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Immunogenicity and safety of Quadrivalent Influenza HA vaccine in Indonesian children: An open-labeled, bridging, clinical study

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ABSTRACT

Background: Influenza B (Yamagata/Victoria lineage) can cause severe forms of respiratory infection among the pediatric population as well as influenza A strains (H3N2/H1N1). Vaccination against all four strains is required to prevent infection and severe outcome. This study is the first study to assess the immunogenicity of Quadrivalent Influenza HA vaccine (QIV) and ascertain safety among children in Indonesia.

Methods: This is an open labeled, single arm, bridging clinical study involving unprimed healthy children 6–35 months of age (Group I) and 3–8 years of age (Group II). Subjects on both groups receiving two doses of QIV with a 28 days interval. Serology tests were performed on baseline and 28 days post-vaccination. Hemagglutination inhibition antibody titers were analyzed for Geometric Mean Titer (GMT), seroprotection, and seroconversion rates. Solicited reactions, unsolicited adverse events, and serious adverse events were observed up to 28 days post-vaccination.

Results: Out of 270 subjects enrolled, 269 subjects completed the study. Immunogenicity analysis were evaluated on 254 subjects. Seroprotection rates were ≥85% for all vaccine strains in both groups. Seroconversion of more than 4 folds for all strains occurred in both groups post-vaccination. In Group I, the increase of GMT for A/H1N1, A/H3N2, B/Texas, and B/Phuket was 12.5, 14.5, 8.2, and 6.4 folds, respectively. In Group II the increase of GMT for A/H1N1, A/H3N2, B/Texas, and B/Phuket was 14, 17, 10, and 8 folds, respectively. The majority of local adverse events (AEs) after the first and second immunizations were immediate injection-site pain (10.4% and 12.6%). The majority of systemic AEs after the first and second immunizations were delayed unsolicited AEs (14.8% and 14.9%). No vaccine-related serious adverse events or deaths were reported.

Conclusion: The investigational QIV was immunogenic with an acceptable safety profile in children 6 months to 8 years of age.

Clinical Trial registration: NCT03336593.

1. Introduction

Influenza is an underappreciated contributor to global mortality and morbidity and has significant economic consequences [1]. It continues to be an important burden of disease, particularly for the pediatric population [2]. Current estimates indicates annual attacks to be 20–30% in children whereas for adults are 5–10% [3], 11.5% of 2210 healthy unvaccinated children 6–35 months of age contracted influenza during the 2014–2016 influenza season [4]. Each year, there are approximately 870.000 children less than 5 years of age hospitalized worldwide due to influenza [4] A meta-analysis report on global burden of respiratory disease suggests that the majority of reported influenza related deaths among children occur in developing countries [5,6]. Influenza A and B are the most important viral strain to cause influenza illness in humans. There are two subtypes of influenza A commonly identified, H1N1 and H3N2. Influenza B on the other hand is divided based on two antigenically distinct lineage, B/Yamagata and B/Victoria [7]. Influenza-related illness caused by Influenza A strains are more frequent in number, however illnesses due to Influenza B strains is

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associated with higher health cost burden, higher hospital admission, and most importantly higher morbidity and mortality among children [7,8,9].

In temperate climates, Influenza infection tends to occur seasonally, with epidemics experienced mainly during the winter. While in the tropical regions, influenza may occur throughout the year, causing outbreaks more irregularly [10]. According to a literature review study in 15 Asia Pacific countries, influenza B was identified and associated with 0–92% of laboratory-confirmed influenza cases in any one season or year [9].

In Indonesia, influenza illness has been reported to be 18.75% for Influenza A and 17.26% for Influenza B, occurring throughout the year with no apparent pattern [11].

Vaccination is currently the most effective intervention to prevent infection and severe outcomes of influenza infection in children. Numerous studies worldwide have consistently reported efficacy and effectiveness of influenza vaccination to reduce the risk of influenza infection among children under 9 years old [12–14]. However, the viral strain undergoes continuous antigenic drifts, causing a renewed pool of susceptible host that requires annual administration of new influenza vaccination [15].

Recommendations for vaccine composition are made annually to ensure protection against current circulating strains. The widely available form of influenza vaccine is the trivalent vaccine consisting of two influenza A strain and one B strain. Since there are two lineages of influenza B strains, this type of vaccine must choose one B strain most probable to circulate in the next year. To ensure broader coverage of all influenza strains, the Quadrivalent Vaccine (QIV) has become available. QIV contains both strains of influenza A and strain from both influenza B lineages. A previous study has reported immunogenic properties and safety of trivalent vaccines among Indonesian children [16]. However, it is not known whether the addition of another B strain will have any effect towards immunogenicity and safety of QIV. This study aims to determine the immunogenicity of Quadrivalent Influenza HA vaccine and to ascertain safety of vaccine administration among children in Indonesia.

