The third dose of the vaccines was like a "booster dose" for the two strains, A/H3N2 and B, which did not change across the two consecutive seasons.

2.1. Assessment of vaccine clinical tolerability and safety

Parents were instructed to record solicited local and systemic reactions on a diary card immediately after vaccination and for the following 7 days. Solicited local reactions were ecchymosis, erythema, induration, swelling, and tenderness (children < 3 years) or injection site pain (children ≥ 3 years). Solicited systemic reactions were sleepiness, diarrhea, vomiting, irritability, change in eating habits, shivering, unusual crying, fever for children <3 years of age; chills, malaise, myalgia, arthralgia, headache, fatigue, fever for children ≥3 years of age. Fever (defined as an axillary temperature ≥ 38 °C), usage of analgesic/antipyretic medication and any other adverse event (AE) were also recorded on the diary cards during the 7 days following each vaccination.

All AEs including serious adverse events (SAEs) and those necessitating a physician's consultation, or leading to premature study discontinuation were collected throughout the entire trial (6 months).

2.2. Assessment of vaccine immunogenicity

Blood samples (5 mL) were obtained immediately prior to (day 1) and 3 weeks (day 22) after vaccination. Sera were stored at −20 °C until laboratory determination of HI antibody titers against the vaccine strains, as described previously [8].

The study parameters considered for humoral immune response for each strain were: the Geometric Mean Titer (GMT) of HI antibodies with 95% confidence interval (CI); the Geometric Mean Ratio (GMR, ratio of post- to pre-vaccination titers) with 95% CI; the seroprotection rate, defined as the percentage of children achieving an HI titer ≥ 40; and the seroconversion rate, defined as the percentage of children achieving either seroconversion (i.e., a post-vaccination HI titer ≥ 40 from a pre-vaccination HI titer < 10) or significant increase in HI titers (i.e., at least a 4-fold rise in post-vaccination HI antibody titer from a pre-vaccination HI titer ≥ 10).

2.3. Statistical analysis

Least squares GMTs and associated 95% CIs for each strain and each vaccine group were determined using analysis of variance (ANOVA) with one factor for vaccine group. Vaccine group differences (post-vaccination ratio of GMTs between vaccines) were assessed using the same approach. All analyses were performed on the logarithmically (base 10) transformed titers.

The chi-square or Fisher's Exact test were performed to analyze differences between proportions of subjects. The 95% CIs for the difference between the two vaccines groups on percentages of subjects achieving seroprotection and/or seroconversion were calculated using the Clopper–Pearson method.

Data were analyzed using the SAS System (Cary, NC, USA). A P-value of <0.05 was considered to indicate statistical significance.

3. Results

Overall, 89 children were enrolled, vaccinated and analyzed for safety.

Demographic and other baseline characteristics were well matched between Sub/MF59 ($N=43$) and split ($N=46$) groups. The mean age and the percentage of females enrolled were similar between the two vaccine groups (33.5 months vs. 33.9 months of age and 44% vs. 46% of females, respectively, in the Sub/MF59 and split group). All subjects were Caucasians.

Overall, 81 subjects were included in the per protocol population (children who received the vaccine dose correctly and provided valuable pre- and post-vaccination serum samples) for the analysis

Table 2
Geometric Mean Ratios, seroprotection and seroconversion rates, by vaccine and age group—per protocol population[a].

