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A clinical study to assess the immunogenicity and safety of a monovalent 2009 influenza A (H1N1) vaccine in an area with low-level epidemics of pandemic influenza☆

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ABSTRACT

We conducted a multi-center, randomized, laboratory-blinded clinical trial in 185 healthy adults (<60 years) and 107 elders (>60 years) to examine the immunogenicity and safety of different doses of an inactivated, monovalent, non-adjuvanted, split vaccine against the 2009 pandemic influenza A (H1N1) virus. The 186 adults were assigned to three treatment groups, i.e., one 15 μg hemagglutination (HA) antigen dose, two 15 μg or 30 μg HA doses in 3 weeks apart, and the 107 elders were treated with two 15 μg or 30 μg doses in 3 weeks apart. Prior to the vaccination, 4.8% subjects had hemagglutination-inhibition (HAI) antibody titers of 1:40 or more. By day 21 post-vaccination of one dose of 15 μg HA, the seroprotective rate was 95.1% and 75.5% in subjects <60 and >65 years of age, respectively; by day 21 post the second 15 μg HA dose, the seroprotective rates were 93.2% and 73.1%, respectively. The seroprotective rates for recipients of 30 μg HA antigen by day 21 were 95.2% for subjects <60 years and 81.1% for subjects >65 years of age, that was boosted to 98.3% and 80.4%, respectively with a second dose of 30 μg HA antigen. No vaccine-related serious adverse events occurred. The data indicated a single 15 μg HA dose of the vaccine induced a protective immune response in most adults, including the elders >60 years of age, and a booster dose at the third week did not render a higher level of antibody response.

1. Introduction

In the spring of 2009, the pandemic influenza A (H1N1) virus was first identified in Mexico and the United States, and continued to spread globally [1,2]. The rapid global spread of the virus prompted the World Health Organization to declare the novel influenza pandemic on June 11, 2009 [3]. Influenza vaccination is the primary method to prevent influenza and its severe complications. A previous study found that vaccination with recent seasonal non-adjuvanted or adjuvanted influenza vaccines provided little or no cross-reactive antibody protection against the 2009 pandemic influenza A (H1N1) in any age group [4]. So there is an urgent need to develop a vaccine against the virus. Early availability of safe and effective vaccines is critical to prevent the pandemic 2009 H1N1 infection and to mitigate the doomed complications. In response to the pandemic, a novel vaccine against the virus strain A/California/07/2009(H1N1) was developed in Taiwan. We conducted a clinical trial in healthy adults and elders to examine the immunogenicity and safety of different doses of this monovalent, split-virus 2009 H1N1 influenza vaccine. The manufacturer of this vaccine already has a licensed influenza vaccine and applies the same process to produce the A (H1N1) vaccines. The objective of this study is to determine safety and effectiveness of this vaccine

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in eliciting an immune response to H1N1 influenza in the healthy adult and elderly populations.

2. Materials and methods

2.1. Study design

This multi-center, randomized, laboratory-blinded, phase 2 clinical trial was conducted in three medical centers in Taipei (National Taiwan University Hospital, Tri-Service General hospital, and Taipei Medical University Wan Fang Hospital). The purpose of this study was to evaluate the immunogenicity and safety of different doses of H1N1 vaccine in healthy adults and elders. The study followed the principles of the Declaration of Helsinki, Good Clinical Practice (as defined by the International Conference on Harmonisation), and Taiwanese regulatory requirements. The human research ethics committee in each study center approved the study and the written informed consent was obtained from each subject. In the adult (<60 years) cohort, all volunteers were randomized in a 1:1:1 ratio to receive 2 doses of triweekly vaccine with 15 μg hemagglutination antigen, 2 doses of triweekly vaccine with 30 μg hemagglutination antigen, and 1 dose of vaccine with 15 μg hemagglutination antigen, respectively. In the elderly (>60 years) cohort, all volunteers were randomized in a 1:1 ratio to receive two doses of triweekly 15 or 30 μg hemagglutination antigen. The actual treatment given to each subject was determined by a randomization scheme generated by the biostatistician through the computer software program that incorporated a standard procedure of generating random numbers. The randomization code for the individual subject was exposed only if the subject was eligible for this study. Safety assessment was recorded both by diary cards and phone contact. Safety was determined by assessment of adverse events in 6 weeks following the first vaccination, serious adverse events and new-onset chronic medical conditions through 7 months post the first vaccination, and reactogenicity to the vaccines in 7 days following each vaccination. Titers of hemagglutination-inhibition (HAI) antibody to the H1N1 antigen were measured on enrollment, 3 and 6 weeks after the first vaccination. All serum samples were analyzed under the blinded condition in the laboratory.