2. Materials and methods

2.1. Study design

This is an experimental open-labeled, single arm, bridging study aiming to assess the immunogenicity and safety of Quadrivalent Influenza HA vaccine (QIV) 28 days after immunization among children in Indonesia. The study is a collaboration between the Department of Child Health, Faculty of Medicine, Universitas Padjadjaran and PT Bio Farma (Persero) Indonesia. Written informed consent obtained from the participants parents prior to any study-specific procedure performed. Ethical approval was obtained from the Research Ethics Committee, Faculty of Medicine, Universitas Padjajaran, in accordance with the Declaration of Helsinki and the International Conference on Harmonization Good Clinical Practice guidelines.

2.2. Study population

Study participants were unprimed children, divided into two age groups: I (6–35 months) and II (3–8 years). Primary inclusion for participations were healthy children aged 6 months to 8 years committed to comply to study instructions and trial schedules. Subjects were not eligible if present with mild, moderate or severe illness with fever (axillary temperature $\geq$37.5 °C). Other exclusion criteria include allergic to egg, chicken protein, or other vaccine components, history of blood disorders contraindicating intramuscular injection, undergoing treatment that may alter immune response within the last 4 weeks, any abnormality or chronic diseases, history of influenza vaccination within the last year, or any vaccination 1 month before or after immunization with QIV.

Subjects were enrolled from 3 primary care centres in Bandung City: Ibrahim Adjie Primary Health Center, Puter Primary Health Center, and Garuda Primary Health Center from October 2017 to June 2018. After obtaining written consent from parents/-guardians, every participant was allocated an inclusion number in chronological order from I001 to I135 for Group I (6–35 months) and II001-II135 for Group II (3–8 years old).

2.3. Study intervention and outcome

The QIV vaccine was formulated by PT Bio Farma (Persero) Indonesia using bulks imported from Japan. The investigational QIV contains 15 μg hemagglutination for each of 4 strains A/California/7/2009(X-179A) pdm09n (H1N1), A/Hong Kong/4801/2014 (X-263) (H3N2), B/Texas/2/2013, and B/Phuket/3073/2013, in a 0.5 ml dose. Participants in group I were given half dose (0.25 ml) of QIV and Group II participants were given full dose (0.5 ml) of QIV on each injection.

The first dose of QIV was administered at visit 1 (Day 0) and the second dose at visit 2 (Day 0 + 28 (−4/+7) days). Vaccines were administered via intramuscular injection at the left anterolateral thigh region for children $\leq$2 years and the left deltoid region for children >2 years. For serologic testing, blood samples were collected at visit 1 prior to vaccination and visit 3, 28 days (−4/+7) after completion of 2 doses of vaccines. Hemagglutination Inhibition (HI) assays were performed by the Immunology Laboratory of Clinical Trial Department of Bio Farma in accordance with the standard methods from Biken Vaccine Institute, validated and approved by the Quality Assurance Division (CDC, 2016). Immunogenic response towards QIV was characterized by seroprotection rate, geometric mean titer (GMT), and seroconversion rate in antibody titer.

To assess the safety of QIV, parents/guardian were provided with a thermometer and observation card to assess and record information on the occurrence and intensity grade of any solicited local reaction (pain, redness, induration, and swelling) or systemic reactions (fever, fatigue and myalgia) up to 28 days post-vaccination. Fever was defined as body temperature of $\geq$38 °C. During each visit, the subject's parents/caregivers were given a specific question to assess occurrence of adverse reactions on subject that not yet able to communicate symptoms experienced, e.g., whether there is any abnormal crying or reduced muscle activity to assess myalgia. Unsolicited adverse events were recorded and any severe symptoms were reviewed by a specific team to assess correlation with vaccine administration. In addition, phone call follow-ups were conducted to ensure compliance. Solicited and unsolicited adverse events were categorized as immediate local and systemic events (within 0–30 min), intermediate local and systemic events (within 31 min to 72 h), or delayed local and systemic events (within 72 h to 28 days).

2.4. Sample size and study analysis

Sample size was determined based on 95% CI and power of the test 80%. The required sample size was 115 in each group with 20% drop out anticipation. With the assumption that not all of the subjects could complete the study, the total number of subjects was added at least 20% from the minimum requirement (N $\times$ 1.2) equal to 134.

Demographic data was expressed as mean, standard deviation (SD) and range values. The immunogenicity analyses were per-

formed on the per-protocol population. Analysis of Geometric Mean Titer (GMT), seroprotection, and seroconversion rates between the vaccine groups were done using Chi-square, McNemar, or Wilcoxon tests. Values of $p < 0.05$ were considered to be indicator of statistically significant differences between groups. The safety analyses were based on the intention-to-treat population analyses.