	Number of subjects (%) and [95% CI]					
	A/H1N1[b]		A/H3N2[b]		B[b]	
	Sub/MF59[c] N = 41[d]	Split[c] N = 40[d]	Sub/MF59[c] N = 41[d]	Split[c] N = 40[d]	Sub/MF59[c] N = 41[d]	Split[c] N = 40[d]
Overall						
SP[e] (day 1)	6 (15%) [6, 29]	2 (5%) [1, 17]	36 (88%)* [74, 96]	16 (40%) [25, 57]	4 (10%) [3–23]	0 (0%) [0, 9]
SP[e] (day 22)	41 (100%) [91, 100]	40 (100%) [91, 100]	41 (100%) [91, 100]	40 (100%) [91, 100]	41 (100%)* [91, 100]	27 (68%) [51, 81]
GMR (day 22/day1)	91 [59, 140]	52 [35, 79]	17 [12, 24]	12 [8.1, 18]	18* [14, 24]	8.14 [5.7, 12]
Serocon. rate[f] (day 22)	39 (95%) [83, 99]	38 (95%) [83, 99]	40 (98%) [87, 100]	34 (85%) [70, 94]	40 (98%)* [87, 100]	27 (68%) [51, 81]
	Number of subjects (%) and [95% CI]					
	A/H1N1[b]		A/H3N2[b]		B[b]	
	Sub/MF59[c] N = 23[d]	Split[c] N = 20[d]	Sub/MF59[c] N = 23[d]	Split[c] N = 20[d]	Sub/MF59[c] N = 23[d]	Split[c] N = 20[d]
<3 year						
SP[e] (day 1)	2 (9%) [1, 28]	1 (5%) [0, 25]	22 (96%)* [78, 100]	10 (50%) [27, 73]	1 (4%) [0, 22]	0 (0%) [0, 17]
SP[e] (day 22)	23 (100%) [85, 100]	20 (100%) [83, 100]	23 (100%) [85, 100]	20 (100%) [83, 100]	23 (100%)* [85, 100]	9 (45%) [23, 68]
GMR (day 22/day 1)	122** [77–94]	43 [25–5]	17*** [11–4]	7.86 [5.01, 12]	19* [14, 27]	4.14 [2.7, 6.35]
Serocon. rate[f] (day 22)	23 (100%) [85, 100]	19 (95%) [75, 100]	23 (100%) [85, 100]	17 (85%) [62, 97]	22 (96%)* [78, 100]	9 (45%) [23, 68]
	Number of subjects (%) and [95% CI]					
	A/H1N1[b]		A/H3N2[b]		B[b]	
	Sub/MF59[c] N = 18[d]	Split[c] N = 20[d]	Sub/MF59[c] N = 18[d]	Split[c] N = 20[d]	Sub/MF59[c] N = 18[d]	Split[c] N = 20[d]
≥3 years						
SP[e] (day 1)	4 (22%) [6, 48]	1 (5%) [0, 25]	14 (78%)* [52, 94]	6 (30%) [12, 54]	3 (17%) [4, 41]	0 (0%) [0, 17]
SP[e] (day 22)	18 (100%) [81, 100]	20 (100%) [83, 100]	18 (100%) [81, 100]	20 (100%) [83, 100]	18 (100%) [81, 100]	18 (90%) [68, 99]
GMR (day 22/day 1)	63 [28, 141]	64 [34, 121]	18 [9.6, 34]	19 [9.7, 36]	17 [11, 26]	16 [11, 24]
Serocon. rate[f] (day 22)	16 (89%) [65, 99]	19 (95%) [75, 100]	17 (94%) [73, 100]	17 (85%) [62, 97]	18 (100%) [81, 100]	18 (90%) [68, 99]

[a] In the per protocol population were included all children who received the correct vaccine dose and provided valuable pre- and post-vaccination serum samples.
[b] Strain.
[c] Vaccine group.
[d] Population.
[e] Seroprotection: HI titers ≥ 40.
[f] Seroconversion rate: seroconversion and/or significant increase; seroconversion—negative pre-vaccination serum (i.e., HI titer < 10) and post-vaccination HI titer ≥40 and significant increase—at least a 4-fold increase in HI titers in subjects who were positive pre-vaccination (i.e., HI titer ≥ 10).
* $P < 0.001$.
** $P < 0.01$.
*** $P < 0.05$ vs. split group.

of immunogenicity (41 in the Sub/MF59 group and 40 in the split group).

3.1. Clinical tolerability and safety

Overall, solicited reactions were more frequently recorded in the Sub/MF59 group, compared with the split group, in particular for solicited local reactions (Table 1). The most commonly reported local reactions in children <36 months were erythema and tenderness (32% Sub/MF59 and 30% split, for both reactions), while in those aged 36 months and above were pain at the injection site (67% and 26%, respectively), erythema and induration (28% Sub/MF59 and 22% split, for both reactions). The most frequent solicited systemic reactions in young children were irritability (36% Sub/MF59 and 22% split) and diarrhea (24% Sub/MF59 and 13% split), while in the older age group (≥36 months) the most common systemic reactions were fatigue (28% Sub/MF59 and 35% split), chills and headache (22% vs. 17% and 17% vs. 9%, respectively, in the Sub/MF59 and split groups). Fever was reported by 16% of younger children in the Sub/MF59 and 9% of those in the split group; in children aged 3 years and above fever was recorded in 2 subjects in each vaccine group.

The only statistically significant difference between groups was found for injection site pain in the older age cohort (≥3 years; Sub/MF59 vs. split group, $P < 0.01$).

In both vaccine groups, solicited reactions were usually mild or moderate in intensity and of short duration. Moreover, a higher incidence of reactions in children aged ≥3 years, compared with younger children, was evident for both vaccine groups (Table 1).

Other adverse events, regardless of vaccine relatedness, collected from day 1 to study termination (day 181), were reported by 70% (30/43) and 76% (35/46) of subjects in the Sub/MF59 and split groups, respectively (Table 1). The most frequent AEs were otitis media (30% in both groups), cough (21% Sub/MF59; 17% split), and upper respiratory tract infection (19% Sub/MF59; 17% split); none of these AEs was classified as severe.