2.2. Subjects

Subjects were eligible to participate the study if they were males or non-pregnant females and age ≥18 years, were willing and able to adhere to visiting schedules as well as all study requirements, were in good physical health, and agreed and signed the informed consent. The exclusion criteria includes: receiving influenza vaccine within the previous 6 months, having history of hypersensitivity to eggs or egg protein or similar pharmacological effects to study medication, having personal or family history of Guillain–Barre' syndrome, experiencing an acute febrile illness within the last 72 h prior to vaccination, having bleeding or any coagulation disorder thus posing a contraindication for intramuscular injection, having influenza-like illness as defined by the presence of fever (temperature ≥38.5 °C) and at least two of the four symptoms (headache, myalgia/arthralgia, sorethroat, and cough), accepting treatment with an investigational drug or device in a clinical study within 3 months before the consent, experiencing immunodeficiency, immunosuppressive or household contact with immunosuppression, having history of wheezing or using bronchodilators within 3 months prior to the study, receiving any inactivated vaccine within 2 weeks prior to the study vaccination or 3 weeks after the immunogenicity evaluation period, receiving live virus vaccine within 1 month prior to or 2 months after the study vaccination, receiving any blood products including immunoglobulin in the prior 3 months, and having underlying condition that may be inappropriate for vaccination in the investigator's opinion.

2.3. Vaccine

The H1N1 vaccine is an inactivated, monovalent, unadjuvanted, and split-virus that was produced by Adimmune Corporation (Taipei, Taiwan). The seed virus was prepared from the reassortant vaccine virus NYMC X-179A (New York Medical College, New York) that was derived from the A/California/7/2009(H1N1) virus, one of the candidate reassortant vaccine viruses recommended by the WHO [5]. The seed virus was propagated in embryonated chicken eggs with the standard techniques producing the seasonal trivalent inactivated vaccine [6]. The virus-containing fluid was harvested, and the virus was purified by zonal centrifugation, splitted by ether, and inactivated by formaldehyde. The final vaccine product consists of 30 μg hemagglutinin antigen per mL, thimerosal 0.1 mg/mL, residual formalin <0.1 μL/mL and polysorbate 80 0.1 μL/mL.

2.4. Immunogenicity

Serum samples were obtained prior to vaccination, as well as 3 weeks and 6 weeks after vaccination. The antibody titers were measured by hemagglutination-inhibition (HAI) assays. The three immunogenicity end points were applied based on the international guidelines to evaluate influenza vaccines [7,8]. The immunogenicity profiles, including the seroprotective rate (the proportion of subjects with antibody level ≥1:40 on HAI assay), the seroconversion rate (the proportion of subjects with a pre-vaccination HAI antibody titer <1:10 and a post-vaccination titer ≥1:40, or a pre-vaccination titer ≥1:10 and an increase in the titer by a factor of four or more) and the geometric mean fold rises of the HAI antibody titer, were analyzed. Subjects were considered to be seronegative if the serum HAI titer was less than 1:10. The reference antiserum to A/California/7/2009 was obtained from National Institute for Biological Standards and Control (NIBSC). Serum samples were treated by receptor-neutralizing enzymes to eliminate nonspecific hemagglutination inhibitors prior to antibody measurement. Duplicates of HAI assays were performed in each sample for validation. Pre- and post-vaccination sera were titrated simultaneously and tested using 2-fold serial dilution starting from 1:10 of the tested serum.

2.5. Statistical analyses

Instead of power calculations, the sample size in this study was based on the requirements of 50 subjects per dosing group established by European guidelines for influenza trials [9]. To compare the baseline characteristics and the medical conditions between the two dosing groups, the Student's *t*-test was applied for continuous variables and the χ^2-test was used for discrete variables. The protection and the conversion rates were determined by exact 95% confidence intervals. The two-sided Fisher's exact test was applied to compare proportions of the groups. Ninety-five percent confidence intervals of the geometric mean titers were obtained by transforming the mean of the log titer with antilog, which were compared by means of one-way analysis of co-variance (ANCOVA) on the log transformed titers with the pre-vaccination level of titer as the covariate. To identify the independent factors associated with the incremental antibody titers of vaccination, multivariate analyses were conducted and the independent variables were selected based on the results of simple logistic regression ($P < 0.1$). Statistical significance for all comparisons was determined at $P < 0.05$.