3. Results

Out of 298 screened subjects, we enrolled 270 healthy subjects, from two age groups: Group I, age 6–35 months and Group II, age 3–8 years old. One subject voluntarily withdrew after the first visit and 15 subjects were excluded from the immunogenicity analysis because visits were out of the determined window period [Fig. 1].

Demographic characteristics of study participants showed fair distribution in gender and age [Table 1]. At the time of enrollment, mean age and standard deviation were, 21.33 ± 8.919 months for group I and 5.10 ± 1.707 years for group II.

Table 1
Demographic characteristics of study participants.

Characteristics	6–35 Months n = 135	3–8 Years n = 135
Sex		
Male	79 (58.5%)	66 (48.9%)
Female	56 (41.5%)	69 (51.1%)
Age		
Mean (SD)	21.3 (8.9)	5.10 (1.7)
Median	22.0	5.2
Range	6–35	3–8

3.1. Immunogenicity

At baseline, most participants in Group I were seronegative for each strain of QIV. For Group II, most participants were seropositive for A/H3N2 and A/H1N1. However, there was a significant increase of antibody titer post-vaccination in both groups against each strain of QIV [Table 2]. The percentage of subjects with

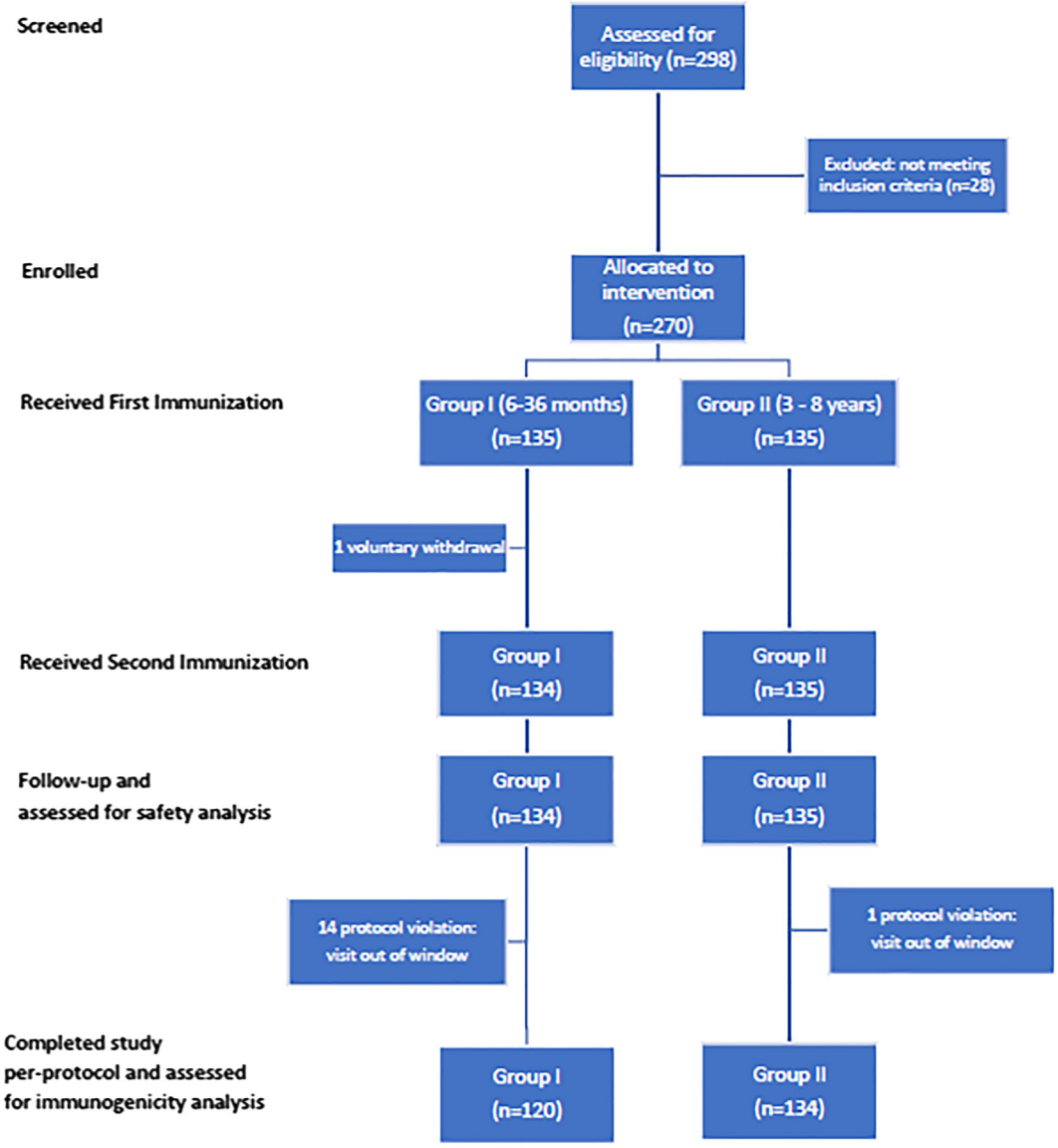

Fig. 1. Study Flow Chart.