Unsolicited AEs considered to be possibly or probably related to vaccination occurred within the first 21 days after vaccine injection, were more frequent in the Sub/MF59 group, and were mostly local or systemic reactions ongoing after the 7-day observational period, known side effects of influenza vaccinations, or common illnesses expected in this population (Table 1).

There were no SAEs or deaths during the study.

3.2. Immunogenicity results

Children primed during the previous influenza season with Sub/MF59 vaccine showed higher pre-vaccination HI antibody titers and higher seroprotection rates, compared with those vacci-

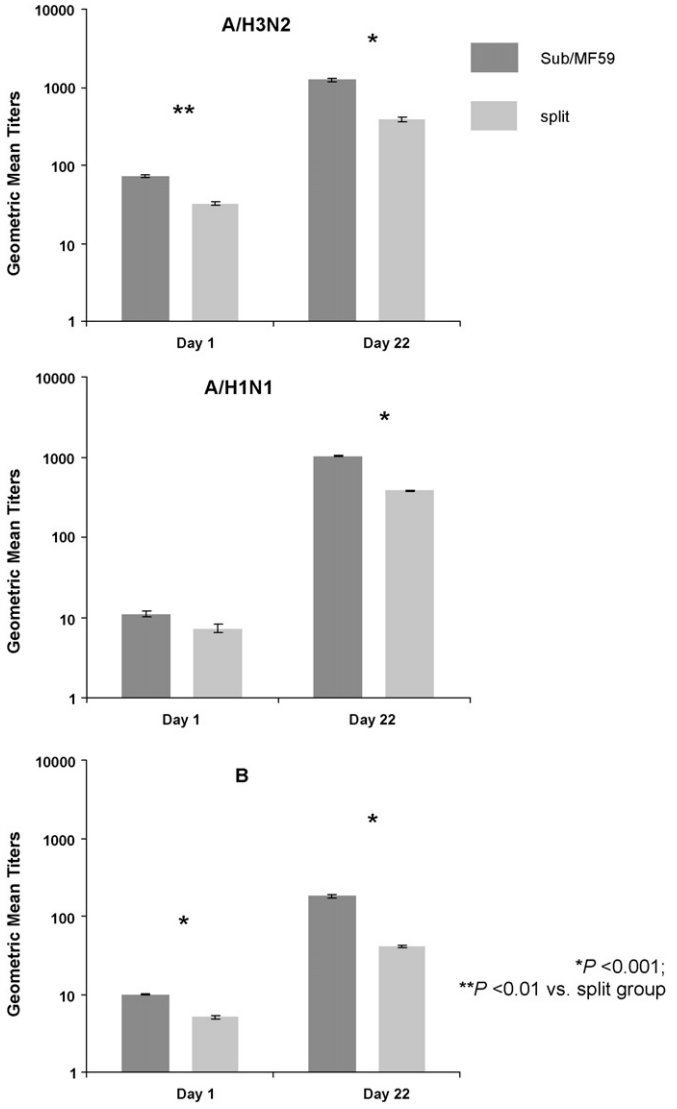

Fig. 1. Geometric Mean Titers in the overall population, by strain and vaccine group—per protocol population.

nated with the conventional split TIV (Fig. 1), the difference was statistically significant against A/H3N2 antigen ($P < 0.01$ vs. split group).

Three weeks after the booster vaccination, immune responses in the Sub/MF59 group were significantly higher than those observed in the comparator group ($P < 0.001$, Sub/MF59 vs. split group; Fig. 1). In the adjuvanted vaccine group, HI antibody titers were generally higher in children <3 years of age than in the older ones, but within the comparator group higher immune responses were induced in children aged ≥3 years.

Seroprotection rates of 100% were achieved by both vaccines against both influenza A strains; however, only Sub/MF59 resulted in 100% seroprotection against influenza B, compared with 68% seroprotection with the split vaccine ($P < 0.001$). The same trend was observed for seroconversion rates (Table 2).

Seroprotection and seroconversion rates in children aged <3 years were generally similar to those in the older age group; lower rates were only seen in the younger split vaccine group for the B strain ($P < 0.001$, Sub/MF59 vs. split group for both seroprotection and seroconversion rates).

4. Discussion

The benefits of influenza vaccination in frail populations, such as the elderly and adults with underlying chronic conditions, have been established in previous studies leading to recommendations for seasonal prophylaxis, whereas only limited data are available on the benefits in younger age groups [4].

Epidemiological studies have consistently shown high rates of hospitalizations, as well as emergency room and out-patient visits attributable to influenza disease among young children [9,10]. Based on such evidences, the US Advisory Committee on Immunization Practices (ACIP) started recommending seasonal influenza vaccination in very young children in 2004, expanding recommendations to include all children aged 6–59 months in June 2006, and further extending to all children and adolescents aged 5–18 years in July 2008 [11,12].