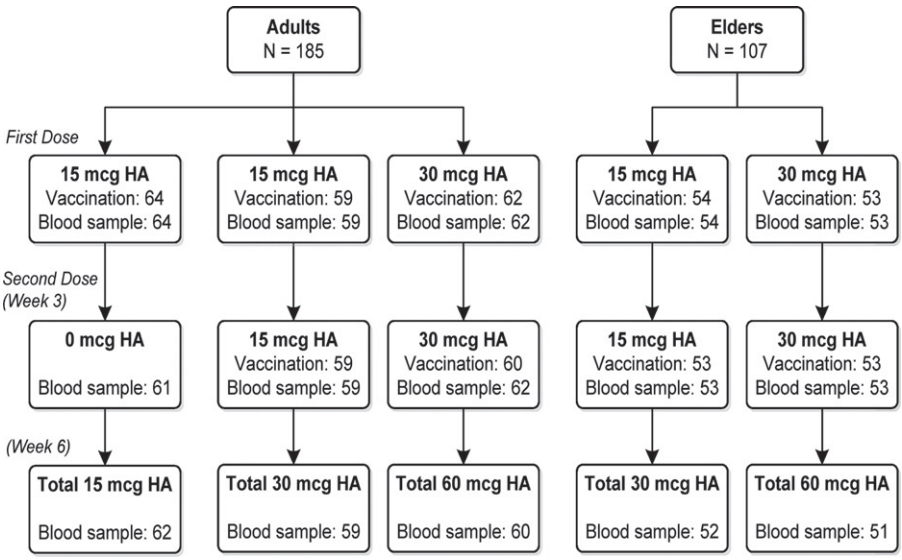

Fig. 1. Enrollment and follow-up of the study subjects.

All statistical analyses were performed by SAS software (version 9.2).

3. Results

3.1. Study subjects

Between September 24 and October 17, 2009, 292 adult subjects were enrolled (Fig. 1) and grouped into 185 subjects (70 males and 115 females) who were <60 years of age and 107 elder subjects (40 males and 67 females) who were >60 years of age. Among them, 123 adults and 54 elders received vaccines of 15 μg hemagglutination per dose, and 62 adult subjects and 53 elderly subjects received vaccines containing 30 μg hemagglutination per dose. Sixty-four of the 123 adults receiving 15 μg hemagglutination per dose only accepted one dose; the others received two doses. Data from all vaccinated subjects were included in the safety analysis. Immunogenicity data on days 21 and 42 were available for 288 and 284 subjects, respectively. The demographic characteristics of all vaccinated subjects were summarized in Table 1. 4 adults and 4 elders dropped out from the study; 4 were due to no blood sampling, 3 withdrew their consent, and one received seasonal influenza vaccine.

3.2. Safety

Injection-site and systemic reactions that occurred during the 7 days after each vaccination were summarized in Table 2. In the adult cohort, 76 (62.3%) subjects receiving 30 μg and 106 (58.2%) subjects who received 15 μg HA had at least one solicited local event. On the other hand, 41 (38.7%) elderly subjects who received 30 μg and 28 (26.2%) subjects receiving 15 μg had at least one solicited local event. The incidence of the solicited local event was higher in adults than in elders. Most reactions were mild to moderate; severe reactions, affecting daily activities and requiring medical attention, occurred in 0.77% of the study subjects.

Unsolicited adverse events were reported in 24.3% of subjects after administration of vaccination (26.5% of adults and 20.6% of elders). In adults, the most common reported unsolicited events following the vaccination included upper respiratory tract infection (9 subjects, 4.9%) and dizziness (8 subjects, 4.3%). In elders, the most frequent unsolicited event was cough (4 subjects, 3.7%). Two subjects reported events leading to emergency room visit on the second day after their first vaccination: cellulitis in one subject and renal stone in the other. Both were not vaccine-related, judged by the investigator.

No death occurred before the release of this report. Three subjects experienced serious adverse events requiring hospitalization,

Table 1
Demographic characteristics of all vaccinated subjects.

	Adults			*P*-Value	Elders		*P*-Value
	15 μg, 1 dose *N* = 64	15 μg, 2 doses *N* = 59	30 μg, 2 doses *N* = 62		15 μg, 2 doses *N* = 54	30 μg, 2 doses *N* = 53	
Sex, no. (%)				0.0713			0.0723
Male	25 (39.1%)	28 (47.5%)	17 (27.4%)		25 (46.3%)	15 (28.3%)	
Female	39 (60.9%)	31 (52.5%)	45 (72.6%)		29 (53.7%)	38 (71.7%)	
Age, year				0.3283			0.6827
Mean ± SD	36.0 ± 9.2	37.6 ± 9.4	38.3 ± 8.8		69 ± 6.3	68.5 ± 5.5	
Median	34	38	36		67	68	
Range	20–60	21–59	22–60		61–86	61–83	
Pre-vaccination antibody titer, no. (%)				0.8638			0.5406
Seronegative	51 (79.7%)	48 (81.4%)	52 (83.9%)		34 (63.0%)	37 (69.8%)	
Seropositive	13 (20.3%)	11 (18.6%)	10 (16.1%)		20 (37.0%)	16 (30.2%)	

Table 2
Proportion of subjects having a solicited local or systemic event within 7 days after each vaccination.