Table 2
Influenza seroprotection rate pre- and 28 days post-immunization.

Description	Group I 6–35 Months (N = 120)		Group II 3–8 Years (N = 134)		All (N = 254)	
	Pre	Post	Pre	Post	Pre	Post
A/California/7/2009 (X179A)(H1N1) pdm09						
≥ 1:40 HI, n	27	117	95	134	122	251
% (95% CI)	22.5 (15.0, 30.0)	97.5 (92.9, 99.1)	70.9 (62.7, 77.9)	100.0 (97.2, 100.0)	48.0 (42.0, 54.2)	98.8 (96.6, 99.6)
A/Hong Kong/4801/2014(X-263) (H3N2)						
≥ 1:40 HI, n	43	118	110	134	153	252
% (95% CI)	35.8 (27.8, 44.7)	98.3(94.1, 99.5)	82.1 (74.7, 87.7)	100.0 (97.2, 100.0)	60.2 (54.1, 66.1)	99.2 (97.2, 99.8)
B/Texas/2/2013						
≥ 1:40 HI, n	26	111	42	131	68	242
% (95% CI)	21.7 (15.2, 39.9)	92.5 (86.4, 96.0)	31.3 (24.1, 39.6)	97.8 (93.6, 99.2)	26.8 (21.7, 32.5)	95.3 (91.9, 97.3)
B/Phuket/3073/2013						
≥ 1:40 HI, n	5	102	46	127	51	229
% (95% CI)	4.2 (1.8, 9.4)	85.0 (77.5, 90.3)	34.3 (26.8, 42.7)	94.8 (89.6, 97.4)	20.1 (15.6, 25.4)	90.2 (85.9, 93.2)

Mc.Nemar test $p^* < 0.001$.

anti-HI titer ≥ 1:40 after 28 days vaccination in Group I was 97.5% for A/H1N1, 98.3% for A/H3N2, 92.5% for B/Texas, and 85.0% for B/Phuket. In Group II, the percentage was 100% for A/H1N1, 100% for A/H3N2, 97.8% for B/Texas, and 94.8% for B/Phuket. There was a statistically significant increase $p < 0.001$ (McNemar test) of seroprotection rate among subjects before and 28 days post-vaccination for all four strains.

Geometric mean titer before and after vaccination showed a significant difference in all strains $p < 0.001$ (Table S1). In Group I, the increase of GMT was 12.5 folds for A/H1N1, 14.5 folds for A/H3N2, 8.2 folds for B/Texas, and 6.4 folds for B/Phuket. In Group II, the increase of GMT was 14 folds for A/H1N1, 17 folds for A/H3N2, 10 folds for B/Texas, and 8 folds for B/Phuket [Fig. 2]. In general, Group II had higher GMT at baseline and after vaccination in all four strains.

In our study, there were two patterns of seroconversions after vaccination of QIV. First, there were subjects that experienced transition from seronegative to seropositive following immunization. Second, there were subjects who had antibody response towards Influenza HA vaccine during baseline then increases ≥4 times post-vaccination [Table 3]. For both B lineage strains (B/Texas and B/Phuket), there are significant differences of seroprotection rate, increasing antibody titer ≥4 times, and transition from seronegative to seropositive between group I and II. Transition from seronegative to seropositive defined as a pre-vaccination titer <1:40 HI units and post-vaccination titer ≥1:40 HI units. Seroconversion was defined as increasing antibody titer ≥4 times and transition from seronegative to seropositive.

3.2. Safety

We recorded several adverse events (AEs) during the study period. Solicited and unsolicited post-vaccination AEs were categorized as immediate (within 30 min), intermediate (30 min to 72 h) and delayed (72 h to 28 days) reactions. Percentage of subjects with reported AEs in Group I was higher than Group II for immediate local reactions. Subjects with immediate local AEs after the first immunization in group I and II were 17% and 16.3%.