The current European situation is quite different. Finland has introduced routine immunization of 6–35 month-old children as part of national immunization schedule, reaching 40% coverage in 2007 (unpublished Finnish National Public Health Institute [KTL] survey). Estonia, Latvia, Slovakia and Slovenia recommend routine immunization of children (with the age limits varying from 6 months to 5 years of age) and Austria recommends influenza vaccination for all age groups [4].

An inherent assumption of expanded vaccination recommendations is that currently available influenza vaccines are efficacious in preventing clinical influenza disease. Although studies have documented immune responses following two doses of TIVs, immunogenicity data, especially vs. the B influenza strain, are generally suboptimal and consequently vaccine efficacy results remain inconclusive, with a field efficacy and effectiveness estimated to be limited in young children [5].

Adjuvants have been developed to improve the performance of vaccines and MF59®, an oil-in-water emulsion containing the naturally occurring squalene oil, has been approved for human use in 1997 as an influenza subunit vaccine adjuvant for the elderly [13]. MF59-adjuvanted influenza vaccine has been shown to provide higher and broader immunogenicity than non-adjuvanted comparators, with a good tolerability and safety profile in vulnerable populations, such as the elderly and adults with underlying chronic conditions, especially those with low pre-vaccination antibody titers [14–17].

In the original proof of concept observer-blind, randomized, controlled study, performed in 269 unprimed children aged 6–<36 months, the MF59-adjuvanted vaccine was able to induce significantly higher immune responses compared with those of a non-adjuvanted split vaccine ($P < 0.001$ for all three vaccine strains), even in the youngest children (<24 months of age) and vs. the B influenza strain, which epidemiologically is particularly relevant in the pediatric community. Moreover the difference between the two vaccines was sustained for 6 months over an influenza epidemic season [7]. Sub/MF59 vaccine also induced significantly higher immune responses against mismatched A influenza strains, when compared with conventional TIV ($P < 0.001$ against A/H3N2 strain recommended for inclusion in the 2005/06 NH formulation and against A/H1N1 included in the 2007/08 NH vaccine formulation). Both vaccines were generally well tolerated and safe, with slightly more mild and transient solicited reactions, especially injection site swelling ($P = 0.033$ vs. split), in the Sub/MF59 group.

Following on from that clinical trial, the current study is the first investigation on the safety and immunogenicity of a third consecutive dose of a MF59-adjuvanted influenza vaccine or control split vaccine, administered as a booster approximately 1 year after two priming doses, in healthy children aged 16–<48 months. As in the proof of concept study, both vaccines were generally well tolerated. There were slightly more mild and transient solicited local

and systemic reactions reported in the Sub/MF59 group, but the only statistically significant difference was for injection site pain for children ≥3 years of age ($P < 0.01$, Sub/MF59 vs. split group). Moreover, irrespective of the vaccine group, a higher number of solicited reactions was reported in children aged 3 years and above, compared with younger children (<3 years).

Unsolicited AEs were consistently more common in the split vaccine group, while at least possibly or probably related AEs were more frequent in the Sub/MF59 group, and were generally solicited local or systemic reactions ongoing after the 7-day observation period, known side effects of influenza vaccinations or common illnesses expected in the pediatric population. No serious adverse effects were reported during the study.

The children vaccinated during the previous influenza season with Sub/MF59, showed higher pre-vaccination HI antibody titers and seroprotection rates compared with those primed with split vaccine, irrespective of the age stratum. This difference was greatest against the A/H3N2 vaccine strain, with 36 of 41 children (88%) having seroprotective titers at baseline in the Sub/MF59 group, and 16 of 40 (40%) in the split vaccine group ($P < 0.001$ vs. split vaccine).

Three weeks after booster vaccination, the MF59-adjuvanted vaccine induced consistently higher HI antibody titers than the split vaccine for all three influenza strains tested ($P < 0.001$ vs. split group). This difference was most pronounced in the youngest children (<3 years of age) and against the B influenza strain. In the overall population, post-vaccination mean fold increases in HI titers were higher in the Sub/MF59 group than in the comparator vaccine group, with differences achieving a statistical significance against all three strains in the younger age cohort.

Irrespective of the age cohort and the vaccine group, post-vaccination seroprotection rates were achieved by all children against A/H1N1 and A/H3N2 vaccine influenza strains. Sub/MF59 vaccine induced statistically significant higher ($P < 0.001$) seroprotection and seroconversion rates against the B/Malaysia strain, when compared with the non-adjuvanted split vaccine in the overall population and in the youngest children (<3 years of age).

The results of this extension study, performed to mimic the ideal field conditions of consecutive seasonal vaccinations, further support the use of MF59®-adjuvanted subunit TIV as a highly immunogenic and well-tolerated vaccine for active immunization against seasonal influenza in healthy children.

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