	Adults		*P*-Value	Elders		*P*-Value
	15 μg	30 μg		15 μg	30 μg	
First vaccination	*N* = 123	*N* = 62		*N* = 54	*N* = 53	
Any solicited local events	74 (60.2%)	41 (66.1%)	0.5210	15 (27.8%)	23 (43.4%)	0.1085
Pain	62 (50.4%)	35 (56.5%)	0.5330	13 (24.1%)	17 (32.1%)	0.3951
Swelling	19 (15.4%)	18 (29.0%)	0.0337	3 (5.6%)	8 (15.1%)	0.1231
Redness	19 (15.4%)	16 (25.8%)	0.1117	0 (0.0%)	9 (17.0%)	0.0012
Ecchymosis	4 (3.3%)	2 (3.2%)	1.0000	3 (5.6%)	2 (3.8%)	1.0000
Decreased limb mobility	6 (4.9%)	9 (14.5%)	0.0418	0 (0.0%)	1 (1.9%)	0.4953
Second vaccination	*N* = 59	*N* = 60		*N* = 53	*N* = 53	
Any solicited local events	32 (54.2%)	35 (58.3%)	0.7133	13 (24.5%)	18 (34.0%)	0.3933
Pain	28 (47.5%)	31 (51.7%)	0.7151	8 (15.1%)	14 (26.4%)	0.2307
Swelling	13 (22.0%)	15 (25.0%)	0.8294	6 (11.1%)	7 (13.2%)	1.0000
Redness	9 (15.3%)	9 (15.0%)	1.0000	3 (5.7%)	5 (9.4%)	0.7157
Ecchymosis	0 (0.0%)	0 (0.0%)		1 (1.9%)	0 (0.0%)	1.0000
Decreased limb mobility	2 (3.4%)	3 (5.0%)	1.0000	0 (0.0%)	4 (7.5%)	0.1179
First vaccination	*N* = 123	*N* = 62		*N* = 54	*N* = 53	
Any solicited systemic events	54 (43.9%)	34 (54.8%)	0.1651	13 (24.1%)	22 (41.5%)	0.0655
Fever (≥38.5 °C)	0 (0.0%)	1 (1.6%)	0.3351	1 (1.9%)	0 (0.0%)	1.0000
Nasal congestion	10 (8.1%)	6 (9.7%)	0.7839	2 (3.7%)	5 (9.4%)	0.2702
Cough	13 (10.6%)	12 (19.4%)	0.1136	7 (13.0%)	6 (11.3%)	1.0000
Sore throat	13 (10.6%)	15 (24.2%)	0.0179	5 (9.3%)	3 (5.7%)	0.7159
Muscle aches	22 (17.9%)	20 (32.3%)	0.0400	4 (7.4%)	6 (11.3%)	0.5265
Headache	17 (13.8%)	15 (24.2%)	0.0993	0 (0.0%)	7 (13.2%)	0.0059
Nausea	7 (5.7%)	5 (8.1%)	0.5400	1 (1.9%)	1 (1.9%)	1.0000
Vomiting	1 (0.8%)	1 (1.6%)	1.0000	0 (0.0%)	1 (1.9%)	0.4953
Malaise	32 (26.0%)	22 (35.5%)	0.2303	3 (5.6%)	11 (20.8%)	0.0235
Eye redness	1 (0.8%)	3 (4.8%)	0.1103	0 (0.0%)	2 (3.8%)	0.2430
Chest tightness	6 (4.9%)	7 (11.3%)	0.1308	4 (7.4%)	4 (7.5%)	1.0000
Respiratory distress	1 (0.8%)	2 (3.2%)	0.2605	0 (0.0%)	0 (0.0%)	
Face edema	3 (2.4%)	1 (1.6%)	1.0000	0 (0.0%)	0 (0.0%)	
Second vaccination	*N* = 59	*N* = 60		*N* = 53	*N* = 53	
Any solicited systemic events	20 (33.9%)	26 (43.3%)	0.3479	9 (17.0%)	19 (35.8%)	0.0463
Fever (≥38.5 °C)	0 (0.0%)	0 (0.0%)		0 (0.0%)	0 (0.0%)	
Nasal congestion	6 (10.2%)	9 (15.0%)	0.5822	4 (7.5%)	4 (7.5%)	1.0000
Cough	5 (8.5%)	9 (15.0%)	0.3945	4 (7.5%)	7 (13.2%)	0.5260
Sore throat	8 (13.6%)	6 (10.0%)	0.5822	3 (5.7%)	2 (3.8%)	1.0000
Muscle aches	7 (11.9%)	8 (13.3%)	1.0000	0 (0.0%)	6 (11.3%)	0.0269
Headache	4 (6.8%)	6 (10.0%)	0.7430	1 (1.9%)	4 (7.5%)	0.3629
Nausea	1 (1.7%)	2 (3.3%)	1.0000	0 (0.0%)	1 (1.9%)	1.0000
Vomiting	0 (0.0%)	1 (1.7%)	1.0000	0 (0.0%)	1 (1.9%)	1.0000
Malaise	9 (15.3%)	14 (23.3%)	0.3538	2 (3.8%)	4 (7.5%)	0.6783
Eye redness	0 (0.0%)	4 (6.7%)	0.1187	1 (1.9%)	2 (3.8%)	1.0000
Chest tightness	1 (1.7%)	4 (6.7%)	0.3644	2 (3.8%)	2 (3.8%)	1.0000
Respiratory distress	0 (0.0%)	0 (0.0%)		1 (1.9%)	0 (0.0%)	1.0000
Face edema	1 (1.7%)	0 (0.0%)	0.4958	0 (0.0%)	0 (0.0%)	