Subjects with immediate local AEs reported after second immunization in group I and II were 25.4% and 14.8%. Apart from this, the

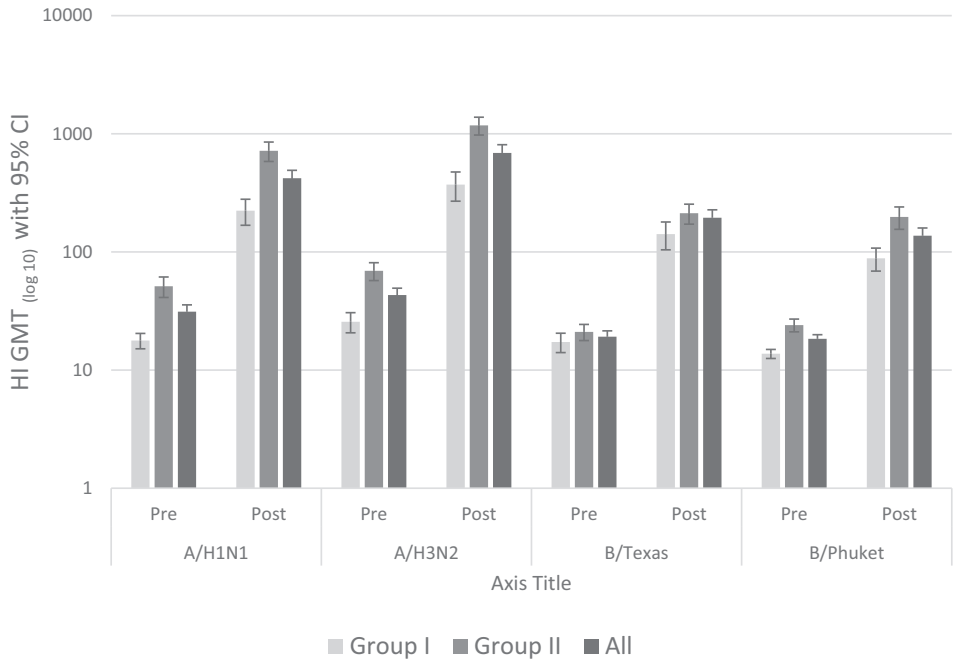

Fig. 2. Hemagglutination Inhibition (HI) Geometric Mean Titers (GMT) in group I and II pre- and 2 days post-vaccination.

Table 3
The difference in seroconversion rate.

Description	Seroprotection Rate ≥ 1:40 HI [a] n (%)				Increasing antibody titer ≥ 4 times [b] n (%)				Transition from seronegative to seropositive[c] n[d] (%)			
	Group I	Group II	Diff (95% CI)	p	Group I	Group II	%Diff (95% CI)	p	Group I	Group II	Diff (95% CI)	p[e]
A/California/7/2009 (X179A) (H1N1)pdm09	117 (97.5)	134 (100)	−2.5 (−7.1, 0.7)	0.104	117 (97.5)	127 (94.8)	2.7 (−2.6, 8.1)	0.342	90 (96.8)	39 (100)	−3.2 (−9.1, 6.0)	0.048
A/Hong Kong/4801/2014 (X-263) (H3N2)	118 (98.3)	134 (100)	−1.7 (−5.9, 1.4)	0.222	115 (95.8)	131 (97.8)	−1.9 (−7.3, 2.8)	0.482	75 (97.4)	24 (100)	−2.5 (−8.8, 11.4)	0.104
B/Texas/2/2013	111 (92.5)	131 (97.8)	−5.3 (−11.6, 0.2)	0.048	111 (92.5)	131 (97.8)	−5.3 (−11.6, 0.2)	0.048	85 (90.4)	89 (96.7)	−6.3 (−14.2, 1.1)	0.046
B/Phuket/3073/2013	102 (85.0)	127 (94.8)	−9.8 (−17.7, −2.4)	0.009	93 (77.5)	120 (89.6)	−12.1 (−21.3, −2.9)	0.009	97 (84.3)	81 (92.0)	−7.7 (−16.4, 1.7)	0.023

Abbreviations: HI, hemagglutination inhibition; CI, confidence interval.
[a] Number of population (N) on seroprotection rate group I = 120; group II = 134.
[b] Number of population (N) on increasing antibody titer group I = 120; group II = 134.
[c] Number of population (N) on transition from seronegative to seropositive for each group and each strain was based on number of seronegative subjects at baseline (pre-vaccination).
[d] n defined as number of subjects with anti-HI titer < 1:40 HI (seronegative) at baseline and ≥ 1:40 HI (seropositive) at post-vaccination.
[e] p value based on Exact Fisher test.

Table 4
Summary of reported adverse event.