One adult subject receiving 30 μg hemagglutination reported a body temperature of 38.1 °C within 7 days after the first vaccination.

including falling down, chest tightness and gastroesophageal reflux disease. These events were unrelated to the study vaccine according to the investigator's medical opinion.

3.3. Immune response

Prior to the vaccination, 14 (4.8%) of the 292 subjects had protective antibody titers (≥1:40), that showed no difference between the age groups ($P = 1.0000$) or groups receiving different HA doses ($P = 0.1735$) (Table 3 and Fig. 2). For subjects ≤60 years of age, seroprotective rate of HAI titers after 3 weeks was 93.3% (95% CI, 87.3–97.1%) for recipients of 15 μg HA dose and 95.2% (95% CI, 86.5–99.0%) for the recipients of 30 μg dose, showing no significant difference between the 15 μg and 30 μg dosing groups ($P = 0.7516$). Among the elderly subjects >60 years, seroprotective rate after 3 weeks of the first vaccination was 75.5% (95% CI, 61.7–86.2%) in the recipients of 15 μg HA dose and 81.1% (95% CI, 68.0–90.6%) in the 30 μg HA dose, again, showing no significant difference of the seroprotection rate between the 15 μg and 30 μg dosing groups ($P = 0.6381$).

The overall seroprotective rate at 3 weeks after a 15 μg HA dose was 93.3% (95% CI, 87.3–97.1%) (Table 3); for the subgroup who did not receive a booster dose, at 6 weeks after the first vaccination, seroprotective rate was maintained at 93.5% (95% CI: 84.3–98.2%), which was not different from the 93.2% (95% CI: 83.5–98.1%) for the subgroup who received a second dose of 15 μg ($P = 1.0000$). Among the adult subjects, positive seroconversion was observed in 92.5% (95% CI, 86.2–96.5%) and 91.9% (95% CI, 82.2–97.3%) of the recipients of 15 μg and 30 μg, respectively, at 3 weeks after the first dose. At 3 weeks after the first dose vaccination, the elder groups receiving 15 μg and 30 μg HA doses showed a seroconversion rate of 71.7% (95% CI, 57.7–83.2%) and 81.1% (95% CI, 68.0–90.6%), respectively; the seemimgly 10% higher rate of positive seroconversion among the recipients of 30 μg was not statistically different from that of the 15 μg dose ($P = 0.3603$).

Among the adult subjects who received two doses of 15 μg, the seroconversion rate was 89.8% before the booster dose and increased to 93.2% after the second dose. On the contrary, the rates of seroconversion were 71.7% at 3 weeks and 71.2% after the second dose in the elder group receiving two doses of 15 μg. Among the

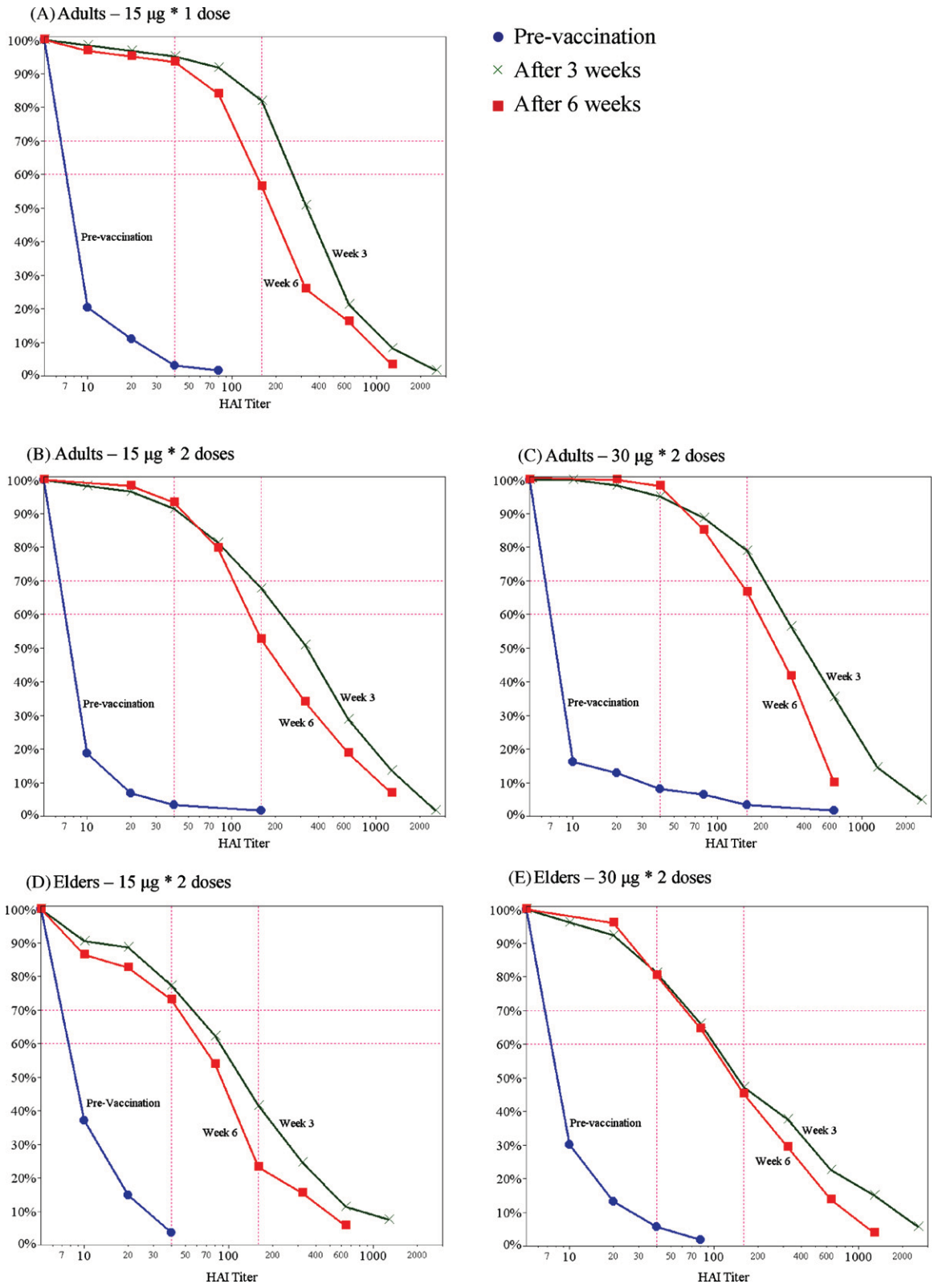

Fig. 2. Cumulative distribution curves for titers of hemagglutination-inhibition antibodies before, 3 weeks and 6 weeks after the first dose of vaccine, in treatment group and age group. (A) Adults – 15 μg × 1 dose; (B) adults – 15 μg × 2 doses; (C) adults – 30 μg × 2 doses; (D) elders – 15 μg × 2 doses; (E) elders – 30 μg × 2 doses. Titers are expressed as the reciprocal of the dilution.

Table 3
Immune response among subjects receiving different doses of study vaccine, according to age group.

Adults	15 μg, 1 dose	15 μg, 2 doses	30 μg, 2 doses
Pre-vaccination (*n*/*N*) at day 0	2/64	2/59	5/62
Seroprotection (titre ≥ 1:40) (%) (95% CI)	3.1(0.4–10.8)	3.4(0.4–11.7)	8.1(2.7–17.8)
Geometric mean titer (95% CI)	6.4(5.6–7.4)	6.3(5.4–7.3)	7.1(5.6–9.0)
3 Weeks (*n*/*N*)	58/61	54/59	59/62
Seroprotection (titre ≥ 1:40) (%) (95% CI)	95.1(86.3–99.0)	91.5(81.3–97.2)	95.2(86.5–99.0)
Geometric mean titer (95% CI)	215.0(162.7–284.1)	193.1(136.2–273.8)	260.2(191.2–354.2)
Geometric mean fold rise (95% CI)	34.3(25.4–46.3)	30.9(21.1–45.3)	36.8(26.1–51.8)
Seroconversion rate (%) (95% CI)	95.1(86.3–99.0)	89.8(79.2–96.2)	91.9(82.2–97.3)
6 Weeks (*n*/*N*)	58/62	55/59	59/60
Seroprotection (titre ≥ 1:40) (%) (95% CI)	93.5(84.3–98.2)	93.2(83.5–98.1)	98.3(91.1–100.0)
Geometric mean titer (95% CI)	130.8(97.8–175.0)	139.8(102.8–190.1)	160.0(127.9–200.2)
Geometric mean fold rise (95% CI)	20.5(15.0–27.8)	22.4(16.0–31.2)	23.4(17.2–31.9)
Seroconversion rate (%) (95% CI)	91.9(82.2–97.3)	93.2(83.5–98.1)	91.7(81.6–97.2)