Description	Group I (N = 135)		Group II (N = 135)		All (N = 270)	
	n	% (95% CI)	n	% (95% CI)	n	% (95% CI)
1st immunization						
Any immediate adverse event[a]						
Any immediate local reaction	23	17.0 (11.6, 24.3)	22	16.3 (11.0, 23.4)	45	16.7 (12.7, 21.6)
Any immediate systemic event	1	0.7 (0.1, 4.1)	1	0.7 (0.1, 4.1)	2	0.7 (0.2, 2.7)
Any intermediate adverse event[b]						
Any intermediate local reaction	8	5.9 (3.0, 11.3)	8	5.9 (3.0, 11.3)	16	5.9 (3.7, 9.4)
Any intermediate systemic event	7	5.2 (2.5, 10.3)	8	5.9 (3.0, 11.3)	15	5.5 (3.4, 9.0)
Any delayed adverse event[c]						
Any delayed local reaction	0	0.0	0	0.0	0	0.0
Any delayed systemic event	28	20.7 (14.8, 28.3)	25	18.5 (12.9, 25.9)	53	19.6 (15.3, 24.8)
2nd immunization	**(N = 134)**		**(N = 135)**		**(N = 269)**	
Any immediate adverse event						
Any immediate local reaction	34	25.4 (18.8, 33.4)	20	14.8 (9.8, 21.8)	54	20.1 (15.7, 25.3)
Any immediate systemic event	0	0.0	0	0.0	0	0.0
Any intermediate adverse event						
Any intermediate local reaction	5	3.7 (1.6, 8.4)	4	3.0 (1.2, 7.4)	9	3.3 (1.8, 6.2)
Any intermediate systemic event	9	6.7 (3.6, 12.3)	4	3.0 (1.2, 7.4)	13	4.8 (2.8, 8.1)
Any delayed adverse event						
Any delayed local reaction	0	0.0	0	0.0	0	0.0
Any delayed systemic event	26	19.4 (13.6, 26.9)	22	16.3 (11.0, 23.4)	48	17.8 (13.7, 22.9)

Abbreviations: CI, confidence interval; N, number of subjects on each group; n, number of subjects reporting the adverse event.
[a] Occurring from 0 to 30 min post immunization.
[b] Occurring from 31 min to 72 h post immunization.
[c] Occurring from 72 h to 28 days post immunization.

reported AEs were similar in both groups and comparable between the first and second injection [Table 4].

Immediate local reactions after first immunization were reported by 16.7% of all subjects, mostly pain (10.4%), redness (7.0%), and induration (0.7%). Immediate systemic reactions after first immunization were reported in 0.7% of subjects, including fever (0.4%) and myalgia (0.4%). Intermediate local reactions were reported in 5.9% of subjects, including pain (5.2%), induration (0.7%), swelling (1.5%), and redness (0.4%). Intermediate systemic reactions were reported in 5.5% of subjects, including fever (1.5%), fatigue (1.5%), myalgia (1.1%), and other unsolicited AEs (1.8%) such as common cold and cough. There were no delayed local reactions reported. However, 19.6% of subjects reported delayed systemic reactions, fever 8.5% and the majority were unsolicited AEs (14.8%) such as common cold, cough, diarrhea, rash and upper respiratory tract infection, varicella, and stomachache.

Immediate local reactions after second immunization were reported by 20.1% of all subjects, mostly pain (12.6%), redness (7.4%), induration (0.4%), and swelling (0.4%). No subject experienced immediate systemic reactions after second immunization. Intermediate local reactions were reported in 3.3% of subjects, including pain (3.3%), redness (0.7%), induration (0.7%), and swelling (0.7%). Intermediate systemic reactions were reported in 4.8% of subjects, including fever (2.2%), fatigue (0.7%), myalgia (1.1%), and other unsolicited AEs (1.1%) such as common cold and vomiting. There was no delayed local reaction reported. However, 17.8% of subjects reported delayed systemic reactions; fever (7.8%) and fatigue (0.4%); the majority were unsolicited AEs (14.9%) such as common cold, cough, rash, vomit, varicella, and dyspepsia.

After the first immunization, the majority of the reported local adverse events were mild (98.5%) and lasted for less than 48 h

Table 5
Duration and intensity of related local and systemic adverse event.

First Immunization (N = 270)	Duration						Intensity					
	<48 h		48–72 h		>72 h		Mild		Moderate		Severe	
	n	%	n	%	n	%	n	%	n	%	n	%
Local (at least one reaction)[a]												
Pain	34	12.6	8	3.0	0	0.0	42	15.6	0	0	0	0
Redness	11	4.1	8	3.0	1	0.4	19	7.0	1	0.4	0	0
Induration	4	1.5	0	0.0	0	0.0	4	1.5	0	0	0	0
Swelling	4	1.5	0	0.0	0	0.0	4	1.5	0	0	0	0
Other	0	0.0	0	0.0	0	0.0	0	0	0	0	0	0
Systemic[b]												
Fever	9	3.3	14	5.2	5	1.8	17	6.3	3	1.1	8	3.0
Fatigue	3	1.1	1	0.4	0	0.0	4	1.5	0	0	0	0
Myalgia	4	1.5	0	0.0	0	0.0	4	1.5	0	0	0	0
Other	5	1.8	17	6.3	23	8.5	38	14.1	7	2.6	0	0
Second Immunization (N = 269)	Duration						Intensity					
	<48 h		48–72 h		>72 h		Mild		Moderate		Severe	
	n	%	n	%	n	%	n	%	n	%	n	%
Local (at least one reaction)[a]												
Pain	37	13.7	6	2.2	0	0.0	43	16.0	0	0	0	0
Redness	19	7.0	3	1.1	0	0.0	19	7.1	3	1.1	0	0
Induration	3	1.1	0	0.0	0	0.0	3	1.1	0	0	0	0
Swelling	3	1.1	0	0.0	0	0.0	3	1.1	0	0	0	0
Other	0	0.0	0	0.0	0	0.0	0	0.0	0	0	0	0
Systemic[b]												
Fever	7	2.6	14	5.2	6	2.2	13	4.8	3	1.1	11	4.1
Fatigue	1	0.4	2	0.7	0	0.0	2	0.7	1	0.4	0	0
Myalgia	2	0.7	1	0.4	0	0.0	3	1.1	0	0	0	0
Other	9	3.3	17	6.3	17	6.3	36	13.4	6	2.2	1	0.4

[a] Solicited local adverse event: pain, redness, induration, and swelling. Pain for group age 6–35 months was graded as mild (reacts when site is touched), moderate (cries when site is touched), and severe (cries when limb is moved). Pain for group age 3–8 was graded as mild (mild pain to touch), moderate (pain with movements), and severe (significant paint at rest). Redness, induration, and swelling intensity were measured using a plastic bangle and categorized as mild (<2.5 cm), moderate (2.5–5 cm), and severe (>5 cm).

[b] Solicited Systemic adverse events: fever, fatigue, myalgia. Fever was graded as mild (38.0–38.4 °C), moderate (38.5–38.9 °C), and severe (>39.0 °C). Fatigue, myalgia, and other systemic events were graded as mild (no interference with activity), moderate (some interference with activity not requiring medical intervention), and severe (prevents daily activity, requires medical intervention).

(75.7%). The majority systemic adverse events after the first immunization were mild (77.7%) with duration of 48–72 h (39.5%).

After the second immunization, the majority of the reported local adverse events were mild (95.7%) and lasted for less than 48 h (87.3%). The majority systemic adverse events after second immunization were mild (71%) with duration of 48–72 h (44.7%) [Table 5].

During the trial period, 4 subjects from Group I were hospitalized. One reported fever, cough, nausea, and vomiting; one reported vomiting and diarrhea; and two subjects were diagnosed with bronchopneumonia. All subjects recovered from their illnesses. A review team concluded that the symptoms were not associated with vaccination.

4. Discussion

Vaccination is the most effective way to prevent infection and severe outcomes caused by influenza viruses, particularly for younger population [12–14]. Quadrivalent influenza vaccines that could potentially provide wider protection against influenza B viruses are becoming available in developing countries, including Indonesia. This study reports the immunogenic response of children towards QIV indicated by antibody titer and the safety of QIV administration. Our study demonstrated a significant increase of antibody titer towards each strain of QIV among children of age 6 months to 8 years.

In our study, at baseline, study participants had a varying degree of seroprotection against each strain of influenza A and as well as influenza B lineages. For group I (6–36 months of age), the seroprotectivity at baseline was similar with finding on recent studies by Pepin et al. (2018) conducted at Latin America, Asia, Africa, and Europe [17] where majority of subjects in group I were seronegative for each strain. After two doses of vaccination, seroprotection at group I for each strain was >85%. Similar with study by Statler al. (2018) at the United States [18], post-vaccination seroprotection in group II were higher for A strains than B strains. At baseline, most subjects in group II (3–8 years of age), were seropositive for both influenza A strains. The finding in group II is consistent with that of Soedjatmiko et al. (2017) reported that assessed immunogenicity of TIV among Indonesian children, in which majority of subjects in this age group were seropositive towards A/H3N2 and A/H1N1 at baseline [16]. This indicates that the subjects, particularly those older than 3 years old, had pre-existing immunity towards all strains of influenza virus, suggesting prior exposure to influenza virus some time in their life.