Elders	15 μg, 2 doses	30 μg, 2 doses
Pre-vaccination (*n*/*N*) at day 0	2/54	3/53
Seroprotection (titre ≥ 1:40) (%) (95% CI)	3.7(0.5–12.7)	5.7(1.2–15.7)
Geometric mean titer (95% CI)	7.3(6.3–8.6)	7.1(6.0–8.5)
3 Weeks (*n*/*N*)	40/53	43/53
Seroprotection (titre ≥ 1:40) (%) (95% CI)	75.5(61.7–86.2)	81.1(68.0–90.6)
Geometric mean titer (95% CI)	79.5(52.9–119.3)	124.0(79.1–194.2)
Geometric mean fold rise (95% CI)	10.7(7.0–16.4)	17.4(11.8–25.7)
Seroconversion rate (%) (95% CI)	71.7(57.7–83.2)	81.1(68.0–90.6)
6 Weeks (*n*/*N*)	38/52	41/51
Seroprotection (titre ≥ 1:40) (%) (95% CI)	73.1(59.0–84.4)	80.4(66.9–90.2)
Geometric mean titer (95% CI)	52.9(36.4–77.0)	97.4(67.2–141.3)
Geometric mean fold rise (95% CI)	7.3(5.0–10.7)	14.3(10.2–19.9)
Seroconversion rate (%) (95% CI)	71.2(56.9–82.9)	80.4(66.9–90.2)

elderly subjects, 3 weeks after the booster dose, the 30 μg recipients had around 9.2% higher rate of seroconversion, though not statistically different ($P = 0.3589$), than those receiving 15 μg. Similarly, adult subjects receiving 15 μg and 30 μg showed no significant difference in the rate of seroconversion ($P = 1.0000$), although the 15 μg recipients had a numerically higher seroconversion rate than those received 30 μg. Both one and two 15 μg doses of the study vaccine produced seroconversion in a majority of adult subjects. Before the booster dose, the rate of seroconversion was observed in 92.5% (95% CI: 86.2–96.5%) of the recipients of 15 μg. Six weeks after the first vaccination, the rate of seroconversion was seen in 91.9% (95% CI: 82.2–97.3%) of those having only one dose of 15 μg, and in 93.2% (95% CI: 83.5–98.1%) of those given two doses of 15 μg. There was no significant difference in the rate of seroconversion ($P = 1.0000$) between the two groups in different dose schedules.

The HAI GMTs and the mean fold rise against 2009 H1N1 virus were also summarized in Table 3. Among the adult subjects, the GMTs prior to the vaccination were 6.3 (ranging from <10.0 to 160) and 7.1 (ranging from <10.0 to 640) for the recipients of 15 μg and 30 μg, respectively. Three weeks after the first vaccination, the GMTs increased by a factor of 32.6 (95% CI, 25.6–41.4) in the 15 μg group and 36.8 (95% CI, 26.1–51.8) in the 30 μg group. There was no significant difference in factor increment between the two dose groups ($P = 0.2523$). Three weeks after the booster dose, the GMTs increased by a factor of 22.4 (95% CI, 16.0–31.2) in the 15 μg dose group and 23.4 (95% CI, 17.2–31.9) in the 30 μg dose group. Among the subjects receiving only one 15 μg of vaccine, the GMTs increased from 6.4 at pre-vaccination to 130.8 at 6-week post-vaccination, representing approximately a 20.5-fold increase (95% CI, 15.0–27.8) from pre-vaccination. There was no significant difference in the GMTs increase at 6 weeks after the first vaccination among the three dose strategy groups. Among the elderly subjects, the GMTs prior to the vaccination were 7.3 (ranging from <10.0 to 40) and 7.1 (ranging from <10.0 to 80) for the recipients of 15 μg and 30 μg, respectively. Three weeks after the first vaccination, the GMTs increased by a factor of 10.7 (95% CI, 7.0–16.4) in the 15 μg dose group and 17.4 (95% CI, 11.8–25.7) in the 30 μg dose group. There was a slight difference in factor increment between the two dose groups ($P = 0.0997$). Three weeks after the booster dose, the GMTs increased by a factor of 7.3 (95% CI, 5.0–10.7) in the 15 μg group and 14.3 (95% CI, 10.2–19.9) in the 30 μg group. A statistically significant difference was found in the 6-week GMTs factor increase between the two dose groups ($P = 0.0106$).