In Indonesia, there were two nationwide study on influenza virus surveillance. In 2003–2007, a laboratory-based surveillance of the influenza virus was conducted across the archipelago. Influenza was identified throughout the year with no specific seasonal pattern. Samples from patients with Influenza-like-illness (ILI) were examined using real time PCR to determine etiology. Among ILI-cases tested positive for influenza, 64.6% were identified as A/H3N2, 34.9% as A/H1N1, and 35.1% were influenza B [19] (Kosasih et al., 2012). In 2013, Indonesia set up a national surveillance system for severe acute respiratory infection. Of those tested positive for Influenza infection, 46% were influenza A/H3N2, 18% A/H1N1pdm09 and 37% influenza B [20]. The findings in our study are consistent with the surveillance data conducted in Indonesia. However, despite the positive seroprotection against several influ-

enza strains at baseline, QIV was still able to elicit significant immunological response by increasing antibody titer to ≥4 times against all strains in both age groups.

The GMT titer in our study showed a diverse response towards influenza A strains and B strains. In general, children aged 3–8 years showed a higher GMT towards all influenza strains during baseline and post-vaccination compared to children aged 6–35 months, this finding is consistent with previous study [21]. The Increase of GMT was most prominent against H3N2 and H1N1 of more than 10 folds. The European Committee for Medicinal Products for Human Use (CHMP) guideline for the development of new vaccines suggest a minimum increase of 2.5 folds of GMT, rate of seroconversion 40% and seroprotection 70% for new vaccines [22]. However, this guideline is only applicable for the adult population. In the pediatric population no guideline is available. Although there are currently no criteria for children, our study shows a high seroprotection rate against all four strains of influenza.

In a randomized placebo-controlled trial, Pepin et al, (2018) demonstrated two full dosage of QIV for children aged 6–35 months. The results indicate the dosage was well accepted within the age group [17]. In our study, children of age 6–35 months received half dose of QIV during each administration. The rate of seroprotection and seroconversion were achieved in approximately 85% of the subjects with significant difference between pre- and post-vaccination.

We also found a significant difference on seroprotection rate and ≥4 times titer increases between Group I and Group II, specifically towards B/Yamagata and B/Victoria. Although less than half of the children in Group II had positive titer against both Influenza B strains during baseline, their immunogenic response is significantly higher than Group I.

QIV was well tolerated in both age groups. Subjects aged 6–35 months reported higher frequency of immediate local reactions compared to subjects aged 3–8 years (17% vs 16.3%). Most frequent solicited local reactions reported were pain and redness, and most frequent solicited systemic reactions were fever, myalgia, and fatigue. Solicited AEs in our study correlates with other studies conducted among children population from other countries. J.B. Cadorna-Carlos et al. (2015) reported ≤1% of children and adult subjects in their study experience fever, malaise, myalgia, and shivering. Injection-site reactions reported were pain, erythema, swelling, and induration [23].

Several mild adverse events were reported in this study. In both age groups, pain and redness at injection site were the most frequent reactions reported. These findings are similar with other vaccine trials conducted in children and adult. Common Local reactions include pain, redness, and induration. These are mild symptoms and rarely persist longer than 24–48 h [24]. Systemic symptoms reported were also consistent with other trials, which are fever and myalgia.

However, among subjects of age 6–35 months, four serious adverse events that require hospitalization were reported. Two subjects reported fever, cough, and rhinorrhea later diagnosed as bronchopneumonia. One subject reported fever, cough, nausea, and vomit. One subject reported vomitting and diarrhea. All subjects were treated and had good outcome. There was no report of any serious adverse event among subjects aged 3–8 years. There is a high prevalence of bronchopneumonia among children in Indonesia. Review by AEFI concluded the symptoms reported by these subjects were not directly linked to QIV.

There was no comparator arm for this component of the study. However, we found the addition of a fourth influenza strain in QIV did not compromise seroprotection against all influenza strains. The investigational QIV was immunogenic with an acceptable safety profile in children of 6 months to 8 years of age.

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Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Meita Dhamayanti, Kusnandi Rusmil, Eddy Fadlyana, Rodman Tarigan, Dwi Prasetyo, Nelly Amalia, Viramitha, K Rusmil, Hadyana Sukandar, Cissy B Kartasasmita, received grant support through their institutions. Rini Mulia Sari and Novilia Sjafri Bachtiar are employees of PT Bio Farma at the time of this study and manuscript preparation.

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Contribution statement

MD was a national principal investigator. MD, KR, EF, RMS, and NSB conceived the study and its design. MD, KR, and EF participated in drafting the manuscript. MD, EF, RT reviewed the design. DP, NA, VKR, RT and EF recruited the subjects and conducted the study. CBK was the medical advisor and reviewed the study and manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

This trial has been approved by Research Ethics Committee Universitas Padjajaran, Bandung (no.887/UN6.C.10/PN/2017). A written form of informed consent was obtained from every child's parent or legal guardian before recruitment.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2019.12.008.

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