4. Discussion

This report details the findings in subjects receiving an inactivated, monovalent, non-adjuvanted split influenza vaccine. Prior to vaccination, only 4.9% of adults group and 4.7% of elder group of our study population had a pre-existing A (H1N1) antibody titer of 1:40 or greater, which were much lower than that had been reported in Australia (22.3–26.8%), USA (20–31%), Germany (12.5–13.1%) and England (4–12%) [10–13]. The pre-existing antibody most likely reflects previous infection of viruses that are antigenically identical or similar to the 2009 H1N1 influenza A virus. In subjects with pre-existing antibody, the vaccine would induce a boosting effect, rather than a primary response to the vaccine. Thus the low prevalence of HAI antibody against H1N1 virus among our study subjects at the time of clinical trial was more conducive to render a true primary immune response to the vaccine, rather than a boosting effect of the vaccine. The elder subjects' immune response to the 2009 H1N1 vaccine was as with the seasonal influenza vaccine to which the elder group's immune response was less favorable [14–16]. The inverse correlation between immunogenicity and age, i.e., subjects over 60 years of age had lower antibody responses than the younger adult group, was reflected in all three criteria of immunogenicity measures as set forth for the seasonal influenza vaccines. However, despite the lower immune response among subjects over 60 years of age, one 15 μg dose was sufficient to induce immune responses that satisfied all three immunogenicity criteria. Despite

of the putative novel antigenic nature of the 2009 H1N1 virus, results of our study corroborates with finding of other studies that a single 15 μg dose of non-adjuvanted split influenza vaccine against the 2009 H1N1 virus could induce a robust immune response in healthy adults [10,13,17], while less robust in elders over 60 years of age yet still fulfilled regulatory standards. In the elder group, vaccine containing 30 μg rendered a slightly higher rate of protective immune responses (81.1%) than that of 15 dose (75.5%), as well as a higher seroconversion rate (81.1% vs. 71.7%) and a higher GMT rise. A gender-stratified analysis suggested that the lower immune response among the elder group was mostly contributed by male subjects, in contrast to the elder female subjects whose immune response rate resembled that of the younger group. While the study sample size was not designed to accommodate a stratified gender analysis, this seemingly anecdotal finding of differences between male and female elders should still be noted for future investigation. Whether a higher dose is needed for elderly, especially for the male elder subject, in order to achieve a more favorable level of immune response has neither been thoroughly investigated for the seasonal influenza vaccine nor for the A (H1N1) 2009 vaccine.

Profiles of the adverse events following vaccination, particularly the frequency and severity of the solicited local and systemic adverse events, were consistent with the previous experience in the seasonal influenza vaccines or other 2009 H1N1 vaccines [10,13,17]. No death, vaccine-related serious adverse events, or adverse events of special interest, such as the optic neuritis, cranial neuropathy, brachial neuropathy, and Guillain–Barre syndrome (GBS), were reported. All participants would be further monitored in a 6-month period after the vaccination. No subjects in our study received a 2009 Northern Hemisphere seasonal trivalent inactivated vaccine. They were not allowed to receive the vaccine until 3 weeks after the immunogenicity evaluation period. In the contrast, forty-five percent of the adult subjects and 40.1% of the pediatric subjects in Australian H1N1 vaccine studies received a 2009 Southern Hemisphere seasonal trivalent inactivated vaccine [10,18]. There were some limitations in our study. First, the sample size was small, and our study is not placebo-controlled trial. Second, we did not record the previous seasonal influenza vaccination status of our subjects. Seasonal influenza vaccine for 2008–2009 was given to 179 (36%) of 497 individuals aged 18–64 years and 217 (62%) of 352 aged 65 years or older for studies in United States [11]. Del Giudice et al. suggested that priming by previous infection or vaccination against seasonal influenza may affects the response to the H1N1 vaccine [19]. However, previous vaccination with seasonal influenza vaccine did not appear to have a marked influence on the immune response in the subgroup analysis of the three H1N1 vaccine trials [11–13]. A study in Italy showed that one dose of MF59-adjuvanted H1N1 vaccine met the licensure criteria for adult and elderly subjects 3 months after seasonal vaccination, or concomitantly with seasonal vaccine in adults, without impacting the tolerability or immunogenicity of either vaccine [20]. In conclusion, this study indicated that a single 15 μg dose of the vaccine induced a protective immune response in most adults, including those who were greater than 60 years of age, and a booster dose at the third week did not render a higher level of immune response.

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